

RESEARCH ARTICLE

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Linseed, microalgae or fish oil dietary supplementation affects performance and quality characteristics of light lambs

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Abstract

The aim of this work was to improve the fatty acid profile of meat from light lamb, frequently reared in Spain and in other Mediterranean countries. A total of 44 light lambs fed different n-3 polyunsaturated fatty acids sources were studied: control (C) (palm oil), extruded linseed (L), extruded linseed mixed with microalgae (LM) and fish oil (FO). The productive performance from 14.7 to 26.2 kg of live weights and meat quality characteristics during refrigerated storage were assessed. Lambs fed FO showed lower feed intake ($p < 0.001$), average daily gain ($p < 0.001$) and higher feed conversion ratio ($p < 0.001$) than lambs from the other treatments. Fish oil meat had the lowest ($p < 0.01$) protein proportion and the highest ($p < 0.001$) C20:5n-3, C22:5n-3 and C22:6n-3 proportions, while L or LM produced the highest ($p < 0.001$) C18:3n-3 deposition. Fish oil meat had higher ($p < 0.05$) L* and lower ($p < 0.001$) a* than meats from the other treatments. After 7 days of storage, FO meat showed the highest TBARS ($p < 0.001$), while C meat showed similar value to 0 days of storage. Additionally, FO underwent higher microbial growth after 7 days of storage as compared to the other treatments. Dietary supplementation with L and LM leads to C18:3n-3 meat enrichment and to a microbial load and colour characteristics similar to those of C, without adverse effect on lamb performance. However the use of FO improves the fatty acid profile of meat, but negatively affects lamb performance and meat quality.

Additional key words: light lamb; lipid oxidation; n-3 polyunsaturated fatty acids; colour stability.

Introduction

Recently, a number of epidemiological studies have associated red and processed meat consumption with the development of two of the major chronic diseases in the Western world: cardiovascular disease and colon cancer (McAfee *et al.*, 2010). Two of the components responsible for these associations are fat content and fatty acid composition. In this respect, ruminant meat

has been criticized for its high fat content, which, in addition, is rich in saturated fatty acids (SFA) because of biohydrogenation in the rumen. Currently the research within the ruminant sector is, therefore, interested in developing feeding strategies for further increasing the nutritional value of meat by adding n-3 polyunsaturated fatty acids (PUFA), or conjugated linoleic acid (CLA) in the animal diet (Scollan *et al.*, 2006). Despite ruminal hydrogenation, several studies

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Received: 19-06-13. Accepted: 19-05-14.

Abbreviations used: APC (aerobic plate counts); C (control treatment); C* (chroma); CLA (conjugated linoleic acid); FAME (fatty acid methyl esters); FM (fresh matter); FO (fish oil treatment); h* (hue-angle); L (linseed treatment); LM (linseed plus microalgae treatment); Mb (deoxymyoglobin); MDA (malonaldehyde); MetMb (metmyoglobin); MUFA (monounsaturated fatty acids); OxyMb (oxymyoglobin); PUFA (polyunsaturated fatty acids); SEM (standard error of mean); SFA (saturated fatty acids); TBARS (thiobarbituric acid reactive substances).

have shown that medium or long-term supplementation with lipid sources rich in n-3 fatty acids modifies the fatty acid profile of meat, thus improving its nutritional value (Cooper *et al.*, 2004). The enhancement of n-3 PUFA levels in meat has been achieved by dietary supplementation of vegetable sources, such as linseed, which is known to provide linolenic acid, C18:3n-3 (Noci *et al.*, 2011), or marine products, such as either fish oil or algae (Cooper *et al.*, 2004), both of which being the major sources of long-chain fatty acids, such as eicosapentaenoic (C20:5n-3) and docosahexaenoic acids (C22:6n-3). The main problem associated with the modification of fatty acid profile of muscle foods is determined by the high susceptibility of unsaturated fatty acids to oxidise, especially those with more than two double bonds. Lipid oxidation together with microbial spoilage, may affect meat colour, flavour (Nute *et al.*, 2007) and texture. Regarding meat colour, the oxidation of oxymyoglobin (OxyMb, responsible for the desirable bright-red colour in meat) to metmyoglobin (MetMb, characterised by a brown-red colour), which seems to be enhanced by lipid oxidation, leads to meat discoloration (Lindhahl *et al.*, 2001).

It should be noted that the mentioned studies were generally performed in lambs older than those frequently reared in Spain and in other Mediterranean countries, which are usually slaughtered below 3 months old. This practice may affect the response to dietary n-3 PUFA supplementation, since the development of an active rumen during the growth and maturation of the young ruminant is also associated with marked changes in glucose (Eisemann *et al.*, 1997), protein (Fortin *et al.*, 2010) and lipid metabolism (Masters, 1964), which are reflected in the meat composition (Payne & Masters, 1971; Fortin *et al.*, 2010). Taking these considerations into account, the objective of the current study was to investigate the effect of dietary supplementation of growing lambs with several sources rich in n-3 fatty acids on the performance, fatty acid composition and some quality parameters of meat stored for 7 days at 4°C under aerobic conditions.

