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Effect of dietary neutral detergent fibre source on lambs growth, meat quality and biohydrogenation intermediates

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ABSTRACT

With this trial we have tested the effects of structural and chemical composition of neutral detergent fibre (NDF) of the diet on lamb fatty acid composition of meat and subcutaneous fat. Twenty lambs, were fed complete diets with low starch and similar NDF content of different origin (ground alfalfa or soybean hulls). Animal performance and product quality were not affected by treatments. Rumen pH increased and parakeratosis intensity decreased with the level of alfalfa in the diet. Increasing the alfalfa proportion in the diet decreased *t*10–18:1 ($P = .023$), increased *t*11–18:1 ($P = .003$) and decreased the *t*10/*t*11 ratio according to a quadratic pattern ($P = .020$). Chemical composition and structure of the diet's fibrous fraction influenced the BI pattern of the final product. Forty percent of alfalfa in diet reduced the severity of *t*10-shift, but for its full resolution, other factors should be considered including forage particle size and buffering capacity of the diet.

1. Introduction

Rumen biohydrogenation (BH) is a set of biochemical processes by which dietary unsaturated fatty acids (FA), mainly oleic (*c*9–18:1), linoleic (18:2 n-6) and linolenic acids (18:3 n-3), are isomerised, hydrogenated and ultimately converted to stearic acid (18:0) by rumen microbiota. The BH pathways yield variable amounts of FA with conjugated double bonds, *cis* and *trans* isomers which appear in meat and milk of ruminants. When fed with high-forage diets the main biohydrogenation intermediates (BI) deposited in their tissues are vaccenic (*t*11–18:1) and rumenic (*c*9,*t*11–18:2) acids (Bessa, Alves, & Santos-Silva, 2015). Both *t*11–18:1 and *c*9,*t*11–18:2 are considered healthy FA and nutritional strategies to enrich them in ruminant products have been extensively researched (Mapiye et al., 2015; Scollan et al., 2014). The supplementation of high-forage diets with C18 polyunsaturated FA is probably the most effective approach to achieve that goal (Bessa et al., 2015). However, in intensive production systems, meat ruminants are usually fed with low fibre and high concentrate cereal based diets, and under such conditions BH pathways change, and the *t*10–18:1 often becomes the overwhelming BI. Contrarily to *t*11–18:1 and *c*9,*t*11–18:2, the *t*10–18:1 might exert deleterious health effect in the consumers (Chikwanha, Vahmani, Muchenje, Dugan, & Mapiye, 2018; Hodgson, Wahlqvist, Boxall, & Balazs, 1996). The occurrence of this shift of the BH pathways, hereafter *t*10-shift, has also been associated with low milk fat syndrome in dairy cows (Griinari & Bauman, 2001). The *t*10-shift can be monitored using the ratio between *t*10–18:1 and *t*11–18:1 (*t*10/*t*11 ratio) in the rumen or tissues, and *t*10/*t*11 ratio values above 1 clearly indicate the occurrence of the *t*10-shift (Bessa et al., 2015). The causes of *t*10-shift are not known, but in the presence of high polyunsaturated FA (PUFA) concentration, the *t*10-shift is associated to high dietary starch (Maia, Bessa, & Wallace, 2009; Zened, Enjalbert, Nicot, & Troegeler-Meynadier, 2013) and low rumen pH (Choi et al., 2005).

The need to reconcile intensive meat production systems with high nutritional value of meat lipids led us to raise the hypothesis that replacing cereals with low starch agro-industrial by-products in finishing diets would prevent the occurrence of the *t*10-shift. Following this approach, we were able to reduce the *t*10/*t*11 ratio in the milk of dairy sheep (Santos-Silva et al., 2016) and lamb meat (Francisco et al., 2017). However, we also reported unexpected results showing that diets with low starch and high fibre contents are compatible with the occurrence of *t*10-shift in growing lambs (Costa et al., 2017). Thus, the proportion

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and nature of fibrous feeds in diets may play an important role in the occurrence of *t*10-shift. We now hypothesized that the effects of neutral detergent fibre (NDF) on BH depend on its structural and chemical composition, and that the low fermentable NDF of alfalfa will be more effective in preventing the *t*10-shift than the high fermentable NDF of soybean hulls. To test this hypothesis we conducted an experiment where lambs were fed diets with similar NDF content but differing in the origin (ie. ground alfalfa or soybean hulls) and evaluated the presence of BI in lamb tissues. In addition, possible collateral effects of diet on lamb growth performance and carcass and meat quality traits were evaluated.

2. Materials and methods

2.1. Animals and management

Twenty crossbred male lambs born in late winter of 2016 from Merino Branco ewes were used for the experiment, and animal handling followed the Directive 2010/63/EU (EU, 2010) concerning to the protection of animals used for scientific experiments. The lambs were born on a farm at Baixo Alentejo (Portugal) and reared with their dams on extensive pasture until weaning at about 60 days of age. Afterwards lambs were fed indoors with commercial concentrate for approximately four weeks. During that period, the lambs were dewormed for gastrointestinal and pulmonary parasites (Seponver® Plus, Ecuphar Veterinaria, Spain) and vaccinated against pasteurellosis and clostridial diseases (Heptavac® P Plus., MSD Animal Health, Portugal), and coccidiosis (Vecoxan® 2.5 mg/ml, Esteve Veterinaria, Spain). Lambs were transported to Estação Zootécnica Nacional, Instituto Nacional de Investigação Agrária e Veterinária (EZN-INIAV), located at Vale de Santarém, Portugal in April, where the experiment was performed. Lambs were housed in individual pens with 1.25 m^2 of area and wood shaving beds and had permanent access to clean water. The experiment started after 7 days of adaptation to the facilities and to the experimental period and lasted for 6 weeks. The average live weight of lambs at the beginning of the experiment was 24.6 ± 2.21 kg (mean ± SD).

Three experimental diets were formulated and prepared at EZN-INIAV feed mill facilities, attempting to obtain similar NDF, crude protein (CP) and ether extract (EE) proportions. The forage source of the diets was dehydrated pelleted alfalfa (*Medicago sativa*) that was balanced with different proportions of soybean hulls (*Glicine* max), and dehydrated citrus and beet pulps. The diets contained 200, 400 or 600 g/kg DM of alfalfa were named as low (LA), medium (MA) and high (HA) alfalfa, respectively. All diets contained 6% soybean oil and were presented to the lambs milled (< 3 mm). Lambs were randomly assigned to individual pens, with seven replicates for diets LA and MA and six replicates for the diet HA. Every day, at 9:00 am, feed refusals were weighed, registered and discarded and feed was provided at 110% of previous days intake. The chemical composition of the diets were averaged from three sets of analysis performed on pooled samples collected at the beginning, middle and end of the experimental period. Metabolizable energy was determined according to (Sauvant, Chapoutot, Peyraud, Meschy, & Doreau, 2002) and the proximal and chemical compositions of the diets are presented in Table 1. During the experimental period, animals were weighed once a week before feeding.

2.2. Slaughter procedures, carcasses evaluation and sample collection

Lambs were slaughtered at the experimental abattoir of EZN-INAV, located 400 m from the lamb barn. Lambs were weighed before transport to the abattoir. After a maximum waiting period of 30 min, lambs were stunned and exsanguinated. The rumen was emptied immediately and ruminal wall was evaluated for the occurrence of parakeratosis using a visual scale, as described by Tamate, Nagatani, Yoneya, Sakata, and Miura (1973). The whole rumen content was collected and homogenized. A sample was strained through 4 layer of cheesecloth, resulting in an aliquot with about 80 ml of rumen liquor, and its pH was immediately measured using a pH meter (Metrohm 744). An aliquot of 2 ml of the strained rumen fluid was also preserved at −20 °C for volatile fatty acids (VFA) analysis.

