JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Long Chain n-3 Polyunsaturated Fatty Acid Concentration and Color and Lipid Stability of Muscle from Heifers Offered a Ruminally Protected Fish Oil Supplement

Peter G. Dunne,[†,‡] Jaroslaw Rogalski,[‡] Stuart Childs,[§] Frank J. Monahan,[∥] David A. Kenny,[∥] and Aidan P. Moloney*[,†]

[†]Animal and Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland

[‡]Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland

[§]Animal and Grassland Research and Innovation Centre, Teagasc, Mellows Campus, Athenry, Co. Galway, Ireland

[∥]School of Agriculture, Food Science & Veterinary Medicine, University College Dublin, Dublin 4, Ireland

Supporting Information

ABSTRACT: Beef heifers ($n = 48$) were offered, daily, a 1.5 kg ration that contained 0, 69, 138, and 275 g of ruminally protected fish oil (RPFO) fortified with vitamin E (5000 IU/kg). The fatty acid profile of neutral (NL) and polar lipids (PL) of neck muscle was analyzed by GC-FID. Minced muscle was displayed in an 80% O_2:20% CO_2 atmosphere under simulated retail display conditions. Muscle α-tocopherol concentrations did not differ. For PL, the proportions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased ($P < 0.05$, linearly and quadratically, respectively). For NL, the proportion of EPA was not affected but DHA increased linearly and quadratically ($P < 0.05$). Supplementation did not affect color stability of ground beef, but lipid oxidation was higher ($P < 0.001$) for 275 g of RPFO on day 10 of display. In conclusion, supplementation with RPFO increased muscle EPA and DHA with no effect on color stability while lipid oxidation was increased only at the highest level of supplementation, after prolonged display.

KEYWORDS: ruminally protected, n-3, omega-3, polyunsaturated fatty acids, beef, color, oxidative stability, eicosapentaenoic acid, docosahexaenoic acid

INTRODUCTION

Epidemiological evidence points to a lower incidence of cardiovascular disease among populations with a high dietary consumption of oily fish and seafood, partly explained by the relative abundance of the n-3 long chain polyunsaturated fatty acids (PUFA) (n-3 LC-PUFA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) in marine foods.[1] There is considerable variation in the mean intake of n-3 LC-PUFA in Western Europe, with a 9-fold difference between the lowest and highest mean national intakes,[2] probably reflecting differences in fish consumption. Apart from fish, ruminant meats can be a relatively good source of n-3 PUFA. Compared to cattle fed concentrates, grass-fed cattle have been shown to have higher, albeit comparatively low, concentrations of n-3 LC-PUFA in muscle,[3,4] especially in polar membrane lipids.[5] Although the fatty acid composition of meat primarily reflects the fatty acid composition of an animal's diet, in ruminants this relationship is complicated by ruminal biohydrogenation (BH) of dietary PUFA. Thus, where a more unsaturated fatty acid composition of ruminant meats is desired, e.g. to enhance nutritional quality for humans, strategies must be adopted to reduce ruminal BH of unsaturated lipids. An array of modification of dietary lipids to prevent BH by rumen microorganisms has been examined.[6] The ruminally protected n-3 PUFA used in most studies has been rich in linolenic acid (18:3n-3), and there have been relatively few reports on the effect of supplementation with a source of ruminally protected (RP) EPA and DHA on muscle concentrations of these fatty acids.

When altering the fatty acid composition of meat for human benefit, consideration must be given to the effects on meat sensory quality, especially during storage and retail display. Muscle foods by virtue of the fact that they contain unsaturated lipids and prooxidant components, are inherently susceptible to lipid oxidation[7] which is strongly associated with color stability. Oxidative changes in muscle lipids are the principal cause of deterioration in the quality of muscle foods and consequent economic losses to the meat industry. Muscle lipids are composed of both polar lipids (PL) (mainly phospholipids located in the cellular and subcellular membranes) and neutral lipids (NL) (consisting mainly of triacylglycerols (TAG) in the adipocytes that are located along the muscle fibers and in the interfascicular region).[8] In beef, long chain (>C18) highly unsaturated and, therefore, highly oxidizable fatty acids of dietary origin are not esterified into TAG but preferentially deposited in membranal phospholipids.[5,9] The fatty acid composition of membranal phospholipids, rather than TAG, therefore largely determines the susceptibility of meat to lipid oxidation.[10] Thus, successful efforts to increase n-3 LC-PUFA in meat would be expected to

Received: January 18, 2011
Accepted: March 30, 2011
Revised: March 25, 2011
Published: March 30, 2011

Table 1. Ingredient Formulation of the Experimental (PU00, PU69, PU138 and PU275) and Balancer Rations

	diet (kg/1000 kg, dry matter basis)					
ingredient	PU00	PU69	PU138	PU275	balancer 1	balancer 2
barley	340	278	220	110	225	420
soyabean meal	5	29	55	105	430	110
molassed sugar beet pulp	345	296	236	118	230	421
molasses					45	20
Palmit 80 prills[a]	310	230	155			
vitamins/minerals[b]					50	20
ground limestone					15	7
sodium chloride					1	1
dicalcium phosphate					4	1
palmitate	310	230	155			
RP n-3LC-PUFA supplement[c]		167	334	667		