Material and methods

Experimental husbandry, growth performance and carcass measurements

A total of 44 weaned male lambs (Manchego breed), with an initial weight of 14.7 ± 0.5 kg, were randomly

assigned to one of the four dietary treatments under study. A detailed description of diets and experimental procedure was described elsewhere (Díaz *et al.*, 2011). Briefly, the ingredients used in the four experimental diets were: corn meal, barley, wheat, soya-bean meal, sunflower meal, limestone, salt and mineral/vitamin premix. Different proportions of the n-3 PUFA sources were added to each dietary treatment: Control (C; containing 3.3% palm oil), Linseed (L; with 12.5% of a mixture composed of 70% extruded linseed and 30% wheat bran), Linseed plus microalgae (LM; a mixture consisting of 10.7% of the cited extruded linseed source and 4% of dehydrated microalgae (*Isochrysis* sp.)) and Fish oil (FO; with 3.3% semi-refined fish oil). The composition and nutritional value of the diets are shown in Table 1. The diets were pelleted (2.5 mm diameter).

The lambs were allocated in individual pens and gradually adapted to the diets for 10 days. Concentrate was offered daily at the rate of 120% of *ad libitum* intake, calculated by weighing-back refusals daily. Barley straw was also offered daily at the rate of 10% of concentrate offered. Lambs had continuous access to water. The animals were fattened for an average period of 40.8 ± 0.7 days, with weekly monitoring of the feed intake and the live weight, to a slaughter weight of 26.2 ± 0.3 kg. Those data were used to calculate the average daily gain, average daily feed intake and feed conversion ratio.

After a 12 h fasting period, lambs were transported to a local slaughterhouse (20 km of distance approx.) where were slaughtered and dressed according to commercial practices. The carcasses were then weighed to obtain the hot carcass weight and, after a 24 h chilling period (4°C), packed separately and transported under refrigeration (2°C) to the INIA's pilot plant for further processing. The carcasses were re-weighed, to obtain the cold carcass weight, and then classified according to their fatness using four-point structured scale (1 = minimum score; 4 = maximum score) following the community scale for classification of carcasses of ovine animals (OJ, 1994).

The dorsal fat thickness was measured at 4 cm from the carcass midline and at 4 cm from the caudal edge of the last rib with a digital calibrator. The carcasses were jointed, and then the loins were removed and cut into 2 cm thick chops. The chops from the left side were directly vacuum-packed in metallic pouches and frozen at -20°C until analysis, remaining as 0 days samples. The chops from the right side were placed

Table 1. Ingredients and analyzed chemical composition of the dietary treatments, control (C), linseed (L), linseed plus microalgae (LM) and fish oil (FO)

	C	L	LM	FO
Ingredients (%)				
Corn	14.2	14.8	17.9	15.7
Barley	31.5	25.5	24.2	30.0
Wheat	25.0	25.0	23.0	25.0
Soya-bean meal (44% CP)	18.0	14.5	13.0	18.0
Sunflower meal	4.8	4.5	4.0	4.8
Limestone	2.2	2.2	2.2	2.2
Salt	0.5	0.5	0.5	0.5
Mineral/vitamin premix ^a	0.5	0.5	0.5	0.5
Palm oil	3.3	—	—	—
Extruded linseed	—	12.5	10.7	—
Marine microalgae	—	—	4.0	—
Fish oil	—	—	—	3.3
Chemical composition (% fresh matter, FM)				
Moisture	10.8	10.6	10.7	10.4
Crude protein	21.1	21.1	21.1	21.0
Ether extract	7.7	8.9	8.6	7.1
Neutral detergent fiber	16.8	16.8	16.5	15.3
Acid detergent fiber	5.9	5.4	5.5	6.0
Ash	9.2	6.5	7.1	8.6
Vitamin E (mg kg ⁻¹ FM)	45	39	42	46
Metabolizable energy ^b (MJ kg ⁻¹ FM)	11.7	12.0	11.7	11.6

^a Vitamins and minerals per g of premix: retinol, 1650 UI; cholecalciferol, 330 UI; dl- α -tocopherol, 5 mg; riboflavin, 0.6 mg; ascorbic acid, 2 mg; niacin, 4 mg; cyanocobalamin 0.002 mg; vitamin K, 0.18 mg; Mn, 12.6 mg; Zn, 22 mg; Cu, 1 mg; I, 0.12 mg; Fe, 4.2 mg; Co, 0.2 mg; Se, 0.03 mg; Mg, 50 mg; and S, 69 mg. ^b Calculated metabolizable energy.

into a tray, overwrapped in an oxygen-permeable polyvinyl chloride film and stored at $2 \pm 1^\circ\text{C}$ in the dark for 7 days. Afterwards, these chops were vacuum-packaged and frozen, as mentioned, until analysis (which was carried out within the next 3 months).

Analyses of chemical composition, fatty acid composition and vitamin E content were carried out in 0 days samples, whereas pH values, microbial counts, lipid oxidation, colour and MetMb were studied at both 0 and 7 days.