Hot carcass weight (HCW) was measured immediately post-slaughter and carcasses were kept in a refrigerated room at 10 ± 1 °C for 24 h to prevent cold shortening. At 24 h, carcasses were evaluated for conformation and fat cover, using the SEUROP classification system for lamb carcasses weighing > 13 kg (EC, 2011), and re-weighed to determine cold carcass weight (CCW). Carcasses were then chilled at 2 ± 1 °C and 72 h after slaughter, kidney and kidney knob channel fat (KKCF) was removed, carcasses were split along the spine and the left sides were separated into eight joints that were individually weighed to estimate the proportion of the higher-priced joints (leg + chump + loin + ribs) as described by Santos-Silva, Bessa, and Santos-Silva (2002). Shoulders were vacuum-packed and frozen at −20 ± 1 °C until dissection to estimate the percentages of muscle, intermuscular and subcutaneous fat (ScF), and bone. Loin joints, containing the *Longissimus lumborum* muscle (LL), were vacuum-packed and kept for 4 days in a refrigerator at 2 ± 1 °C before being frozen at −20 ± 1 °C, for 30 days, until shear force and cooking loss determination. The *Longissimus thoracis* (LT) was isolated from rib joints. Colour coordinates were evaluated on a 1 cm chop after 1 h blooming at room temperature (day 0 of storage). Other LT chops with similar thickness were placed on polystyrene trays and over-wrapped with oxygen permeable polyvinyl chloride film, and maintained at 2 °C under continuous light for

Table 1
Ingredients, chemical composition and fatty acid (FA) profile of the experimental diets.

Item	Diets[1]		
	LA	MA	HA
Ingredients, g/kg			
Dehydrated alfalfa	200	400	600
Soybean hulls	338	235	90
Dehydrated citrus pulp	70	56	30
Dehydrated beet pulp	120	54	40
Soybean meal	187	170	155
Soybean oil	60	60	60
Calcium carbonate	13	13	13
Sodium bicarbonate	5	5	5
Salt	4	4	4
Premix[2]	3	3	3
Chemical composition, g/kg DM			
DM[3]	907.0	914.0	917.3
CP	177.1	168.9	182.8
Ether extract	79.3	76.3	71.0
FA	75.9	72.1	74.8
Starch	55.5	55.4	58.3
Sugar	94.9	102.4	108.7
NDF	437.4	432.5	417.8
ADF	285.9	284.5	271.1
ADL	25.4	36.8	40.7
ME (kJ/kg DM)	13.35	13.01	12.81
FA profile, g/kg total FA			
14:0	11.0	9.3	25.6
16:0	127.1	123.5	123.7
18:0	41.0	41.2	41.4
*c*9-18:1	229.0	224.7	225.1
*c*11-18:1	19.8	19.8	19.8
18:2n-6	512.7	516.8	505.1
18:3n-3	59.4	64.7	59.3

[1] LA- Low alfalfa; MA – Medium alfalfa; HA – High alfalfa.
[2] Premix composition – Vit A – 4000000 UI; Vit D3 – 1100000 UI; Vit E 7.5 g/kg; Vit B1 and B2 – 250 mg/kg; Zn – 35 g/kg; Fe – 12.5 g/kg; Mn – 17.5 g/kg; I – 200 mg/kg; Co – 250 mg(kg; Se – 100 mg/kg.
[3] g/kg feed.

7 days. On day 7 of storage, meat colour was measured and each chop was vacuum-packed and stored at −80 °C for lipid oxidation analysis. The *epimysium* was removed from the remaining portion of the LT, and the LT was ground with a food processor (Moulinex-123 A320R1) (3 × 5 s) and vacuum-packed and stored at −20 °C until chemical analysis and pH determination. Subcutaneous fat (ScF) samples were also collected for analysis of FA at the level of the 12th vertebra. Subcutaneous fat colour was evaluated on the inner surface of the leg, near the tail insertion.

2.3. Analytical procedures

2.3.1. Feed, rumen and muscle chemical analysis

Feed was analysed for dry matter (DM), crude protein (CP), ether extract (EE), starch, NDF, acid detergent fibre (ADF) and lignin (ADL) and lipid composition following the procedures described by Francisco et al. (2017). Rumen volatile FA profile was determined by gas chromatography, as described by Oliveira, Alves, Santos-Silva, and Bessa (2016).

Meat DM was measured on LT as described by Santos-Silva et al. (2016). Intramuscular lipids were extracted according to Folch, Lees, and Stanley (1957) as described by Jerónimo, Alves, Prates, Santos-Silva, and Bessa (2009). Fatty acids from intramuscular and ScF lipids were transesterified according to Raes, De Smet, and Demeyer (2004), using sodium methoxide in methanol, followed by hydrochloric acid in methanol (1:1 *v*/v). Fatty acid methyl esters were analysed using a Shimadzu GC 2010 Plus chromatograph (Shimadzu, Kyoto, Japan), equipped with a flame-ionization detector and fused silica capillary column (SP-2560 (100 m × 0.25 mm internal diameter × 0.20 μm film thickness, Supelco, Bellefonte, PA, USA)). The injector and detector temperatures were 250 °C and 280 °C, respectively. The initial oven temperature of 50 °C was held for 1 min, increased at 50 °C/min to 150 °C and held for 20 min, increased at 1 °C/min to 190 °C and then increased at 2 °C/min to 220 °C and held for 40 min. Helium was used as carrier gas at a flow rate of 1 ml/min and the split ratio was 50:1. Nonadecanoic acid (19:0) was used as internal standard to quantify muscle lipid FA methyl esters. Fatty acids were identified by comparison of the FAME retention times with those of authentic standards (FAME mix 37 components from Supelco Inc., Bellefont, PA, USA) and by comparison with published chromatograms (Alves & Bessa, 2009; Vahmani, Rolland, Gzy, L, & Dugan, 2016). In addition, a few FAME extracts of the muscle or ScF samples were hydrogenated (Alves et al., 2013) or derivatized into dimethyloxazoline derivatives (Alves & Bessa, 2014) and subsequently analysed by electron impact mass spectrometry using a Shimadzu GC–MS QP2010 Plus (Shimadzu, Kyoto, Japan) system to reveal and identify more clearly the branched chain FA and the BI.

Lipid oxidative stability in meat was assessed by quantification of thiobarbituric acid reactive substances (TBARS) following the method described by Grau, Guardiola, Boatella, Barroeta, and Cordony (2000). Briefly, 2 g of meat was homogenized in 1 ml of 0.3% aqueous ethylenediaminetetraacetic acid disodium salt (EDTA), 8 ml of 5% aqueous trichloroacetic acid (TCA) and 5 ml of 0.8% butylated hydroxytoluene (BHT) in hexane using an Ultra-Turrax T25 digital homogenizer (IKA Werbe GmbH & Co. KG, Staufen, Germany) for 30 s at 19000 rpm. The homogenates were centrifuged for 5 min at 1400*g* and the top hexane layer discarded. The bottom layer was filtered, and TCA was added to filtrate (5% aqueous) to make up a volume of 10 ml. A 2.5 ml aliquot from the bottom layer was mixed with 1.5 ml of 0.8% aqueous 2-thiobarbituric acid (TBA) and incubated at 70 °C for 30 min. Following incubation, the mixture was cooled under tap water and the absorbance was measured at 532 nm in a Double-beam UV–Vis scanning spectrophotometer (Helios alpha spectrophotometer, Thermo Scientific, Bremen, Germany). The 1,1,3,3 tetraethoxypropane standard curve was used for calculating the TBARS concentration and the results were expressed as mg of malonaldehyde (MDA)/kg of meat.