[a] Content of palmitic acid (16:0) = 0.8; myristic acid (14:0) < 0.02; stearic acid (18:0) = 0.04−0.07; oleic acid (18:1) = 0.08−0.12; linoleic acid (18:2) < 0.03, product of Trouw Nutrition, 36 Ship St., Belfast BT15 1JL, Northern Ireland. [b] The mineral/vitamin mix included in the balancer rations contained the following ingredients: vitamin A, 3.2×10^5 IU (international units)/kg; vitamin D_3, 8.0×10^4 IU/kg; vitamin E, 1×10^4 IU/kg; cobalt carbonate, 100 mg/kg; manganese oxide, 2.5×10^3 mg/kg; cupric sulfate, 1.25×10^3 mg/kg; zinc oxide, 4×10^3 mg/kg; calcium iodate, 320 mg/kg; sodium selenite, 30 mg/kg (Mixrite (I), Ltd., Bennettsbridge, Co. Kilkenny, Ireland). [c] The RP n-3 LC-PUFA (EPA/DHA) supplement contained 44.66 g of crude oil/100 g, 0.10 g of crude protein/100 g, 0.03 g of crude fiber/100 g and 50 g of ash/100 g. The crude oil fraction contained 330 g of EPA, 220 g of DHA and 650 g of total n-3 fatty acids. Vitamin E, as *dl*-α-tocopheryl acetate, was added to the RP n-3 LC-PUFA supplement to achieve a final concentration of 5.0×10^3 IU/kg. Fatty acid composition of the supplement and of the concentrates reported by Childs et al.[14]

increase the susceptibility of lipids, particularly phospholipids, to oxidative deterioration thereby necessitating concomitant antioxidant supplementation (usually vitamin E).[11−13]

The challenge therefore is to improve fatty acid composition, i.e. to increase in particular n-3 LC-PUFA, while not decreasing oxidative stability which could compromise the sensory quality characteristics of the meat. The objectives of the present study were to (a) determine the effect of incremental inclusion of RP n-3 LC-PUFA (EPA and DHA) in rations offered to beef heifers on the concentrations of n-3 LC-PUFA in muscle and (b) to determine the color and oxidative stability of the beef, when ground and packaged in a high oxygen modified atmosphere under simulated retail display conditions. We hypothesized that such supplementation would (a) increase concentrations of EPA and DHA in muscle lipids and (b) decrease the stability of color and lipids in beef.

MATERIALS AND METHODS

Materials. All procedures conducted in this experiment were performed in accordance with the European Communities (Amendment of Cruelty to Animals Act 1876) Regulations 2005 (S.I. No. 613/2005) administered by the Department of Health & Children of the Irish Government and complied with all relevant Irish national and EU legislation. Continental crossbred heifers (n = 48) were assigned to treatments in a completely randomized design. All heifers were offered grass silage ad libitum for approximately 6 weeks before the experiment commenced. Thereafter, daily at 0900 h heifers were offered a 1.5 kg fresh weight of bolus ration (approximately 850 g of dry matter (DM)/kg) that contained the proprietary RP n-3 LC-PUFA supplement of EPA and DHA (Nutreco Holding N.V., Amersfoort, The Netherlands) mixed with 0.375 kg of molasses. Rumen protection was achieved via encapsulation in a pH sensitive matrix which remained intact at rumen pH but was broken down at the lower pH in the abomasum releasing the constituents for absorption. Fish oil (FO) derived from anchovy, sardine and salmon and distilled to concentrate the n-3 PUFA content was used. The bolus ration contained 0 (control), 69, 138, and 275 g of RP n-3 LC-PUFA per kg (PU00, PU69, PU138 and PU275, respectively) (Table 1) and heifers on each treatment were fed their respective rations individually using Calan Broadbent feeders (Calan, Inc., Northwood, NH 03261, USA). A high crude protein (CP) (26.2 g/100 g) balancer ration (1.0 kg; balancer 1, Table 1) was fed concomitantly to all heifers to compensate for the low CP of the treatment rations. Each afternoon 3.5 kg (3.028 kg DM) of another balancer ration (balancer 2, Table 1) was offered to all heifers with 1.5 kg (1.24 kg DM) of straw. Diets were formulated to be isoenergetic, isolipidic and isonitrogenous. The chemical composition of the rations, including the fatty acid profile, was reported by Childs et al.[14] The proportion of EPA was 0.01, 4.25, 13.01, and 29.26 g/100 g of fatty acid methyl esters (FAME) for PU00, PU69, PU138 and PU275, respectively. The corresponding values for DHA were 0.00, 2.97, 9.10, and 19.03 g/100 g of FAME. Vitamin E, as *dl*-α-tocopheryl acetate, was added to the RP n-3 LC-PUFA supplement at a rate of 5,000 IU per kg and to the vitamin/mineral supplement used in the balancer rations at a rate of 10,000 IU per kg (manufacturer's specification; 1 mg of *dl*-α-tocopheryl acetate is equivalent to 1 IU of vitamin E activity).