Analyses of muscle: chemical composition, fatty acid profile and vitamin E content

Fat, protein, and moisture were determined using a near-infrared spectrophotometer (XDS NIR Rapid Content™ Analyser, FOSS, Hillerød, Denmark), applying calibration equations previously calculated. The WINISI software was used for the data analysis

and calibrations were performed by modified partial least squares regression. Ash was estimated as the difference.

The intramuscular fat was extracted as described by Hanson & Olley (1963). The fatty acids were derivatized into fatty acid methyl esters (FAME) by using 14% boric trifluoride in methanol according to the method of Morrison & Smith (1964), and then analysed by gas chromatography with flame ionization detection (Agilent, Santa Clara, CA, USA). Separation was carried out in an Omegawax 320 capillary column (30 m \times 0.32 mm i.d., 0.25 μm film thickness) with polyethylene glycol as the stationary phase (Supelco, Bellefonte, PA, USA). Gas chromatography conditions were as follows: oven temperature 200°C held for 60 min with helium as carrier gas (11 psig). Both injector and detector temperatures were 260°C . Samples were injected (0.2 μL) in the split mode with a 1:50 split ratio. Individual FAME were identified by comparing their retention times with those from standards (FAME

Mix, PUFA No. 2 and PUFA No. 3; Supelco, Bellefonte, PA, USA). The results were expressed as a percentage of the total fatty acids. The CLA isomers were computed as the major peak in the chromatographic region where the standard (conjugated linolenic FAME; Sigma, St Louis, MO, USA) elutes.

The concentration of vitamin E (α -tocopherol) in muscle was determined in duplicate, following the procedure described by Cayuela *et al.* (2003). A high performance liquid chromatograph with fluorescence detector (Perkin Elmer, Waltham, MA, USA) equipped with a silica column (150 \times 4.6 mm, 5 μ m particle size) and a 10 μ m silica guard column (Kromasil KR100-5-150 and KR100-10-10C5, respectively, Symta, Madrid, Spain) was used. The mobile phase consisted of isooctane-tetrahydrofuran (97:3) working in isocratic mode at a flow rate of 1 mL min⁻¹. Fluorescence detection was carried out at 297 and 321 nm as excitation and emission wavelengths, respectively. Quantification was performed by means of a calibration curve of α -tocopherol standards ranging from 0.2 to 1 μ g mL⁻¹. Results were expressed as mg α -tocopherol kg⁻¹ muscle.

Measurements of pH, colour and pigments of meat

Triplicate pH values were measured with a penetrating electrode coupled to a portable pH meter (HI 9025, Hanna Instruments, S.L., Gipuzkoa, Spain). Instrumental colour was evaluated at 0 (1 h blooming after cutting) and after 7 days of storage, using a CM-2600d spectrophotometer (Minolta Camera Co., Osaka, Japan) with illuminant D65, visual angle 10° and measurement aperture 8 mm. Colour was measured on the surface of chops and three different locations were scanned and averaged for statistical analyses. Calibration was performed by using standard white tiles prior to the colour measurement. The colour coordinates were expressed as L*, a*, and b*. Chroma (C*) and hue-angle (h*) values were calculated as $C^* = (a^{*2} + b^{*2})^{0.5}$ and $h^* = \tan^{-1}(b^* / a^*)$, respectively. The proportion of MetMb, deoxymyoglobin (Mb) and OxyMb on the meat surface was performed as described by Krzywicki (1979) with some modifications for lamb meat, using 473, 525, 572 and 690 nm as wavelengths. Reflectance values at wavelengths not given by the instrument (473, 525 and 572 nm) were calculated using linear interpolation.

Lipid oxidation analysis

Lipid oxidation, assessed as thiobarbituric acid reactive substances (TBARS) value, was determined in duplicate according to the method described by Maraschiello *et al.* (1999). The results were expressed as mg MDA kg⁻¹ muscle.

Microbiological examination

Aerobic plate counts (APC) were determined as described by De la Fuente *et al.* (2007). Microbial counts were expressed as base-10 logarithms of colony forming units per cm⁻² of surface area (log₁₀ cfu cm⁻²).

Statistical analysis

Data were subjected to analysis using SAS package (SAS® 9.2, SAS Inst. Inc. Cary, NC, USA). Performance data, carcass characteristics, muscle composition, vitamin E content and fatty acid composition were analyzed using a one-way ANOVA with the dietary treatments as a fixed factor. Values of pH, colour parameters, pigments, TBARS, and APC counts were analyzed using a 4 \times 2 factorial design in which the dietary treatments (4), the storage period (2) and the interaction between both of them were the fixed factors. Differences among means were determined by Dunn-Sidak test (Sokal & Rohlf, 2003, p. 239) ($p < 0.05$).