2.3.2. Meat quality determinations

Meat quality was assessed based on colour, pH, cooking loss and Warner-Bratzler shear force. The colour was measured using a Minolta CR-300 Chromometer (Konica Minolta, Japan) according to the CIELAB system, where L^* is lightness, a^* redness and b^* yellowness. Determinations were made using the C illuminant and a 2° standard observer. Colour saturation index (Chroma, C^*) was calculated as $(a^{*2} + b^{*2})^{1/2}$ and the colour Hue angle (H^*) as (tan-1(b^*/a^*) x (180/π)) (AMSA, 2012). Meat colour variation (ΔE) during 7 days of storage were determined as follows: $\Delta E\ (7–0) = ((\Delta L^*\ (7–0))^2 + (\Delta a^*\ (7–0))^2 + (\Delta b^*\ (7–0))^2)^{1/2}$. Muscle pH was determined according to ISO2917/1999. The pH was measured in a suspension of 5 g of minced meat in 50 ml of a solution of potassium chloride 0.1 M, using a pH meter (Metrohm 744) equipped with a combined glass electrode. Meat cooking loss and Warner-Bratzler shear force were determined on LL, isolated from the left loin joints that were thawed for 20 h at 2 ± 1 °C and cooked in a non ventilate electric oven, with the resistors covered by aluminium foil, at 170 ± 5 °C until an internal temperature of 71 ± 1 °C was reached. *Longissimus lumborum* temperatures were monitored individually with thermocouples (Thermometer, Omega RDXL4SD, Manchester, USA) and were cooled in a refrigerator at 2 ± 1 °C and weighed after 20 h (AMSA, 2015). The cooking loss was determined as the difference between the weights of the intact LL, before and after cooking and expressed as a percentage of initial weight. To determine maximum shear force (N), 1 cm^2 sections of LL muscle were sheared across the grain using a standard Stable Micro Systems Warner-Bratzler shear device, with 1.016 mm thickness blade, mounted in a Texture Analyser (TA.XT *plus* Texture Analyser, Stable Micro Systems, Surrey, UK) equipped with a compression load cell of 25 kgf. Sections were sheared using a crosshead speed of 2 mm/s. Data were collected using Texture Exponent version 6.1 software (Stable Micro Systems, Surrey, UK). The maximum force for each sample was averaged from a minimum of twelve to a maximum of 27 tests recorded.

2.4. Statistical analysis

Data were analysed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Individual lambs were the experimental unit and the level of significance was set at $P < .05$. Variance homogeneity was tested at $P = .01$, and when significant accommodated in the model using the group option within the repeated statement of the Proc Mixed.

Weekly BW data were subjected to regression analysis to estimate ADG (*i.e.* slopes). Daily DMI and weekly feed conversion ratio were analysed using a repeated measures model including diet as the main effect and day as the repeated measure, considering a first order autoregressive (AR(1)) covariance structure.

Body weight, rumen pH, carcass and meat quality traits were analysed as a completely randomized experimental design, considering diet as the single fixed effect.

For FA composition, besides diet the model also included the adipose depot and the interaction between those two fixed effects, using an unstructured (UN) covariance structure.

Linear and quadratic polynomial orthogonal contrasts were used to test the effects of increasing dehydrated alfalfa in the diet. Slaughter weight, hot and cold carcass weights were adjusted to lamb initial weight; shoulder composition data were adjusted to hot carcass weight. When the effect of diet was significant, the least square means were compared, using pairwise Tukey's comparison test.

Spearman's rank coefficient was used to correlate rumen parakeratosis level and pH, using the Graphpad Prism ® 5.03 software (Graphpad Software Inc., La Jolla, CA).

Table 2
Effect of dietary neutral detergent fibre (NDF) composition on nutrient intake and growth performance of lambs.

	Diets[1]			*P*-value	
	LA	MA	HA	*Linear*	*Quad*
Intake, g/d					
DM	1352 ± 49.8[a]	1471 ± 45.4[ab]	1531 ± 49.0[b]	0.013	0.605
CP	239 ± 8.8[a]	249 ± 7.9[a]	280 ± 8.5[b]	0.002	0.295
Sugar	128 ± 5.1[a]	151 ± 5.1[b]	166 ± 4.0[c]	< 0.001	0.591
Starch	75.1 ± 2.86[a]	81.6 ± 2.85[a]	89.3 ± 2.14[b]	< 0.001	0.830
NDF	591 ± 22.2	631 ± 22.2	640 ± 15.2	0.069	0.540
ADF	386 ± 14.2	417 ± 14.5	415 ± 10.0	0.100	0.339
ADL	34.6 ± 1.43[a]	49.5 ± 1.73[b]	62.1 ± 1.54[c]	< 0.001	0.554
ME (MJ/d)	18.06 ± 0.810	19.16 ± 0.809	19.63 ± 0.552	0.106	0.733
Total fatty acids	107 ± 4.0	112 ± 3.9	109 ± 2.6	0.721	0.354
14:0	1.16 ± 0.049[a]	1.06 ± 0.049[a]	2.73 ± 0.082[b]	< 0.001	< 0.001
16:0	13.6 ± 0.49	13.9 ± 0.49	13.5 ± 0.32	0.794	0.584
18:0	4.40 ± 0.162	4.62 ± 0.162	4.51 ± 0.107	0.579	0.379
*c*9-18:1	24.5 ± 0.89	25.2 ± 0.89	24.5 ± 0.58	0.973	0.512
*c*11-18:1	2.12 ± 0.078	2.22 ± 0.078	2.16 ± 0.051	0.0.685	0.350
18:2n-6	54.9 ± 2.03	57.9 ± 2.02	55.0 ± 1.31	0.984	0.215
18:3n-3	6.37 ± 0.244[a]	7.24 ± 0.243[b]	6.46 ± 0.154[a]	0.751	0.005
Initial BW, kg	25.3 ± 0.81	24.9 ± 0.81	23.3 ± 0.88	0.114	0.532
Slaughter BW, kg	36.4 ± 0.98	36.8 ± 0.96	37.5 ± 1.09	0.434	0.887
ADG, g/d	282 ± 10.9	283 ± 10.9	304 ± 11.8	0.178	0.487
Feed conversion ratio[2]	4.57 ± 0.266	4.56 ± 0.279	5.02 ± 0.290	0.261	0.485
Rumen parameters					
pH	5.45 ± 0.091[a]	5.49 ± 0.091[a]	5.79 ± 0.098[b]	0.023	0.281
Volatile fatty acids					
Total, mmol/L	74.4 ± 2.80	78.1 ± 2.80	67.2 ± 3.97	0.174	0.065
Molar percentages					
2:0	52.5 ± 1.01[ab]	54.8 ± 1.01[b]	51.1 ± 1.10[a]	0.377	0.028
3:0	32.6 ± 1.11	29.8 ± 1.11	31.7 ± 1.66	0.665	0.140
iso-4:0	4.01 ± 0.860	2.73 ± 0.860	3.52 ± 0.447	0.614	0.312
4:0	10.4 ± 0.81	11.7 ± 0.81	12.1 ± 1.62	0.393	0.707
2:0/3:0 ratio	1.63 ± 0.081	1.86 ± 0.081	1.65 ± 0.143	0.898	0.075

Means within a row with different superscripts differ ($P < .05$).
[1] LA- Low alfalfa; MA – Medium alfalfa; HA – High alfalfa.
[2] kg dry matter intake/kg weight increase.