Methods. *Slaughter, Muscle Sample Recovery and Storage.* After 7 weeks on treatment, heifers were slaughtered humanely at a commercial abattoir. Samples of neck muscle were recovered within 1 h postmortem and placed in polythene bags, vacuum-packed within 30 min and immediately transferred to an insulated container with dry ice. Samples were subsequently stored at −80 °C for 6 months. One month prior to simulated retail display, samples were transferred to Ashtown Food Research Centre and stored at −30 °C. Portions of frozen muscle (50−100 g) for fatty acid analysis were removed by electrical band saw, vacuum-packed (Webomatic vacuum-packaging systems SuperVax, ThyssenKrupp Schulte GmbH, Düsseldorf 40041, Germany) and replaced in the −30 °C freezer. Eighteen hours before mincing and display, the remaining quantities of muscle were placed in a dark chill room and thawed at 2 °C.

Sample Preparation, Modified Atmosphere Packaging and Simulated Retail Case Display. Prior to mincing, portions of thawed neck muscle were recovered for α-tocopherol (10 g) and proximate compositional analysis (10 g), immediately vacuum-packed and stored at −30 °C

until analysis. All product contact surfaces and utensils were washed with hot water, sanitized using a proprietary liquid sanitizer (Diversey Lever, Ltd., Northampton NN3 8PD, U.K.) and rinsed. Disposable gloves were worn throughout. Sections of neck muscle were minced by passing through a mincer with 5 mm holes. The mincer was dismantled, cleaned, sanitized, rinsed and dried between samples. When minced, samples were divided into six approximately equal portions (30 g), formed into patties of 2.54 cm thickness in a plastic mold and dispensed into styrofoam trays (187 × 145 × 40 mm, Lin Fresh, Linpac Plastics, Ltd., Knottingley, West Yorkshire WF11 0BL, U.K.) to which absorbent pads had been added. Trays were sealed under oxygen impermeable barrier film (Versatile Packaging, Ltd., Castleblayney, Co. Monaghan, Ireland) (oxygen transmission rate: 8 cm^3 O_2/m^2/24 h at 23 °C and 75% relative humidity) following evacuation and flushing with 80% O_2:20% CO_2 (Food Fresh, BOC Gases, Dublin, Ireland) in a modified atmosphere packaging (MAP) machine (Foodpack 400 V/G, Ilpra S.p.A., Corso Pavia 30, Vigevano 27029, Pavia, Lombardia, Italy). Prior to packaging the minced beef, the gaseous composition of inflated empty trays was checked using an automated MAP testing device (MAPtest 3050 Packaging atmosphere analyzer, Hitech Instruments Ltd., Luton, Hertfordshire LU4 8EF, U.K.). Trays were randomly positioned in an open-fronted retail display cabinet with a regular automatic defrost cycle (Cronos fan-assisted cabinet, Crisobanc Refrigeration, Crisobanc S.p.A., 35038 Torreglia, Padova, Italy) under permanent fluorescent lighting (Philips TL-D 58W, Philips Electronics (Ireland) Ltd., Fonthill Rd., Dublin 22, Ireland; luminous flux of 2800 lm, lux = 3616) and permanently shielded by an insulating blind, to maintain a uniform temperature distribution throughout. Temperature in the cabinet was monitored using three needle thermocouples (Thermocouple type T 219-1748, Radionics, Ltd., Dublin 12, Ireland) positioned on the lowest, middle and highest shelves. The thermocouples were attached to an automatic data logger (Grant 1250 series squirrel data logger, Grant Instruments, Ltd., Barrington, Cambridgeshire CB2 5QZ, U.K.) programmed to record temperature at 1 min intervals (Figure 1 in the Supporting Information). Approximately 3 h after MAP packaging, the sealed tray was opened, the minced beef was removed and wrapped in polyvinyl chloride (PVC) film and the L (lightness), a (redness) and b (yellowness) values of the minced beef were measured (day 0) using a benchtop Hunter lab UltraScan XE spectrocolorimeter with Universal Software Version 2.2.2 (Hunter Associates Laboratory, Inc., 11491 Sunset Hills Rd., Reston, VA 20190-5280, USA) which was calibrated using its light trap and standard white calibration tile, prior to measurement. Five readings were made on non-overlapping areas of the minced beef using the small optical port (∅ = 0.95 cm), and average values were reported as final readings. All measurements were made in the Hunter Lab color space. Diffuse illumination (D_{65}, 10°) with an 8° viewing angle was used, and the specular component was excluded. Similarly, on days 1, 3, 5, 7, and 10 of display the color coordinates (Hunter L, a, b) were measured. Muscle hue angle (H) and saturation (C) were calculated as $\tan^{-1}(b/a)$ and $\sqrt{(a^2 + b^2)}$, respectively. Final conversion of hue angle from radians to degrees was achieved by multiplying $\tan^{-1}(b/a)$ by $180/\pi$.[11] The spectrocolorimeter was used in reflectance mode, and K/S values were recorded at 10 nm intervals between 400 and 750 nm. K/S values were calculated at 525 and 572 nm by linear interpolation.