Results

Animal performance and carcass characteristics

Lamb performance and carcass characteristics are shown in Table 2. No differences among dietary treatment was observed for initial live weight, however final live weight was lower in FO lambs. Differences attributable to dietary treatments were observed for growing lambs performance ($p < 0.001$), FO lambs showing lower feed intake, average daily gain and higher feed conversion ratio than lambs from the other treatments. Hot and cold carcass weights were affected by dietary treatment ($p < 0.001$) with FO lambs having lower weights. Carcass yield was also significantly

Table 2. Animal performance and carcass characteristics of growing lamb meat as affected by the following dietary treatments: control (C), linseed (L), linseed plus microalgae (LM) and fish oil (FO)

	Dietary treatments				SEM ¹ n = 11	Sign. ²
	C	L	LM	FO		
Initial live weight (kg)	13.24	13.00	13.05	12.60	1.11	NS
Final live weight (kg)	28.07 ^a	29.10 ^a	27.87 ^a	22.58 ^b	3.11	***
Average feed intake (kg day ⁻¹)	0.858 ^a	0.756 ^a	0.761 ^a	0.540 ^b	0.120	***
Average daily gain (kg day ⁻¹)	0.336 ^a	0.355 ^a	0.324 ^a	0.205 ^b	0.064	***
Feed conversion ratio (kg feed kg ⁻¹ gain)	2.90 ^b	2.41 ^b	2.71 ^b	3.16 ^a	0.44	***
Carcass measurements:						
Hot carcass weight (kg)	13.58 ^a	13.62 ^a	13.14 ^a	10.32 ^b	1.61	***
Cold carcass weight (kg)	13.26 ^a	13.24 ^a	12.85 ^a	10.14 ^b	1.58	***
Chilling losses (%)	2.37	2.89	2.21	1.83	1.14	NS
Carcass yield (%)	47.16 ^a	45.48 ^b	46.12 ^{ab}	44.55 ^b	1.91	*
Dorsal fat thickness (mm)	2.85	3.19	3.11	2.57	0.97	NS
Fat score ³	2.90	3.22	3.01	2.88	0.65	NS

¹ Standard error of the mean. ² Statistical significance: NS, non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ³ Four-point structured scale (1 = minimum; 4 = maximum score). ^{a, b} means within rows with different superscript differ significantly ($p < 0.05$).

affected by the dietary treatment ($p < 0.05$), with C lambs being the highest while L and FO lambs showing the lowest.

Muscle composition

The chemical, fatty acid composition and vitamin E concentration of muscle are given in Table 3. The percentage of fat in muscle was not affected by the dietary treatment, but the protein proportion was lower in FO meat ($p < 0.01$). Most of the studied fatty acids showed significant differences depending on the dietary treatment. Meat from the FO treatment showed the highest proportion in C14:0 ($p < 0.01$), C16:0 ($p < 0.001$) and SFA ($p < 0.001$), whereas the levels of C18:0 were lower ($p < 0.01$) than those of C. The meat from C and LM treatments showed the highest ($p < 0.001$) proportions of both C18:1 and MUFA and the meat from FO had the lowest. With regard to PUFA, C18:2n-6 and total n-6 proportions were the lowest in FO meat ($p < 0.001$ and $p < 0.01$, respectively), whereas C18:3n-3 was especially high ($p < 0.001$) when linseed was included in the diet (L and LM treatments). Dietary treatment also affected CLA proportion ($p < 0.05$), LM and FO meats having the highest proportions. The meat from FO treatment showed the highest ($p < 0.001$) proportions of C20:5n-3, C22:5n-3, C22:6n-3 as well as of the sum of n-3

($p < 0.001$). Consequently, the total PUFA content was the highest ($p < 0.01$) in n-3 supplemented diets (L, LM and FO).

An effect ($p < 0.001$) of the dietary treatment on the deposition of vitamin E within the muscle was observed, with C meat showing the highest concentration (0.83 mg kg⁻¹ muscle) and FO the lowest (0.56 mg kg⁻¹ muscle).

Values of pH, colour parameters and pigment proportions

The effects of the dietary treatment, the storage period and their interaction on pH, colour parameters and pigment proportions are shown in Table 4. The values of pH were not affected by dietary treatment, however storage time produced a decrease in the pH ($p < 0.001$) after 7 days of storage. Concerning colour, differences among treatments were observed for L* ($p < 0.05$), a* ($p < 0.001$) and h* ($p < 0.001$). The meat from FO showed higher L* and h*, and lower a* values than meat from lambs fed the other diets. With regard to storage, b*, C* and h* were significantly affected ($p < 0.001$, $p < 0.01$ and $p < 0.001$, respectively), with increasing values of all of them throughout storage. The MetMb proportion was affected by the dietary treatment ($p < 0.01$), FO meat showing the lowest proportion. As expected, MetMb, OxyMb and Mb

Table 3. Muscle composition as affected by the following dietary treatments: control (C), linseed (L), linseed plus microalgae (LM) and fish oil (FO)