3. Results

3.1. Intake and growth performance

Daily feed intake and growth performance of lambs are presented in Table 2. Increasing alfalfa proportion in the diet linearly increased DM intake ($P = .013$), ranging from 1352 g/d for LA diet to 1531 g/d for the HA diet. Intake of CP ($P = .002$), sugar ($P < .001$), starch ($P < .001$), ADL ($P < .001$) and of 14:0 ($P < .001$) were also higher for the HA than the LA diet. Quadratic effects were only observed for 14:0 and 18:3 n-3 intake. Diet did not influence growth rate of lambs nor feed conversion ratio, which averaged 290 ± 38.5 g and 4.72 ± 1.564, respectively. The results of rumen parameters are presented in Table 2. Rumen pH increased linearly ($P = .023$) with the increase of alfalfa in the diet, and HA (5.79) had a higher value than the other two diets (≈ 5.47). For volatile FA composition of rumen content, only a quadratic effect was observed for acetate ($P = .028$), with a higher value for MA diet. The evaluation of parakeratosis lesions revealed that with the LA diet, 43% of the lambs presented moderate (level 2) and 57% strong lesions (level 3) in rumen wall. In groups fed with MA and LA diets there were no lambs with level 3 lesions, 29% and 3% presented moderate lesions (level 2), 57% and 33% presented weak lesions (level 1) and 14% and 33% showed normal rumen wall (level 0), respectively.

3.2. Carcass traits and meat quality of lambs

Diet did not affect hot carcass weight, cold carcass weight or dressing percentage averaging 18.2 ± 1.60 kg, 17.5 ± 1.61 kg and 49.4 ± 2.63%, respectively (Table 3). For conformation, 15% of the carcasses were graded as U (very good); 70% as R (good) and 15% as O (fair). For fat cover, most of the carcasses (70%) were graded in classes 2 or 3 (slight and average, respectively) and 30% in class 4 (high). The percentage of higher priced cuts in carcasses were unaffected by alfalfa proportion in diets, averaging 54.5 ± 0.96%. Also, carcass composition did not differ among diets (Table 3). The mean KKCF content was 2.38 ± 0.759% and the average tissue composition of the shoulder was 60.8 ± 1.79% of muscle, 19.0 ± 1.53% of bone, 11.1 ± 1.36% of intermuscular fat and 9.1 ± 2.12% of ScF. Meat quality results are presented in Table 3 and were generally not influenced by diet. Muscle pH, intramuscular fat, cooking losses and shear force averaged 5.65 ± 0.075, 126.5 ± 10.40 mg/g DM, 30.1 ± 2.36% and 27.0 ± 3.52 N/cm^2, respectively. Average values for meat colour parameters were 41.6 ± 1.95 for L^*, 18.6 ± 1.23 for a^*, 5.71 ± 0.951 for b^*, 19.5 ± 1.37 for C^* and 17.0 ± 2.15 for H^*. Only a small linear increase was observed for for L^* as the proportion of alfalfa increased ($P = .043$). For colour, L^*, a^* and b^* averaged, 77.5 ± 3.98, 3.88 ± 0.834 and 5.48 ± 1.35, respectively. Colour stability of meat, determined after 7 days of storage was not influenced by diet, with an average ΔE(7) of 6.4 ± 1.84. Meat lipid oxidation at 7 days of storage did not differ among diets, averaging 1.28 ± 0.508 mg MDA/kg of meat.

3.3. Fatty acid composition of muscle and subcutaneous fat

Total FA content did not differ among treatments and averaged

Table 3
Effect of dietary neutral detergent fibre (NDF) composition on carcass traits and meat quality of lambs.

	Diets[1]			*P*-value	
	LA	MA	HA	*Linear*	*Quad*
Carcass traits					
Hot carcass wt, kg	18.1 ± 0.36	18.3 ± 0.35	18.1 ± 0.40	0.917	0.645
Cold carcass wt, kg	17.4 ± 0.37	17.6 ± 0.36	17.4 ± 0.41	0.986	0.554
Dressing, %	49.9 ± 1.00	49.6 ± 1.00	48.5 ± 1.12	0.388	0.727
KKCF[2], %	2.37 ± 0.311	2.54 ± 0.309	2.22 ± 0.347	0.757	0.531
Higher priced cuts[3], %	55.1 ± 0.36	54.4 ± 0.36	54.1 ± 0.40	0.090	0.660
Shoulder composition (%)					
Muscle	60.7 ± 0.56	60.4 ± 0.56	61.3 ± 0.62	0.508	0.421
Bone	19.2 ± 0.53	18.5 ± 0.53	19.5 ± 0.59	0.779	0.201
Intermuscular fat	11.1 ± 0.41	10.9 ± 0.41	11.3 ± 0.46	0.720	0.518
Subcutaneous fat	8.94 ± 0.695	10.21 ± 0.692	7.90 ± 0.778	0.346	0.056
Subcutaneous fat colour[4]					
L*	79.6 ± 1.44	75.9 ± 1.44	76.7 ± 1.56	0.185	0.229
a*	3.39 ± 0.298	4.14 ± 0.298	4.16 ± 0.322	0.097	0.330
b*	5.40 ± 0.530	5.24 ± 0.530	5.84 ± 0.573	0.582	0.579
Longissimus muscle					
pH	5.65 ± 0.027	5.61 ± 0.027	5.69 ± 0.030	0.323	0.135
Intramuscular fat (mg/g DM)	130.0 ± 10.40	122.1 ± 10.40	127.7 ± 11.24	0.881	0.611
Shear force, N/cm [5]	28.7 ± 1.27	25.7 ± 1.27	26.48 ± 1.37	0.219	0.261
Cooking loss (%) [5]	30.1 ± 0.94	30.0 ± 0.94	30.2 ± 1.02	0.982	0.856
Color[4]					
L*	40.6 ± 0.69[a]	41.4 ± 0.69[ab]	42.8 ± 0.74[b]	0.043	0.713
a*	19.1 ± 0.27	18.2 ± 0.27	18.6 ± 0.82	0.613	0.217
b*	5.78 ± 0.359	5.32 ± 0.359	6.06 ± 0.388	0.609	0.195
Colour stability (0 to 7d) [6]					
ΔE	7.01 ± 0.709	5.88 ± 0.709	6.32 ± 0.766	0.503	0.384
TBARS[7]	1.22 ± 0.200	1.38 ± 0.200	1.23 ± 0.216	0.960	0.523

[a-b]Means within a row with different superscripts differ (P < .05).
[1] LA – Low alfalfa; MA – Medium alfalfa; HA – High alfalfa.
[2] Kidney knob channel fat, expressed as % of cold carcass weight.
[3] Leg + chump + loin + ribs, expressed as % of cold carcass weight.
[4] At day 0 of storage.
[5] At day 4 of storage.
[6] Colour stability between days 0 and 7 of storage.
[7] Thiobarbituric acid reactive substances (TBARS) at day 7 of storage, expressed as mg of malonaldehyde / kg of meat.