Measurement of Oxidative Stability of Intramuscular Lipids. Following color measurement on days 0, 5 and 10, PVC film was removed from the minced beef, which was immediately vacuum-packed and frozen at −30 °C until measurement of oxidative stability. This was assessed by determination of secondary products of lipid oxidation as 2-thiobarbituric acid reactive substances (TBARS) using a modification of the filtration method of Siu and Draper.[15] Minced beef (2.5 g) was weighed into a 50 mL centrifugation tube (Sarstedt AG & Co., Rommelsdorfer Strasse, Postfach 1220, D-51582 Nümbrecht, Germany), to which butylated hydroxytoluene (10 mg) (Sigma Chemical Co., Ltd., Poole, Dorset, U.K.) was added. While held on ice, the sample was homogenized in trichloroacetic acid (TCA) solution (10 mL, 5% w/v) using an Ultraturrax T25 homogenizer (Janke & Kunkel GmbH, IKA Werke GmbH & Co. KG, D-71219, Staufen, Germany) and centrifuged for 10 min at 12800g (Beckman Optima XL-100K Ultracentrifuge, Beckman Coulter, Inc., Fullerton, CA 92834-3100, USA), and the supernatant was filtered through Whatman No. 4 filter paper. The filtered supernatant was transferred to a screw cap glass test tube, mixed with 2-thiobarbituric acid (TBA, 0.03M, 3 mL) and heated in a boiling water bath for 1 h to develop the rose-pink color by reaction between malondialdehyde (MDA) and TBA. The absorbance was measured at 532 nm (Shimadzu UV-1700 Pharma Spec UV−visible spectrophotometer, Shimadzu Scientific Instruments, 7102 Riverwood Dr., Columbia, MD 21046, USA) against a blank containing TBA (0.03M, 3 mL) and TCA (3 mL, 5% v/v). The TBARS values were calculated using an external standard technique from a standard curve (1.5−25 μM) of MDA, prepared by acidification of 1,1,3,3-tetramethoxypropane (TMP) and expressed as mg of MDA per kg of sample. The final conversion of TMP concentration to MDA concentration was accomplished by multiplying the number of μM TMP equivalents per gram of sample by the molecular weight of MDA.[16]

Measurement of Muscle α-Tocopherol Concentration and Chemical Composition. Extraction of α-tocopherol from muscle tissue was performed according to the method of Buttriss and Diplock.[17] α-Tocopherol in samples and standards was determined using high performance liquid chromatography, incorporating a C18 column (250 × 4.6 mm i.d., Primesphere 5 μm particle size; Phenomenex, Macclesfield, Cheshire, SK10 2BN, U.K.) fitted with a guard column (Security Guard, Phenomenex, U.K.) and a mobile phase of methanol: water (99:1, v/v) at a flow rate of 2 mL/min. Elution of samples and standards was performed using a Thermo Separation Products HPLC 3000 system incorporating PC1000 software and a UV3000 detector, set at 292 nm (Thermo Separation Products, Hemel Hempstead, HP2 4TG, Herts., U.K.). Quantification of α-tocopherol in samples was achieved using an external standard technique from a standard curve (1−4.5 μg/mL) of α-tocopherol while recovery was determined by adding a known concentration to duplicate sample homogenates prior to extraction and comparing peak heights of spiked samples with their respective unspiked controls. Moisture and fat contents were determined using an automated, integrated microwave moisture and methylene chloride fat extraction method with a CEM microwave moisture/solid analyzer (AVC 80, CEM Corp., Matthews, NC 28105 USA) and a CEM (model FES-80) automatic fat extraction system according to Bostian et al.[18]

Extraction, Separation and Derivatization of Fatty Acids from Intramuscular Lipids. Fatty acids were extracted from muscle tissue as described by Noci, Monahan, French and Moloney.[19] The total weight of extracted lipid was recorded, and approximately 50 mg of the lipid extracts was applied to solid-phase extraction cartridges with 500 mg of aminopropyl packing (Bond-Elut 500 mg, 3 mL reservoir; Varian Instruments Inc., 3120 Hansen Way, Palo Alto, CA 94304-1030, USA) previously conditioned by a 5 × 3 mL flush with chloroform to ensure solvation of the bonded phase and removal of potential impurities. The neutral lipid (NL) was eluted with 5 × 1 mL of chloroform, and the eluate was collected in preweighed Pyrex screw cap test tubes (10 mm × 125 mm). The cartridges were washed with 1 mL of 1:1 chloroform/methanol (v/v), followed by 3 mL of methanol to elute the polar lipid (PL) fraction. The NL and PL fractions were dried at 40 °C to constant weight under a stream of nitrogen, and the weight of each fraction was recorded. The separated lipid classes were dissolved in 300 μL of toluene and stored overnight at −30 °C for preparation of FAME, in a two-step sequential base- followed by acid-catalyzed trans-methylation followed by sample cleanup as described by Noci et al.[19]

Gas Chromatographic Analysis of Intramuscular Lipid FAME. Gas chromatographic analysis of FAME was performed using a BPX70

Table 2. Fatty Acid Composition, as Proportion of Total FAME, in the Muscle Neutral Lipid Fraction of Heifers Offered Increasing Amounts of a RP n-3 LC-PUFA Supplement[a]