	Dietary treatments				SEM ¹ n = 11	Sign. ²
	C	L	LM	FO		
Chemical composition (%)						
Fat	3.38	2.81	2.85	3.63	0.92	NS
Protein	22.9 ^a	22.6 ^a	23.0 ^a	21.8 ^b	0.79	**
Moisture	72.9 ^b	73.6 ^a	73.3 ^{ab}	73.7 ^a	0.67	*
Ash	0.82	0.96	0.89	0.89	0.19	NS
Fatty acids (% of identified FA)						
C14:0	2.33 ^b	2.58 ^b	2.60 ^b	3.29 ^a	0.55	**
C16:0	23.33 ^b	22.28 ^b	22.64 ^b	27.30 ^a	1.24	***
C16:1	2.61 ^a	2.17 ^c	2.28 ^{ab}	2.40 ^b	0.20	***
C17:0	1.42	1.44	1.49	1.44	0.21	NS
C17:1	1.05 ^a	0.76 ^b	0.76 ^b	0.62 ^c	0.14	***
C18:0	13.69 ^a	13.00 ^{ab}	11.86 ^c	12.20 ^{bc}	1.24	**
C18:1	43.88 ^a	41.59 ^b	43.92 ^a	37.69 ^c	2.23	***
C18:2n-6	7.03 ^a	8.17 ^a	7.35 ^a	5.00 ^b	1.38	***
CLA ³	0.33 ^b	0.45 ^{ab}	0.60 ^a	0.59 ^a	0.23	*
C18:3n-3	0.38 ^c	2.49 ^a	1.79 ^b	0.29 ^c	0.36	***
C20:4n-3	0.02 ^c	0.05 ^b	0.05 ^b	0.15 ^a	0.03	***
C20:4n-6	2.21	2.10	1.96	2.18	0.59	NS
C20:5n-3	0.17 ^c	0.60 ^b	0.52 ^b	1.80 ^a	0.29	***
C22:4n-6	0.25 ^a	0.19 ^b	0.15 ^{bc}	0.13 ^c	0.05	***
C22:5n-3	0.41 ^d	0.83 ^b	0.67 ^c	1.18 ^a	0.17	***
C22:6n-3	0.22 ^c	0.53 ^{bc}	0.62 ^b	2.65 ^a	0.37	***
SFA ⁴	41.23 ^b	39.84 ^{bc}	39.10 ^c	44.91 ^a	1.67	***
MUFA ⁵	47.67 ^a	44.69 ^b	47.10 ^a	41.04 ^c	2.40	***
PUFA ⁶	11.10 ^b	15.47 ^a	13.80 ^a	14.05 ^a	2.49	**
Σn-3	1.20 ^d	4.50 ^b	3.65 ^c	6.07 ^a	0.89	***
Σn-6	9.50 ^a	10.45 ^a	9.46 ^a	7.31 ^c	1.90	**
Vitamin E (mg kg ⁻¹ muscle)	0.83 ^a	0.69 ^b	0.61 ^{bc}	0.56 ^c	0.12	***

¹ Standard error of mean. ² Statistical significance: NS, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ³ CLA: conjugated linolenic acid isomers. ⁴ SFA: saturated fatty acids (C10:0 + C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0). ⁵ MUFA: monounsaturated fatty acids (C16:1 + C17:1 + C18:1 + C20:1). ⁶ PUFA: polyunsaturated fatty acids. ^{a,b,c,d} Different letters within the same row indicate significant differences ($p < 0.05$).

proportions were influenced by the storage period ($p < 0.001$). At 7 days, both MetMb and OxyMb were significantly higher whereas Mb was significantly lower than those at 0 days. The pH value, colour parameters and pigment proportion did not show significant interactions between dietary treatment and storage.

Lipid oxidation

An interaction ($p < 0.001$) between the dietary treatment and the storage period on TBARS values was

observed (Fig. 1a). No differences among diets were observed for TBARS at 0 days (0.07 mg MDA kg⁻¹ muscle of mean), however, after 7 days of refrigerated storage, an increase in L (0.8 mg MDA kg⁻¹ muscle), LM (1.4 mg MDA kg⁻¹ muscle) and FO (2.1 mg MDA kg⁻¹ muscle) samples was observed.

Microbial load

Microbial counts were affected by dietary treatment ($p < 0.01$) as depicted in Fig. 1b. Control meat showed lower microbial counts at days 0 and 7 than FO meat.

Table 4. Values of pH, colour parameters and pigment proportions of samples at 0 and 7 days of refrigerated storage, as affected by the following dietary treatments: control (C), linseed (L), linseed plus microalgae (LM) and fish oil (FO)

	Dietary treatments (D)				Storage, days (S)		SEM ¹ (n = 11)	Significance ²		
	C	L	LM	FO	0	7		D	S	D × S
pH	5.67	5.66	5.63	5.71	5.73	5.61	0.13	NS	***	NS
L*	39.57 ^b	39.75 ^b	39.53 ^b	42.72 ^a	39.69	41.09	3.89	*	NS	NS
a*	8.22 ^a	7.13 ^a	7.85 ^a	5.94 ^b	7.35	7.22	1.55	***	NS	NS
b*	11.25	10.44	11.20	11.23	10.17	11.89	1.55	NS	***	NS
C*	13.98	12.67	13.71	12.77	12.61	13.96	1.97	NS	**	NS
h*	53.78 ^b	55.87 ^b	54.98 ^b	62.37 ^a	54.53	58.97	4.32	***	***	NS
MetMb ³	26.15 ^a	23.17 ^{ab}	24.41 ^a	21.14 ^b	12.63	34.82	4.68	**	***	NS
Mb ⁴	42.37	49.34	43.16	52.63	70.36	23.40	16.79	NS	***	NS
OxyMb ⁵	31.48	27.49	32.43	26.22	17.02	41.79	13.37	NS	***	NS

¹ Standard error of mean. ² Statistical significance: NS, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ³ Metmyoglobin (%).