666 ± 69.0 and 104 ± 27.0 mg/g DM for ScF and LT respectively (Table 4). The FA composition of ScF and LT are presented in Table 4 and BI are detailed in Table 5. Fatty acid composition of both ScF and LT were similarly affected by diet. In both tissues, the major FA was *c*9–18:1, followed by 16:0 and 18:0. The *c*9–18:1 and 18:0 proportions were not influenced by diet. The *c*9–18:1 averaged 282 ± 27.9 and 319 ± 22.4 mg/g FA for ScF and LT respectively. The 18:0 proportions were 160 mg/g FA, for both tissues. The proportion of 16:0 linearly decreased ($P = .016$) with alfalfa inclusion, from 208 ± 1.2 to 179 ± 1.3 mg/g FA in ScF and from 240 ± 4.7 to 219 ± 5.1 mg/g FA in LT. The increase of alfalfa proportion in the diet linearly increased the sum of C18 FA ($P < .001$), the sum of n-6 PUFA ($P = .015$) and the tended to increase Total PUFA ($P = .05$). These results were mainly influenced by 18:2 n-6, which increased linearly ($P = .034$) with the level alfalfa in the diet.

The sum of BI did not differ among diets but relevant differences were detected in the proportions of several individual BI. The major BI in the ScF and meat of lambs fed all the diets was the *t*10–18:1 followed by the *t*11–18:1. Increasing the alfalfa proportion in the diet led to linear decreases in *t*10–18:1 ($P = .023$) and *t*10,*c*12–18:2 ($P = .002$), a quadratic decrease in the *t*10/*t*11 ratio ($P = .020$), and linear increases in *t*11–18:1 ($P = .003$), *t*9–18:1 ($P = .012$), *t*15–18:1 ($P = .017$), *c*12–18:1 ($P = .005$), *t*16–18:1 ($P = .016$), *c*15–18:1 ($P = .012$), *c*9,*t*11–18:2 ($P = .003$), the sum of CLA*tt* isomers ($P = .055$) and the sum 18:2BI ($P < .001$).

Fat depot location had a high impact on the FA composition. In a total of 48 FA that were quantified, 42 were affected by tissue. Globally, ScF when compared with LT intramuscular fat, presented lower proportions of saturated FA (SFA) ($P = .044$), n-6 ($P < .001$) and n-3 ($P < .001$) PUFA and higher proportions of terminal branched chain FA (TBCFA) ($P < .001$), non-terminal branched chain FA (NTBCFA) ($P < .001$) and total *cis*-monounsaturated FA (cis-MUFA) ($P = .001$) (Table 4). Subcutaneous fat presented higher proportions of all BI (Table 5), except *t*15 and *c*12–18:1, which were not affected by depot location. The t10/t11 ratio was also not affected by the tissue type ($P = .263$).

4. Discussion

Voluntary DM intake increased linearly with the level of dehydrated alfalfa in the diet (Table 2), but the Metabolizable Energy intake was not affected (results not shown). This suggests that DM intake was mainly regulated by energy satiety mechanisms. However, probably the growth rate of the lambs for all diets was below their genetic growth potential (Costa et al., 2017), and so other regulatory mechanisms of intake would likely be involved.

The rumen pH was quite low (bellow 5.8) for all diets suggesting the occurrence of sub-acute rumen acidosis. Such low rumen pH values are favoured by the fact that complete diets are fed ground, and thus the small forage particle size did not properly stimulate rumination and salivation. In practical ruminant nutrition, the effects of rumen pH are often confounded with that of dietary content of starch and other high fermentable carbohydrates. Moreover, the low rumen pH values observed were consistent with the severity of parakeratosis lesions on

Table 4
Effect of dietary neutral detergent fibre (NDF) composition on lipids and fatty acid (FA) profile (g/100 g of total FA) in *Longissimus thoracis* muscle of lambs.