	nominal dietary supply (g/day)				
fatty acid	PU00 (0)	PU69 (69)	PU138 (138)	PU275 (275)	significance[b]
12:0	0.17 (0.031)	0.20 (0.031)	0.30 (0.031)	0.17 (0.035)	Q*
14:0	3.41 (0.270)	3.61 (0.267)	3.76 (0.199)	3.78 (0.313)	NS
14:1	1.08 (0.262)	0.67 (0.179)	0.61 (0.111)	0.73 (0.122)	NS
15:0	0.59 (0.083)	0.75 (0.085)	0.78 (0.071)	0.76 (0.068)	NS
16:0	25.26 (1.382)	25.40 (1.544)	27.32 (0.550)	24.88 (1.866)	NS
16:1	5.97 (0.758)	4.42 (0.440)	4.70 (0.429)	3.24 (0.546)	L**
17:0	0.92 (0.109)	1.01 (0.149)	1.22 (0.044)	1.29 (0.093)	L*
17:1	0.30 (0.144)	0.77 (0.156)	0.86 (0.162)	0.54 (0.188)	NS
18:0	10.79 (0.561)	11.75 (1.036)	12.90 (0.528)	10.71 (1.139)	NS
18:1n9*trans*	1.60 (0.315)	1.23 (0.447)	2.20 (0.352)	2.30 (0.358)	L*
18:1n9*cis*	34.50 (1.242)	29.76 (3.713)	33.34 (1.242)	29.93 (2.777)	NS
18:2n6*trans*	0.30 (0.108)	0.37 (0.098)	0.32 (0.100)	0.47 (0.096)	NS
18:2n6*cis*	1.46 (0.114)	1.65 (0.109)	1.48 (0.094)	1.45 (0.161)	NS
18:3n6	0.01 (0.006)	0.01 (0.010)	ND[h]	0.01 (0.007)	–
18:3n3	0.39 (0.087)	0.52 (0.103)	0.47 (0.082)	0.68 (0.121)	L*
20:0	0.22 (0.126)	0.18 (0.109)	ND	0.10 (0.054)	–
20:1n9	0.13 (0.028)	0.09 (0.033)	0.13 (0.077)	0.13 (0.036)	NS
20:3n6	0.09 (0.056)	0.19 (0.186)	0.003 (0.003)	0.41 (0.153)	–
20:3n3	0.15 (0.113)	0.03 (0.017)	0.01 (0.009)	0.03 (0.021)	–
20:4n6	0.01 (0.010)	0.01 (0.010)	0.01 (0.009)	0.12 (0.074)	–
22:1n9	0.04 (0.071)	0.03 (0.087)	0.15 (0.071)	0.30 (0.039)	L**
20:5n3	0.22 (0.128)	0.31 (0.148)	0.14 (0.115)	0.41 (0.128)	NS
22:6n3	0.03 (0.032)	0.06 (0.037)	0.05 (0.029)	0.27 (0.032)	L*, Q**
total SFA[c]	41.91 (1.429)	43.50 (2.517)	46.09 (1.136)	42.99 (1.732)	Q^+
total MUFA[d]	43.64 (1.089)	36.97 (4.124)	41.58 (1.535)	37.12 (2.939)	NS
total PUFA[e]	2.65 (0.237)	3.16 (0.392)	2.48 (0.179)	3.85 (0.451)	L*
n-6 PUFA[f]	1.86 (0.186)	2.24 (0.238)	1.82 (0.108)	2.46 (0.299)	L^+
n-3 PUFA[g]	0.79 (0.209)	0.92 (0.296)	0.66 (0.105)	1.40 (0.194)	L*
PUFA:SFA	0.06 (0.007)	0.08 (0.014)	0.05 (0.004)	0.09 (0.013)	L^+, Q^+
n-6:n-3 ratio	4.31 (1.453)	3.33 (1.421)	2.51 (0.456)	2.04 (0.281)	L^+

[a] Mean values are shown; data in parentheses are SEM. [b] NS = not significant. L and Q are linear and quadratic effects, respectively, of increasing dietary inclusion of RP n-3 LC-PUFA. Significance designated as $^+P < 0.10$, $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; – indicates that this fatty acid was not detected in a sufficient number of samples to be statistically analyzed. [c] Sum of all even chain FA from 10:0 to 24:0 + 15:0 and 17:0. [d] Sum of 14:1, 16:1, 17:1, all 18:1, 20:1n9 and 22:1. [e] Sum of total n-6, total n-3. [f] Total n-6 PUFA = sum of 18:2, 18:3n6, 20:3n6 and 20:4. [g] Total n-3 PUFA = sum of 18:3n3, 20:3n3, 20:5n3 and 22:6n3. [h] ND: not detected.

bis-cyanopropyl siloxane-silphenylene cross-linked polymer column (120 m × 0.25 mm i.d., 0.2 μm film thickness, SGE, Ringwood, Victoria 3134, Australia) in a Varian 3400CX GC (Varian Instruments Inc., 3120 Hansen Way, Palo Alto, CA 94304-1030, USA) with a flame ionization detector (FID) and a split/splitless injection port (#1078), used in the split mode (split ratio of 30:1). The inlet liner was a deactivated 4 mm i.d. split sleeve (cat. no. 20781). The injector and the FID were kept at constant temperatures of 250 and 300 °C, respectively. The column oven temperature was held at 50 °C for 1 min, increased at 20 °C/min to 160 °C, increased to 220 at 4 °C/min and held for 5 min, to 240 at 4 °C/min and held for 10 min. Methyl tricosanoate (23:0 me, 1 mg/mL, 500 μL per sample) was used as internal standard. For peak identification, a standard mix of 37 component FAME (47885U Supelco 37 component FAME mix, 10 mg/mL, in methylene chloride; Supelco Inc., Bellefonte, PA 16823, USA) was used. Methyl esters standards of EPA *all-cis*-5,8,11,14,17 and DHA *all-cis*-4,7,10,13,16,19 (Sigma-Aldrich, St. Louis, MO 63103, USA) were also employed to confirm the identity of the respective FAME. The carrier gas was H_2. Chromatograms were recorded and analyzed using the Varian Saturn GCMS software package (Varian), which also controlled oven temperature. For conversion of methyl eicosapentaenoate (EPA-me) and methyl docosahexaenoate (DHA-me) to their respective fatty acid concentrations, as mg of EPA and mg of DHA per 100 g of muscle, the formula of Golay et al.[20] with the stoichoimetric factors 0.974 and 0.976, respectively, was used. Typical chromatograms illustrating the elution of EPA-me and DHA-me in NL and PL are shown in Figure 1 in the Supporting Information.