⁴ Deoxymyoglobin (%). ⁵ Oxy myoglobin (%). ^{a,b} Different letters within the same row indicate significant differences between dietary treatments ($p < 0.05$).

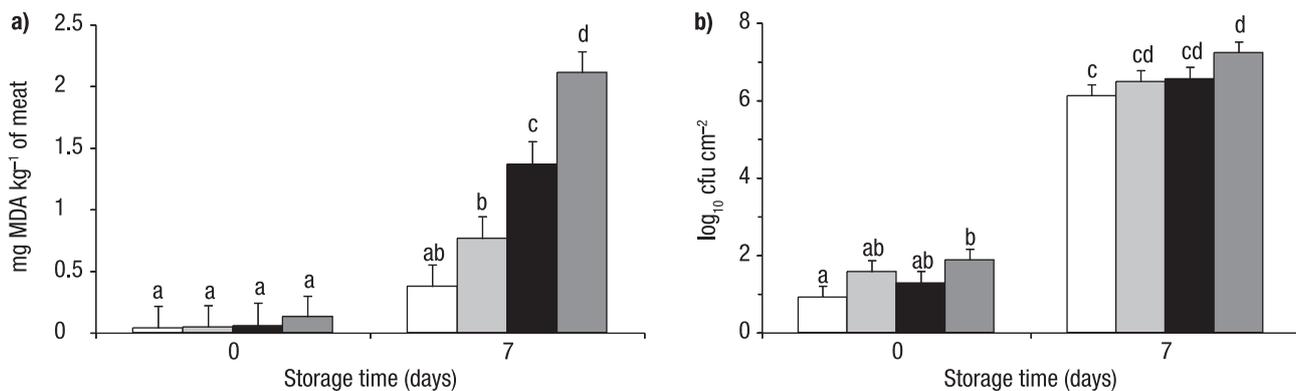


Figure 1. a) Thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde (MDA) kg^{-1} meat) and b) microbial load (\log_{10} cfu cm^{-2}) of lamb chops at 0 and 7 days of aerobic refrigerated storage as affected by dietary treatments: □ control, ■ linseed, ■ linseed and microalgae, and ■ fish oil. Letters (a, b, c, d) indicate significant differences ($p < 0.05$) between samples from different diets and storage times.

The microbial load was also affected by the storage period ($p < 0.001$), with an increase throughout storage being observed.

Discussion

Animal performance and carcass characteristics

Concerning the feed intake, lambs from the FO treatment ate 0.320, 0.216 and 0.221 less kg concentrate per day, as compared to C, L, and LM, respectively. Previous studies in lambs have reported signi-

ficant decreases in feed intake when using fish oil as a supplement in the diet (Kitessa *et al.*, 2001; Wachira *et al.*, 2002; Annett *et al.*, 2011). The depression in feed intake due to fish oil supplementation has been attributed to increases in some biohydrogenation intermediaries that are potentially toxic for rumen microbiota (Kitessa *et al.*, 2001), and to a significant reduction in fibre digestion and microbial growth in the rumen (Wachira *et al.*, 2002). The decrease in feed intake could be also because of a decreased palatability of the fish oil diet. According to the results obtained for the intake, lambs from the FO treatment showed lower average daily gain and final live weight, which was reflected in lower carcass weights than those from the other treatments. In contrast, Cooper *et al.* (2004)

did not observe any effect of the oil supplements on intake, growth performance or carcass weights of heavier lambs. The lower carcass yield was observed, as expected, in FO lambs, since this parameter depends on the slaughter weight. Similarly, Berthelot *et al.* (2010) did not find any effect of linseed supplementation on carcass yield of Romane male lambs but slaughter weight did not differ among treatments. Nevertheless, in our study, in spite of the mentioned differences on the slaughter weight among diets and considering that the calculated metabolizable energy of the diets were similar in the four treatments (Table 1), no significant differences were observed in the fatness measurements, although dorsal fat thickness and fat score mean values were lower in FO carcasses.

Muscle composition

Muscle from FO lambs had lower protein proportion than those from the other dietary treatments. Similarly, Cooper *et al.* (2004) stated that fish oil supplementation had a negative effect on carcass protein deposition. However, other studies have demonstrated that fish oil supplementation increases fat levels in lambs due to a higher proportion of propionate in the rumen, which favour lipogenesis (Wachira *et al.*, 2002).

The higher proportion of C16:0 and the lower proportion of C18:0 in FO meat found in our study were in agreement with Wachira *et al.* (2002), who have previously reported higher levels of C16:0 and lower levels of C18:0 in lambs fed fish oil enriched diets. Scollan *et al.* (2001) also observed higher levels of C16:0 in the neutral fraction and lower levels of C18:0 in the polar fraction of beef fed fish oil, and suggested a replacement of C18:0 by C18:1*trans* since the later seems to behave like a SFA *in vivo*.

On the contrary, C18:1 was less abundant in meat from animals fed fish oil. This fact could be explained because of C18:1*n-9* from phospholipids might be excluded by other more unsaturated fatty acids (Scollan *et al.*, 2001).