	Subcutaneous fat			Intramuscular fat			*P values*			
	LA[1]	MA[2]	HA[3]	LA[1]	MA[2]	HA[3]	*Diet contrast*		*Depot*	*Diet* depot*
							Linear	*Quad.*		
FA, mg/g DM	708 ± 24.6	647 ± 24.6	641 ± 26.5	106 ± 10.3	94 ± 10.3	112 ± 11.1	0.131	0.209	< 0.001	0.181
FA profile										
LC-SFA[4]										
10:0	0.47 ± 0.059	0.23 ± 0.059	0.38 ± 0.063	0.77 ± 0.073	0.67 ± 0.073	0.64 ± 0.078	0.116	0.049	< 0.001	0.410
12:0	0.61 ± 0.046	0.46 ± 0.046	0.51 ± 0.050	0.62 ± 0.053	0.69 ± 0.053	0.63 ± 0.058	0.451	0.691	0.003	0.059
14:0	20.9 ± 1.85	15.8 ± 1.85	16.3 ± 2.00	17.9 ± 0.76	19.0 ± 0.76	16.9 ± 0.83	0.068	0.631	0.828	0.109
15:0	6.78 ± 1.193	8.06 ± 1.193	5.55 ± 1.289	2.71 ± 0.126	2.74 ± 0.126	2.66 ± 0.136	0.478	0.207	< 0.001	0.406
16:0	208 ± 12.2	169 ± 12.2	179 ± 13.2	240 ± 4.7	237 ± 4.7	219 ± 5.1	0.016	0.298	< 0.001	0.157
17:0	19.1 ± 2.74	23.6 ± 2.74	19.1 ± 2.96	9.5 ± 0.46	8.9 ± 0.46	8.9 ± 0.50	0.888	0.237	< 0.001	0.393
18:0	156 ± 14.8	149 ± 14.8	176 ± 16.0	158 ± 6.2	157 ± 6.2	165 ± 6.7	0.260	0.292	0.937	0.731
20:0	0.77 ± 0.067	0.78 ± 0.067	0.93 ± 0.073	0.76 ± 0.035	0.74 ± 0.035	0.82 ± 0.037	0.058	0.209	0.213	0.663
sum	413 ± 8.0	368 ± 25.4	398 ± 27.5	431 ± 3.5	425 ± 8.0	414 ± 8.7	0.324	0.289	0.044	0.359
T-BCFA[5]										
iso-14:0	0.02 ± 0.054	0.13 ± 0.054	0.02 ± 0.058	0.23 ± 0.044	0.26 ± 0.044	0.28 ± 0.048	0.614	0.191	< 0.001	0.401
iso-15:0	1.13 ± 0.123	1.16 ± 0.123	1.23 ± 0.133	0.58 ± 0.089	0.66 ± 0.089	0.70 ± 0.096	0.470	0.994	< 0.001	0.938
anteiso-15:0	1.99 ± 0.150	1.83 ± 0.150	1.59 ± 0.162	0.91 ± 0.085	0.86 ± 0.085	0.89 ± 0.091	0.188	0.997	< 0.001	0.167
iso-16:0	1.90 ± 0.281	2.39 ± 0.281	1.64 ± 0.304	0.94 ± 0.068	0.92 ± 0.068	0.98 ± 0.073	0.630	0.137	< 0.001	0.154
iso-17:0	2.63 ± 0.148	2.67 ± 0.148	2.88 ± 0.159	1.85 ± 0.127	1.91 ± 0.127	2.20 ± 0.137	0.137	0.522	< 0.001	0.745
anteiso-17:0	7.27 ± 1.120	8.79 ± 1.120	6.45 ± 1.210	3.02 ± 0.195	2.73 ± 0.195	2.99 ± 0.211	0.623	0.256	< 0.001	0.300
Sum	14.9 ± 1.52	17.0 ± 1.52	13.8 ± 1.64	7.5 ± 0.53	7.3 ± 0.53	8.0 ± 0.57	0.814	0.334	< 0.001	0.230
NT-BCFA[6]										
14:0-Me[7]	5.74 ± 1.523	7.21 ± 1.523	4.41 ± 1.645	0.52 ± 0.060	0.47 ± 0.060	0.39 ± 0.065	0.524	0.270	< 0.001	0.490
16:0-Me[8]	11.0 ± 2.96	15.2 ± 2.96	10.2 ± 3.20	1.43 ± 0.140	1.34 ± 0.140	1.15 ± 0.151	0.814	0.223	< 0.001	0.479
Sum	16.7 ± 4.47	22.4 ± 4.47	14.6 ± 4.83	1.9 ± 0.19	1.8 ± 0.19	1.5 ± 0.20	0.709	0.237	< 0.001	0.492
cis-MUFA[9]										
*c*9–14:1	0.49 ± 0.069	0.44 ± 0.069	0.33 ± 0.075	0.40 ± 0.042	0.47 ± 0.042	0.38 ± 0.045	0.190	0.358	0.962	0.287
*c*7–16:1	2.86 ± 0.173	2.80 ± 0.173	2.94 ± 0.187	2.06 ± 0.096	2.09 ± 0.096	2.16 ± 0.103	0.616	0.699	< 0.001	0.880
*c*9–16:1	8.95 ± 0.731	7.19 ± 0.731	7.15 ± 0.790	10.7 ± 0.666	10.8 ± 0.666	9.26 ± 0.719	0.082	0.991	< 0.001	0.197
*c*9–17:1	7.10 ± 1.866	10.8 ± 1.87	6.49 ± 2.016	4.26 ± 0.218	4.27 ± 0.218	4.05 ± 0.236	0.766	0.096	0.002	0.278
*c*9–18:1[10]	276 ± 10.9	290 ± 10.9	280 ± 11.7	317 ± 8.9	324 ± 8.9	316 ± 9.6	0.890	0.342	< 0.001	0.889
*c*11–18:1	10.2 ± 0.37	10.3 ± 0.37	10.3 ± 0.40	11.8 ± 0.43	10.6 ± 0.43	11.1 ± 0.47	0.553	0.344	0.006	0.159
Sum	458 ± 10.4	462 ± 10.4	455 ± 11.3	439 ± 8.5	432 ± 8.5	431 ± 9.2	0.642	0.920	0.001	0.772
n-6 PUFA										
18:2n-6	53.6 ± 4.12	62.2 ± 4.12	68.3 ± 4.45	74.0 ± 5.98	85.0 ± 5.98	90.2 ± 6.46	0.034	0.715	< 0.001	0.933
18:3n-6	0.23 ± 0.024	0.20 ± 0.024	0.25 ± 0.026	0.44 ± 0.044	0.42 ± 0.044	0.57 ± 0.047	0.106	0.111	< 0.001	0.261
20:2n-6	0.35 ± 0.073	0.52 ± 0.073	0.43 ± 0.078	0.65 ± 0.053	0.63 ± 0.053	0.62 ± 0.058	0.812	0.371	< 0.001	0.173
20:4n-6	1.11 ± 0.154	1.09 ± 0.154	1.44 ± 0.167	11.7 ± 1.41	11.4 ± 1.41	12.5 ± 1.53	0.572	0.604	< 0.001	0.930
Sum	56.0 ± 4.26	64.7 ± 4.26	71.2 ± 4.60	89.2 ± 7.39	99.8 ± 7.39	106.5 ± 7.98	0.048	0.819	< 0.001	0.968
n-3 PUFA										
18:3n-3	7.18 ± 0.515	8.32 ± 0.515	8.58 ± 0.556	5.85 ± 0.413	6.53 ± 0.413	6.73 ± 0.446	0.099	0.544	< 0.001	0.402
20:3n-3	0.36 ± 0.014	0.35 ± 0.024	0.45 ± 0.025	1.27 ± 0.132	1.20 ± 0.132	1.38 ± 0.420	0.336	0.312	< 0.001	0.917
20:5n-3	0.09 ± 0.022	0.09 ± 0.022	0.12 ± 0.024	1.18 ± 0.110	1.14 ± 0.110	1.28 ± 0.119	0.416	0.444	< 0.001	0.764
22:5n-3	0.68 ± 0.078	0.62 ± 0.078	0.81 ± 0.085	2.68 ± 0.238	2.51 ± 0.238	2.87 ± 0.257	0.401	0.220	< 0.001	0.889
22:6n-3	0.07 ± 0.031	0.10 ± 0.031	0.13 ± 0.033	0.41 ± 0.057	0.45 ± 0.057	0.42 ± 0.061	0.587	0.697	< 0.001	0.698
sum	8.3 ± 0.58	9.1 ± 0.58	9.6 ± 0.63	10.1 ± 0.63	10.6 ± 0.63	11.3 ± 0.68	0.114	0.879	< 0.001	0.596
Total PUFA[11]	64.0 ± 4.82	73.9 ± 4.82	80.9 ± 5.21	99.3 ± 7.86	110 ± 7.86	118 ± 8.49	0.050	0.822	< 0.001	0.983
C18 FA[12]	675 ± 11.8	683 ± 20.5	716 ± 5.4	675 ± 5.0	680 ± 5.0	696 ± 5.4	0.001	0.442	0.396	0.410

[1] LA- Low alfalfa.
[2] MA – Medium alfalfa.
[3] HA – High alfalfa.
[4] Linear chain Saturated Fatty Acids.
[5] Terminal branched chain FA (iso and anteiso).
[6] Non terminal branched chain FA.
[7] Includes fatty acids with a linear chain length of 14 carbon atoms containing a methyl group at carbons -6, -8, -4 or -10.
[8] Includes fatty acids with a linear chain length of 16 carbon atoms containing a methyl group at carbons -6, -8, -4 or -12.
[9] Sum of all cis-Monounsaturated FA excluding the biohydrogenation intermediates.
[10] Coelutes with t13/t14 – 18:1.
[11] Sum of n-6 and n-3 PUFA.
[12] Sum of all C18 FA

rumen mucosa ($\rho = -0.56$; $P = .001$), that can be a better indicator of acidotic rumen conditions over the experimental period than just a single measurement of pH rumen after slaughter (Kleen, Hooijer, Rehage, & Noordhuizen, 2003).

Experimental diets did not affect carcass or meat quality parameters. The results obtained in the present trial were in agreement with other reports for Merino Branco lambs reared in similar conditions and with similar carcass weights (Santos-Silva, Mendes, Portugal, & Bessa, 2004). In LT, the average values obtained three days after slaughter for lightness (L^*) were above 40 and for redness (a^*) above 18

Table 5
Effect of dietary neutral detergent fibre (NDF) composition on C18 biohydrogenation intermediates (BI) (g/100g of total fatty acids) present in LM of lambs.