Statistical Analysis. Statistical analysis of data was performed using SAS. Lipid and vitamin E data were subjected to ANOVA using PROC GLM with treatment as the only main effect, and the response to increasing dietary RP n-3 LC-PUFA inclusion was tested using orthogonal polynomial contrasts (CONTRAST statement, SAS). Color (lightness, redness, yellowness, saturation and hue angle) and TBARS data were analyzed according to a split-plot design using a model that included treatment in the main plot and display time and treatment × display time interaction in the subplot. The treatment effect and the treatment × display time interaction were partitioned into linear and quadratic contrasts. In all

Table 3. Fatty Acid Composition, as Proportion of Total FAME, in the Muscle Polar Lipid Fraction of Heifers Offered Increasing Amounts of a RP n-3 LC-PUFA Supplement[a]

	nominal dietary supply (g/day)				
fatty acid	PU00 (0)	PU69 (69)	PU138 (138)	PU275 (275)	significance[b]
12:0	0.24 (0.089)	0.13 (0.072)	0.07 (0.030)	0.049 (0.019)	L*
14:0	0.64 (0.171)	0.57 (0.161)	0.62 (0.178)	1.02 (0.231)	NS
14:1	0.08 (0.036)	0.06 (0.046)	0.06 (0.035)	0.30 (0.196)	NS
15:0	5.84 (1.686)	7.63 (2.643)	6.38 (1.777)	3.91 (1.604)	NS
15:1	0.10 (0.037)	0.11 (0.077)	0.10 (0.077)	0.07 (0.031)	Q*
16:0	26.79 ab (1.463)	24.25 ab (1.834)	28.71 b (1.401)	22.13 a (1.401)	*
16:1	1.96 (0.253)	0.79 (0.302)	1.49 (0.231)	1.49 (0.2410)	Q*
17:0	0.59 (0.150)	0.47 (0.211)	0.44 (0.091)	0.71 (0.045)	NS
17:1	0.37 (0.127)	0.34 (0.094)	0.20 (0.091)	0.35 (0.084)	NS
18:0	5.01 (0.578)	4.22 (0.398)	4.37 (0.712)	5.57 (0.495)	NS
18:1n9*trans*	0.16 (0.085)	0.17 (0.115)	0.42 (0.197)	1.35 (0.209)	L*
18:1n9*cis*	21.22 (1.438)	16.69 (1.957)	13.76 (2.159)	15.12 (1.602)	L*
18:2n6*trans*	0.45 (0.202)	ND[h]	0.16 (0.119)	0.22 (0.127)	–
18:2n6*cis*	11.03 (0.627)	10.87 (0.786)	8.13 (0.600)	6.15 (0.600)	L***
18:3n6	0.01 (0.007)	0.01 (0.006)	0.01 (0.007)	0.01 (0.008)	NS
18:3n3	0.92 (0.165)	0.79 (0.148)	0.61 (0.132)	0.95 (0.113)	NS
20:3n6	0.67 (0.235)	0.72 (0.248)	0.57 (0.115)	0.93 (0.139)	NS
20:3n3	1.94 (0.427)	2.68 (0.599)	1.76 (0.384)	2.69 (0.304)	NS
20:4n6	0.24 (0.243)	ND	ND	ND	–
22:1n9	0.51 (0.421)	0.74 (0.730)	2.08 (0.344)	3.25 (0.311)	L***
20:5n3	1.88 (0.665)	4.22 (0.833)	4.84 (0.589)	7.09 (0.636)	L***
22:6n3	0.73 (0.173)	1.52 (0.217)	1.19 (0.154)	1.17 (0.166)	Q*
total SFA[c]	39.63 (1.953)	37.49 (2.794)	42.10 (1.652)	34.10 (2.578)	L^+, Q^+
total MUFA[d]	23.72 (1.624)	18.20 (1.850)	18.19 (1.700)	18.69 (1.767)	L^+, Q^+
total PUFA[e]	19.77 (2.118)	25.10 (2.738)	22.58 (1.933)	26.40 (2.018)	L^+
n-6 PUFA[f]	12.42 (1.215)	11.64 (0.563)	8.92 (0.457)	7.40 (0.420)	L***
n-3 PUFA[g]	1.87 (0.347)	1.73 (0.282)	3.10 (0.534)	5.00 (0.512)	L***
PUFA:SFA ratio	0.53 (0.069)	0.71 (0.115)	0.56 (0.062)	0.84 (0.098)	L*
n-6:n-3 ratio	6.38 (0.929)	8.09 (1.579)	4.40 (0.873)	1.61 (0.175)	L***