As expected, meat from lambs fed n-3 enriched diets (L, LM and FO) contained higher PUFA proportions in comparison with C. According to the literature, the levels of PUFA can be increased in lamb meat by means of linseed, fish oil and marine algae dietary treatments, since the former is a source of C18:3n-3 and the rest of them are rich in long-chain PUFA (Cooper *et al.*, 2004; Noci *et al.*, 2011). Concerning

n-3 PUFA, C18:3n-3 was especially high when linseed was supplemented, the percentage of this fatty acid being 6.5-4.7-fold higher in L and LM than in C meat. Noci *et al.* (2011) reported a 3-fold increase in the C18:3n-3 proportion in muscle lipids from lambs supplemented with linseed (oil or seed).

Although long-chain n-3 PUFA were not detected in the extruded linseed source (Díaz *et al.*, 2011), their proportion was higher in L than in C meat, thus suggesting a biosynthetic pathway involved. In this sense, Noci *et al.* (2011) observed an increase in the proportion of long chain n-3 PUFA with an increase in the 18:3n-3 supply. However, in the present study, the highest deposition of long-chain n-3 PUFA in meat has been observed through diet incorporation, since FO meat showed the highest proportions of C20:5n-3 and C22:6n-3. The literature indicates that diets incorporating marine fat sources result in significantly higher amounts of both eicosapentaenoic and docosahexaenoic acids in lamb (Wachira *et al.*, 2002; Cooper *et al.*, 2004) than diets containing linseed. Indeed, Wachira *et al.* (2002) observed approximately 3 times higher long-chain n-3 PUFA contents in lambs fed fish oil diet (3.6% fish oil) than in animals fed control diet. In our study, long-chain n-3 PUFA was more than 10 times higher in meat from FO lambs than in C meat. The differences in n-3 PUFA deposition found in our study, as compared with the aforementioned, could be due to several factors, including basal diets, breeds and level of inclusion (Wachira *et al.*, 2002). Another important factor could be the age of the animals. Most studies have used heavy lambs, whereas those used in our study were young lambs (supplemented from approximately 45 until 90 days of age and slaughtered at around the 30% their potential mature live weight). Since the consumption of solid feed from 49 to 84 days of age stimulates the rumen's morphological development but not the organ's metabolic development (Lane *et al.*, 2000), less pronounced ruminal PUFA hydrogenation would be expected in light lambs.

The lowest proportions of C18:2n-6 and of total n-6 PUFA in FO meat found in our study were in accordance to Ashes *et al.* (1992), who stated that ruminants are able to preferentially incorporate C20:5n-3 and C22:6n-3 into phospholipids and do so by substitution of C18:1 and C18:2n-6.

The CLA proportion was higher in the meat from lambs fed the three n-3 enriched diets in comparison with that of the control. Many authors have demonstrated that dietary supplementation with n-3 PUFA

results in increasing CLA contents in meat (Scollan *et al.*, 2001), mainly from a higher supply of trans-vaccenic acid which serves as a substrate for endogenous CLA synthesis (Noci *et al.*, 2011).

Concerning vitamin E, the minimum amount in order to meet lambs' nutritional requirements was added to all the dietary treatments. Therefore low concentrations of vitamin E were found in both concentrate and meat samples. The effect of the dietary treatment on the deposition of vitamin E within the muscle was in accordance with the fatty acid composition of muscle which depended on the dietary fat source. The negative association between PUFA and vitamin E content in the muscle has been explained by Nute *et al.* (2007), who observed that both fish oil and marine algae supplements produced the lowest muscle vitamin E content, thus suggesting the use of vitamin E *in vivo* to prevent essential PUFA oxidation in the muscle tissues. Taking this hypothesis into account, the reduction of vitamin E in n-3 enriched samples was expected in our study. Therefore, the high levels of long-chain n-3 PUFA in FO meat could be related with the low vitamin E content.

Values of pH, colour parameters and pigment proportions

Meat from FO treatment showed lighter and less red meat than those from lambs fed the other diets. Lindahl *et al.* (2001) found that the pigment content (haematin) was the most important factor for the variation in L*, a* and h* values, with regression coefficients of -0.45, 0.50 and -0.46, respectively. Similarly, Ngapo & Gariépy (2006) stated that one of the main factors responsible for the colour of fresh beef meat is the pigment content, which is positively correlated with the dietary iron concentration. In this sense, FO meat had the lowest protein proportion in muscle, possibly the lowest myoglobin content (globular protein present in the sarcoplasmic fraction of the muscle), and also the lowest iron content (1.79, 1.45, 1.59 and 1.39 mg/100 g of muscle in C, L, LM and FO, respectively, unpublished data), therefore being the less red meat. The fatty acid profile of the experimental diet could also have affected the meat pigment content and the iron absorption. In this respect, Chao & Gordon (1983) concluded that a diet high in fish oil fatty acid administered as a unique source of fat, can cause metabolic alterations, including iron depletion. Droke

et al. (2003) observed, in a study in Caco-2 cell line, that iron uptake was dependent on the degree of saturation of the fatty acids, the higher the unsaturation the lower the iron uptake.