	Subcutaneous fat			Intramuscular fat			*P values*			
	LA[1]	MA[2]	HA[3]	LA[1]	MA[2]	HA[3]	*Diet contrast*		*Depot*	*Diet*depot*
							Linear	*Quad.*		
18:1 isomers										
*t*4-	0.62 ± 0.054	0.66 ± 0.054	0.62 ± 0.058	0.18 ± 0.018	0.17 ± 0.018	0.16 ± 0.019	0.907	0.472	< 0.001	0.885
*t*6 − /*t*7 − /*t*8-	5.99 ± 0.439	6.26 ± 0.439	6.93 ± 0.475	3.67 ± 0.193	3.53 ± 0.193	4.23 ± 0208	0.040	0.306	< 0.001	0.803
*t*9-	3.60 ± 0.282	4.39 ± 0.282	5.35 ± 0.671	2.47 ± 0.282	2.59 ± 0.101	3.23 ± 0.305	0.012	0.504	< 0.001	0.350
*t*10-	104.6 ± 15.79	75.1 ± 10.50	65.0 ± 17.06	60.9 ± 7.99	37.8 ± 5.60	35.3 ± 8.63	0.023	0.268	0.001	0.866
*t*11-	12.3 ± 1.26	22.7 ± 3.71	37.4 ± 8.06	9.1 ± 0.13	16.7 ± 2.13	23.2 ± 4.01	0.003	0.804	0.040	0.462
*t*12-	8.33 ± 0.646	8.75 ± 0.646	9.47 ± 0.698	5.20 ± 0.299	5.00 ± 0.299	5.79 ± 0.323	0.110	0.473	< 0.001	0.802
*t*15-	3.04 ± 0.444	3.53 ± 0.444	3.92 ± 0.480	2.61 ± 0.198	3.06 ± 0.198	3.56 ± 0.214	0.017	0.955	0.168	0.985
*c*12-	6.65 ± 0.743	9.27 ± 1.790	10.44 ± 1.934	4.80 ± 0.743	6.93 ± 0.743	8.40 ± 0.803	0.005	0.645	0.055	0.975
*c*13-	1.69 ± 0.102	1.48 ± 0.102	1.37 ± 0.110	1.26 ± 0.089	1.07 ± 0.089	1.15 ± 0.096	0.107	0.389	< 0.001	0.280
*t*16–[4]	2.91 ± 0.357	3.56 ± 0.357	4.08 ± 0.386	1.56 ± 0.238	2.21 ± 0.238	2.46 ± 0.257	0.016	0.683	< 0.001	0.785
*c*15-	1.84 ± 0.124	1.62 ± 0.124	1.40 ± 0.134	1.03 ± 0.092	0.97 ± 0.092	0.88 ± 0.099	0.012	0.938	< 0.001	0.434
Sum	152 ± 12.6	137 ± 12.6	146 ± 13.6	92.8 ± 6.16	80.0 ± 6.16	88.2 ± 6.66	0.704	0.338	< 0.001	0.991
18:2 isomers										
*t*8,*c*13-	0.11 ± 0.018	0.14 ± 0.018	0.14 ± 0.0.019	0.10 ± 0.013	0.12 ± 0.013	0.12 ± 0.014	0.372	0.538	0.018	0.644
*c*9,*t*13/*c*9,*t*14–[6]	0.39 ± 0.039	0.43 ± 0.039	0.42 ± 0.042	0.32 ± 0.0.029	0.31 ± 0.029	0.30 ± 0.031	0.870	0.780	< 0.001	0.511
*c*9,*t*11[7]-	4.79 ± 0.821	9.06 ± 0.210	10.91 ± 0.186	3.52 ± 0.332	5.99 ± 0.821	7.42 ± 1.857	0.003	0.346	0.019	0.431
*t*9,*c*11-	0.85 ± 0.281	1.26 ± 0.281	0.58 ± 0.303	0.58 ± 0.058	0.37 ± 0.058	0.29 ± 0.060	0.203	0.195	0.011	0.261
*c*9,*t*15/*c*9,*t*12-	0.07 ± 0.015	0.10 ± 0.015	0.10 ± 0.016	0.05 ± 0.007	0.06 ± 0.007	0.06 ± 0.007	0.234	0.370	< 0.001	0.334
*t*10,*c*15 − /*t*11,*c*15-	4.10 ± 0.562	3.48 ± 0.562	3.07 ± 0.608	2.72 ± 0.299	2.07 ± 0.299	1.72 ± 0.323	0.119	0.811	< 0.001	0.992
CLA *t*/*t*[5]	0.84 ± 0.066	0.86 ± 0.066	1.13 ± 0.071	0.59 ± 0.060	0.53 ± 0.060	0.63 ± 0.065	0.055	0.156	< 0.001	0.060
*t*10,*c*12-	1.59 ± 0.216	1.24 ± 0.216	0.89 ± 0.234	0.93 ± 0.102	0.54 ± 0.102	0.44 ± 0.110	0.002	0.631	< 0.001	0.767
*t*11,*c*13–[8]	0.02 ± 0.002 [b]	0.03 ± 0.002 [bc]	0.04 ± 0.003 [c]	0.01 ± 0.003 [a]	0.01 ± 0.003 [ab]	0.01 ± 0.003 [ab]	0.008	0.676	< 0.001	0.008
Sum	8.07 ± 1.298	12.4 ± 1.30	13.5 ± 1.40	5.62 ± 0.817	7.43 ± 0.817	8.76 ± 0.883	< 0.001	0.334	< 0.001	0.448
18:3 isomer										
*c*9,*t*11,*c*15–[9]	0.33 ± 0.061	0.47 ± 0.061	0.68 ± 0.066	1.20 ± 0.120	1.07 ± 0.120	1.35 ± 0.130	0.068	0.289	< 0.001	0.080
BI	181 ± 13.1	172 ± 13.1	182 ± 14.1	119 ± 6.8	106 ± 6.8	116 ± 7.4	0.904	0.278	< 0.001	0.985
*t*10/*t*11ratio	10.0 ± 2.63	3.8 ± 0.62	4.4 ± 2.84	7.8 ± 1.56	2.5 ± 0.62	2.5 ± 0.69	0.026	0.020	0.259	0.959

abc Within the same row, means with different superscripts are statistically different ($P < .05$).
[1] LA – Low alfalfa.
[2] MA – Medium alfalfa.
[3] HA – High alfalfa.
[4] Coelutes with *c*14–18:1.
[5] Sum of *trans*/*trans* conjugated linoleic acid isomers.
[6] Coelutes with 17-cyclo and *t*8,*c*12–18:2.
[7] Includes *t*7,*c*9–18:2 as minor isomer.
[8] Coelutes with 21:0; [9] coelutes with 20:3n-9.

in all treatments, and would be likely acceptable by consumers (Khliji, van de Ven, Lamb, Lanza, & Hopkins, 2010). The colour of meat and fat from ruminants may be affected by diet, and animals fed a high proportion of forage can have darker and redder meat with more yellow fat compared to others raised in confinement and fed with concentrate-based diets (Priolo, Micol, & Agabriel, 2001; Ripoll, Albertí, & Joy, 2012). In addition, in the present trial, the lambs were reared in confinement and were fed with diets with high levels of concentrate (40 to 80%). Moreover, the forage component was dehydrated alfalfa, which has much lower content of carotenoids than fresh grasses due to changes induced by the conservation process (Nozière et al., 2006).

The average shear force obtained in the present trial is comparable to others reported for Merino Branco LL processed in similar conditions (Santos-Silva et al., 2002). These values correspond to very tender meat (Sañudo et al., 2003), expressing the benefits of aging for 7 days under refrigerated conditions (Ripoll et al., 2012).

The major causes of the loss of quality of meat during conditioning are oxidation of lipids and myoglobin, namely by the development of unpleasant off-flavours, and by discoloration (Buckley, Morrissey, & Gray, 1995). The ΔE characterizes the global colour changes during meat storage (Mancini & Hunt, 2005). In the present trial ΔE was not affected by treatments and is comparable with those previously reported by Jerónimo et al. (2012) and Francisco et al. (2015) for Merino Branco LT with the same conditioning period. According to Abril et al. (2001) such differences in colour are easily distinguishable by consumers.

The TBARS are a common index used to evaluate the oxidation status of meat and relate to the extent of FA oxidation, measuring the secondary oxidation product malonaldehyde. The average value obtained in this experiment (1.28 ± 0.508) is comparable with results obtained in meat of lambs fed high-concentrate diets, supplemented with PUFA-rich vegetable oil and processed and stored under the same condition (Francisco et al., 2015; Francisco et al., 2017). In our experiment, the level of PUFA in meat was similar for the three diets and could not contribute to differences in lipid oxidation. Campo et al. (2006), reported 2 mg/kg as a threshold value for TBAR for consumer acceptability of beef. In spite of between species differences, the values observed in our experiment were below 2 mg/kg, suggesting the lamb maintained its eating quality 10 days after slaughter.