[a] Mean values are shown; data in parentheses are SEM. [b] NS = not significant. L and Q are linear and quadratic effects, respectively, of increasing dietary inclusion of RP n-3 LC-PUFA. Significance designated as $^+P < 0.10$, $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$; – indicates that this fatty acid was not detected in a sufficient number of samples to be statistically analyzed. Within rows, values assigned different superscripts differ significantly ($P < 0.05$). [c] Sum of all even chain FA from 10:0 to 24:0 + 15:0 and 17:0. [d] Sum of 14:1, 16:1, 17:1, all 18:1, 20:1n9 and 22:1. [e] Sum of total n-6, total n-3. [f] Total n-6 PUFA = sum of 18:2, 18:3n6, 20:3n6 and 20:4. [g] Total n-3 PUFA = sum of 18:3n3, 20:3n3, 20:5n3 and 22:6n3. [h] ND: not detected.

cases, P values <0.05 were accepted as statistically significant. Relationships between EPA and DHA concentrations or their ratio to vitamin E concentration in muscle with muscle redness or TBARS concentration after 10 days of display were explored using linear regression.

RESULTS

Vitamin E and Lipid Content of Muscle. There was no effect of treatment on either lipid or vitamin E content. Mean muscle lipid content (g/100 g) was 2.87, 2.29, 2.96, and 3.89 for PU00, PU69, PU138 and PU275, respectively. Mean α-tocopherol concentration (μg/g) was 5.81, 6.95, 5.94, and 7.15 μg/g for PU00, PU69, PU138 and PU275, respectively.

Fatty Acid Composition. In the muscle NL fraction (Table 2), the proportion of EPA was not affected, but the proportion of DHA increased ($P < 0.05$, linear and quadratic) with increasing dietary inclusion of RP n-3 LC-PUFA. In the muscle PL fraction (Table 3), the proportion of both EPA and DHA increased ($P < 0.05$, linear and quadratic, respectively) with increasing dietary inclusion of RP n-3 LC-PUFA. In the muscle NL fraction, the concentration of EPA was not affected but the concentration of DHA was increased ($P < 0.05$, linear) by increasing dietary inclusion of RP n-3 LC PUFA (Figure 1A). In the muscle PL fraction, the concentration of EPA was increased ($P < 0.05$, linear) but the concentration of DHA was not affected (Figure 1B). The total EPA concentration in muscle lipids averaged 18.8, 19.8, 31.4, and 53.6 mg/100 g ($P < 0.05$, linear) for PU0, PU69, PU139 and PU275, respectively. The corresponding values for DHA concentrations were 7.1, 8.2, 9.2, and 13.7 ($P < 0.05$, linear). The sum of EPA and DHA in muscle lipids averaged 25.9, 28.0, 40.6, and 67.3 mg/100 g ($P < 0.05$, linear) for PU00, PU69, PU139 and PU275, respectively.

With regard to other fatty acids, the effects of increasing the dietary supply of RP n-3 LC-PUFA were confined to only a limited number of individual fatty acids in both lipid fractions. In the muscle NL fraction (Table 2), dietary inclusion of RP n-3 LC-PUFA led to increases ($P < 0.05$) in the proportions of 12:0

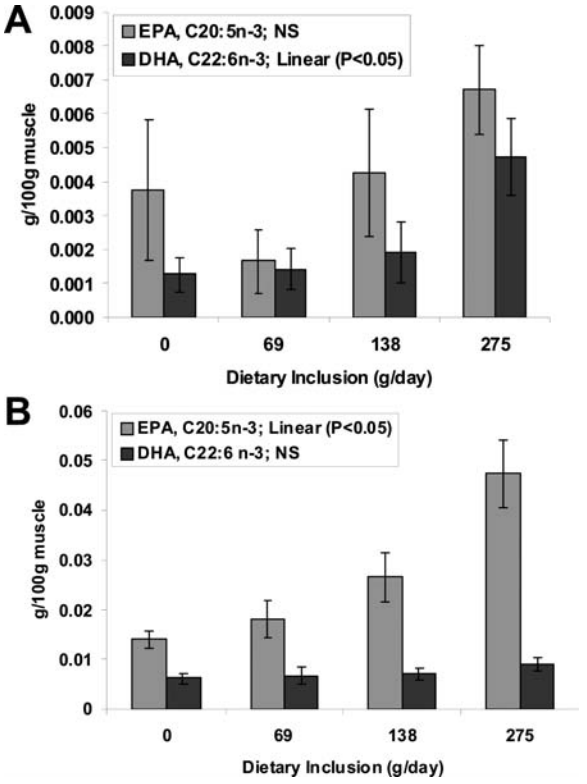

Figure 1. The concentration of eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in (A) neutral and (B) polar lipid fractions. Error bars represent the standard error about each mean.