The lowest MetMb proportion found in FO meat was in accordance with the colour parameters (L*, a* and h*) observed in our study. The MetMb fraction seems to be the second most important factor for the variation in the a* and L* values (Lindahl *et al.*, 2001). Faustman *et al.* (1992) observed that the initial MetMb formation was significantly correlated with the total iron or non-heme iron. As above mentioned, lower iron absorption with increasing the degree of unsaturation of the fatty acids in diet has been reported (Chao & Gordon, 1983). Again, the higher long-chain n-3 PUFA content in FO diet could explain the lower MetMb proportion in FO meat. Nevertheless, the observed increase in MetMb was not dependent on diet, since a significant interaction dietary treatment x storage period was not observed. In this sense, an increase in MetMb in FO meat, due to their higher lipid oxidation (see TBARS values further on), would be expected. In fact, many authors have stated that lipid and pigment oxidation are closely coupled (Younathan & Watts, 1959; Nute *et al.*, 2007). However, according to Faustman *et al.* (2010), not all studies that have measured lipid and myoglobin oxidation in meat have found an interconnection between these two processes. In our study, the lower muscle protein proportion (and possibly the lower pigment proportion) and iron content in FO samples might explain these results.

Lipid oxidation

The highest TBARS values were found in FO meat stored for 7 days, with concentrations above 2 mg MDA kg⁻¹ meat, which is considered to be the cut off value above which rancidity might be detected by consumers (Younathan & Watts, 1959). Regarding with linseed supplementation, the values showed by both L and LM samples at the end of the study exceeded the threshold for detection of off-flavours (0.5 mg MDA kg⁻¹ meat), value above which rancidity may be detected by a trained sensory panel (Lanari *et al.*, 1995). It should be noted that, although the PUFA levels in meat from the three n-3 enriched dietary treatments did not differ significantly, the levels of lipid oxidation among them were clearly different. Meat from lambs fed fish oil underwent greater extent of lipid oxidation after 7

days of storage, which seems to be due to its significantly higher n-3 long-chain PUFA proportions (Table 3). In agreement, previous studies have reported an enhancement of lipid oxidation, assessed as TBARS, in supplemented fish oil lamb (Nute *et al.*, 2007). According to Elmore *et al.* (1999), highly unsaturated fatty acids, such as C20:4n-3, C20:5n-3 and C22:6n-3, readily form free radicals that will then propagate and also catalyze the breakdown of more saturated fatty acids. In addition, vitamin E concentration, as discussed above, was low in samples from all dietary treatments, although, this concentration was especially low when either microalgae or fish oil were used as dietary supplements (Table 3). An insufficient vitamin E content to counteract the high oxidation levels in PUFA-enhanced meats may therefore contribute to explain the poorer lipid stability of n-3 meats (Ponnampalam *et al.*, 2012).

Microbial load

The initial microbial load was low, ranging from 0.91 to 1.88 log₁₀ ucf cm⁻² (Fig. 1b), in comparison with the value set by the Decision 2001/471/EC (OJ, 2001) as the acceptable initial microbial load for sheep carcasses (3.5 log₁₀ ucf cm⁻²). The APC found at the beginning of our study confirms that good hygiene practices were undertaken during our experiment. The higher microbial load of FO meat could be explained by the higher moisture content of these samples. On the other hand, fatty acids and their corresponding esters are a group of chemicals found in nature with proven antimicrobial activity (Kabara *et al.*, 1972). The studies dealing with the effect of the fatty acid composition of the diet on the microbial quality of animal products (such as meat, meat products or even cheese) are scarce and, to our knowledge, none have been carried out on ruminant meat. Among the examples found in the literature, ground pork patties from swine supplemented 12% of high-oleic acid sunflower oil showed, after 4 days of refrigerated storage, significantly lower APC as compared to patties from swine fed a control diet (Acuff *et al.*, 1990). In dry-fermented sausage (in Spanish, “salchichón”), Rubio *et al.* (2007) reported that the lowest microbial growth was found in “salchichón” containing the highest SFA and C16:1 proportion. Finally, Schaffer *et al.* (1995) established that the rate and extent of decline of some microorganisms (*Salmonella* spp., and

Listeria monocytogenes) in cheese from cow milk was correlated with the accumulation of specific fatty acids (C12:0, C14:0, C18:1 and C18:2). In our study, higher C16:1, C18:1 and C18:2n-6 proportions, not only individually but also given as a sum, were observed in C meat (around 56.01%) than in FO meat (48.62%), fact that might be partially responsible for the lower microbial counts observed in control samples. Considering these results, it can be concluded that the use of fish oil as supplement in growing lambs is effective in increasing the long-chain fatty acids content but impairs lamb's performance and meat quality. The higher long-chain n-3 PUFA content of meat from fish oil supplementation yields less attractive meat as far as the colour is concerned, together with higher lipid oxidation values and microbial counts. However, the use of linseed alone or mixed with microalgae leads to linolenic acid enrichment and to a microbial load and colour characteristics similar to those of the control diet, without the adverse effect on lamb performance.

Acknowledgements

We would like to thank to H. Mendoza from the Instituto Tecnológico de Canarias for microalgae supply. This research was funded by an INIA project (RTA2005-00071-C3).

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