The fatty acid composition was evaluated in ScF and intramuscular fat of LT and the statistical model used revealed no significant interactions between diet and fat tissue. As expected, the ScF had lower proportions of SFA and PUFA, and higher proportion of *cis*-MUFA, BI and terminal and non terminal- BCFA compared to the LT (Costa et al., 2017). It is well established that there is a gradient in the melting point of fatty tissues related to body location, with lower values for external ScF when compared to intermuscular or visceral fat. The high proportion of BCFA in the ScF is in line with this general standard because it

has been found to reduce melting point of the fat (Berthelot, Normand, Bas, & Kristensen, 2001). Nevertheless, the proportion of nonterminal-BCFA in ScF remained low and similar across diets, as reported and discussed by Costa et al. (2017).

The main objective of the experiment was to test the hypothesis that incorporating forage NDF into oil-supplemented finishing diets would have a differential effect on the deposition of BI in lamb meat, when compared to high digestible NDF from soybean hulls. Lambs fed ground dehydrated alfalfa diets supplemented with unsaturated vegetable oil, consistently display high proportions of desirable BI, like *t*11–18:1 and *c*9,*t*11–18:2 in the rumen and meat (Alves, Santos-Silva, Cabrita, Fonseca, & Bessa, 2013; Jerónimo et al., 2009; Jerónimo et al., 2010; Jerónimo et al., 2010; Santos-Silva et al., 2004). However, when finishing lambs with high starch diets, particularly if supplemented with oil, the pattern of BI deposited in tissues change, and the undesirable *t*10–18:1 accumulates (Bessa, Portugal, Mendes, & Santos-Silva, 2005; Oliveira, Alves, Santos-Silva, & Bessa, 2017). This change is due to an extensive shift in the rumen BH pathways, the *t*10-shift, although its deleterious consequence on product quality is yet weakly understood (Bessa et al., 2015). In fact, such extensive alteration in rumen BH pathways must be the result of extensive changes in rumen microbiota, but the exact nature of such changes remains to be established (Enjalbert, Combes, Zened, & Meynadier, 2017).

In a previous experiment, we fed lambs with low-starch and high oil diets, where cereals were replaced by industrial by-products. It was observed that a high NDF diet containing soybean hulls was not effective in preventing the *t*10–18:1 accumulation (Costa et al., 2017). Thus, we hypothesized that the rate of NDF degradation in the rumen should affect the rumen BH pathways, and that the reduction of forage NDF degradability rate could promote the *t*11–18:1 synthesis. The results presented here confirm that hypothesis as replacing soybean hulls NDF by alfalfa NDF in isoNDF diets led to a linear decrease of *t*10–18:1 and *t*10,*c*12–18:2 (Alves & Bessa, 2014; Bravo-Lamas, Barron, Kramer, Etaio, & Aldai, 2016) and to a linear increase in *t*11–18:1 and *c*9,*t*11–18:2. The *t*10/*t*11 ratio decreased with dietary alfalfa level from 10.0 to 3.8 and from 7.8 to 2.5 in ScF and LT respectively, following a quadratic pattern. In spite of the *t*10–18:1 being the major BI intermediate present in the fat and meat of most of the lambs, the intensity of the *t*10-shift was clearly reduced when the alfalfa proportion was increased in the diet, mainly from 200 to 400 g/kg.

In fact, in the present experiment and in that reported by Costa et al. (2017), the rumen pH was low and there was evidence of rumen parakeratosis lesions indicative of sustained low rumen pH during the experimental periods. In the present experiment, the dietary content of starch and sugar were low and similar between diets, thus the differences in rumen pH observed are probably related to differences in the rate of NDF fermentation and of cations exchange capacity of the dietary ingredients (Dijkstra et al., 2012). If the rumen pH is the main driver of the *t*10-shift, then production strategies to avoid it must be directed to prevent low rumen pH.

We report again the occurrence of *t*10 shifted animals fed with low-starch, high-NDF diets, as evaluated by *t*10/*t*11 ratio in ScF and meat, confirming our previous results (Costa et al., 2017). Surprisingly, even the diet containing 600 g/kg of alfalfa and no cereals was unable to avoid the *t*10-shift in 66% of the lambs. However, in previous experiments, oil supplemented diets containing 500 g/kg of dehydrated alfalfa did not induce *t*10-shift in lambs (Francisco et al., 2017). In all of these experiments we used ground feed ingredients that might not promote enough salivation to ensure rumen pH buffering. Nevertheless, the critical need for dietary physically effective NDF is certainly associated with diet fermentability as when oil is used to supplement 100% ground alfalfa basal diets the *t*10/*t*11 ratio in the rumen, abomasum and tissue of lambs is consistently very low (Alves, Santos-Silva, et al., 2013; Jerónimo, Alves, Dentinho, et al., 2010).

The difficulty in establishing consistent response patterns between dietary components (like starch, sugar or NDF) and the occurrence of *t*10-shift reinforces the hypothesis that low rumen pH is the main driving force determining the establishment of the *t*10-shift. In fact, in the present experiment and in that reported by Costa et al. (2017), the rumen pH was low and there was evidence of rumen parakeratosis lesions indicative of sustained low rumen pH during the experimental periods. In the present experiment, the dietary content of starch and sugar were low and similar between diets, thus the differences in rumen pH observed are probably related to differences in the rate of NDF fermentation and of cation exchange capacity of the dietary ingredients (Dijkstra et al., 2012). If the rumen pH is the main driver of the *t*10-shift, then production strategies to avoid it must be directed to prevent low rumen pH.

One of the consequences of the *t*10-shift in ruminants is the low concentration of *c*9,*t*11–18:2 in meat, even in animals fed oil supplemented diet (Bessa et al., 2015; Oliveira et al., 2016, 2017). This is clearly demonstrated in the present experiment, as diet LA presents a very low *c*9,*t*11–18:2 proportion in ScF and meat (0.48 and 0.35 g/100 g FA respectively), and increased with dietary alfalfa inclusion following the well established linear relationship with the *t*11–18:1 proportion (Palmquist, St-Pierre, & McClure, 2004).

Besides the effects on BI, increasing alfalfa proportion in the diet led to a linear decrease in 16:0 and a linear increase the 18:2 n-6 despite the fact that the intake of 16:0 and 18:2 n-6 being similar among treatments. The 16:0 is the major end product of the endogenous *de novo* FA synthesis, and that finding might indicate a slight stimulation of those pathways in lambs fed soybean hulls compared to alfalfa, although not reflected in the amount of intramuscular lipid and FA content.

The linear increase of 18:2n-6, coupled with the absence of effects on 18:0 and on the sum of BI, suggest that the BH of 18:2n-6 was reduced as alfalfa was incorporated in the diet, and likely in part related to increased DM intake and rumen passage rate.

5. Conclusions

The BI pattern of meat and ScF was affected by the composition and structure of the fibrous fraction in complete diets for lambs. Increasing the proportion of alfalfa, and thus of ADL, in the diet, did not compromise growth performance and carcass and meat quality, but depressed the intensity of the *t*10-shift following a quadratic pattern. Forty percent of alfalfa in diet reduced the severity of *t*10-shift, but for its full resolution other factors should be considered including forage particle size and the buffering capacity of the diet.

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