(quadratic), 17:0 (linear), 18:1 n9 *trans* (linear), 18:3 n-3 (linear), 22:1 n-9 (linear), saturated fatty acids (SFA, quadratic, $P < 0.1$), PUFA (linear), n-6 PUFA (linear $P < 0.1$), n-3 PUFA (linear) and the PUFA:SFA ratio (linear, quadratic ($P < 0.1$)). Dietary inclusion of RP n-3 LC-PUFA led to decreases ($P < 0.05$) in the proportion of 16:1 (linear) and the n-6:n-3 PUFA ratio (linear, $P < 0.1$). In the muscle PL lipid fraction (Table 3) dietary inclusion of RP n-3 LC-PUFA increased ($P < 0.05$) the proportions of 18:1n-9 *trans* (linear), 22:1n-9 (linear), PUFA (linear, $P < 0.1$), n-3 PUFA (linear) and the PUFA:SFA ratio (linear). Dietary inclusion of RP n-3 LC-PUFA decreased ($P < 0.05$) the proportion of 12:0 (linear), 15:1 (quadratic), 16:1 (quadratic), 18:1n9 *cis* (linear), 18:2n6 *cis* (linear), SFA (linear, quadratic, $P < 0.1$), monounsaturated fatty acids (MUFA, linear, quadratic, $P < 0.1$), n-6 PUFA (linear) and the n-6:n-3 PUFA ratio (linear).

Indices of Color Stability during Simulated Retail Display. There was no effect of treatment or treatment × display time interaction for Hunter *L* value (lightness), *a* value (redness), *C* value (saturation) and *H* value (hue angle). There was a treatment × display time interaction ($P = 0.038$) for *b* value whereby the PU00 *b* value was lowest on day 3, and higher than PU275 on day 10 of display (Figure 2A). Mean *a* and *C* values decreased ($P < 0.001$) as time on display increased (Figure 2B, 2C) while mean *H* value increased ($P < 0.05$) as time on display increased (Figure 2D). Lightness decreased ($P < 0.05$) between day 1 and day 3 but increased ($P < 0.05$) subsequently (data not shown). The mean value of $[(K/S)_{572}/(K/S)_{525}]$ at day 0 was higher ($P < 0.05$) than on day 1; it increased ($P < 0.05$) on day 3 but decreased ($P < 0.05$) subsequently (Figure 3A). The mean value of $[(K/S)_{610}/(K/S)_{525}]$ at day 0 was lower ($P < 0.05$) than on day 1; it decreased ($P < 0.05$) on day 3 but increased ($P < 0.05$) subsequently (Figure 3B).

Lipid Oxidation during Simulated Retail Display. There was a treatment × display time interaction ($P = 0.005$) for TBARS (Figure 4). On day 10 of display, PU275 had a higher ($P < 0.05$) TBARS value, but TBARS values were similar between treatments at the other display times.

There was no significant relationship ($P > 0.05$) between EPA and DHA concentrations alone or combined in muscle PL and muscle redness or TBARS concentration after 10 days of display. Similarly there was no significant relationship ($P > 0.05$) between the ratio of these PUFA to vitamin E concentrations and muscle redness or TBARS concentration after 10 days of display.

DISCUSSION

One objective of ruminant nutritionists over recent decades has been to increase PUFA, particularly LC n-3 PUFA in ruminant products.[21] If sufficient LC n-3 PUFA is supplied in the diet, such as when FO is included, a proportion will escape BH,[22] but if substantial quantities are to be delivered to tissues, protection is required. The extent to which unprotected EPA and DHA from FO have undergone BH has varied from 0.76 and 0.72, respectively, in sheep,[23] to 0.92 and 0.91, respectively, in steers.[24]

While many studies have investigated the efficacy of RP lipid supplementation on muscle fatty acids, the focus has largely been on increasing PUFA per se or the PUFA:SFA ratio. Where increasing the n-3 PUFA concentration has been the objective, RP plant oils rich in 18:3n-3 have generally been used. Few studies have examined supplementation with RP lipids rich in EPA and DHA. The first objective of the present study was to investigate the efficacy of incremental inclusion of RP EPA and DHA in the diet at increasing their concentrations in muscle lipids. Of the protection approaches examined, the most effective seems to be encapsulation of oil in a matrix of formaldehyde-treated protein.[25] The RP technology used in the present study has the advantage that formaldehyde is not used, but no data are available on its efficacy in enriching bovine muscle with EPA and DHA. However, the 6- and 2-fold linear increases in EPA and DHA concentration, respectively, in plasma of these animals, reported previously[14] indicate that a substantial amount of the dietary n-3 LC-PUFA did not undergo ruminal BH. In the present study, concentrations of EPA achieved in muscle PL were in excess of concentrations that would typically be observed on a grass-based diet, even at the moderate (138 g/day) supplementation level used here. Grass feeding is used as a benchmark since, in the absence of supplementation with lipid-rich feedstuffs, the highest PUFA (including LC-PUFA) concentrations are observed when grass is fed.[9,26,27] Thus, Dannenberger et al.[9] reported EPA concentrations of 20.3 and 4.4 mg/100 g and DHA concentrations of 6.8 and 2.5 mg/100 g in muscle from grass- and concentrate-fed bulls, respectively. Argentine beef was reported to contain 15 and 4 mg EPA/100 g and 12 and 6 mg of DHA/100 g for pasture and feedlot beef, respectively,[27] while beef from the United States was reported to contain 8 and 4 mg of EPA/100 g and 1.49 and 1.46 mg of DHA/100 g, for pasture and concentrate-fed steers, respectively.[28] The relatively high levels of EPA and DHA (18.8 and 7.1 mg/100 g, respectively) observed for the control group in the present study may reflect the provision of ad libitum grass silage to all heifers in

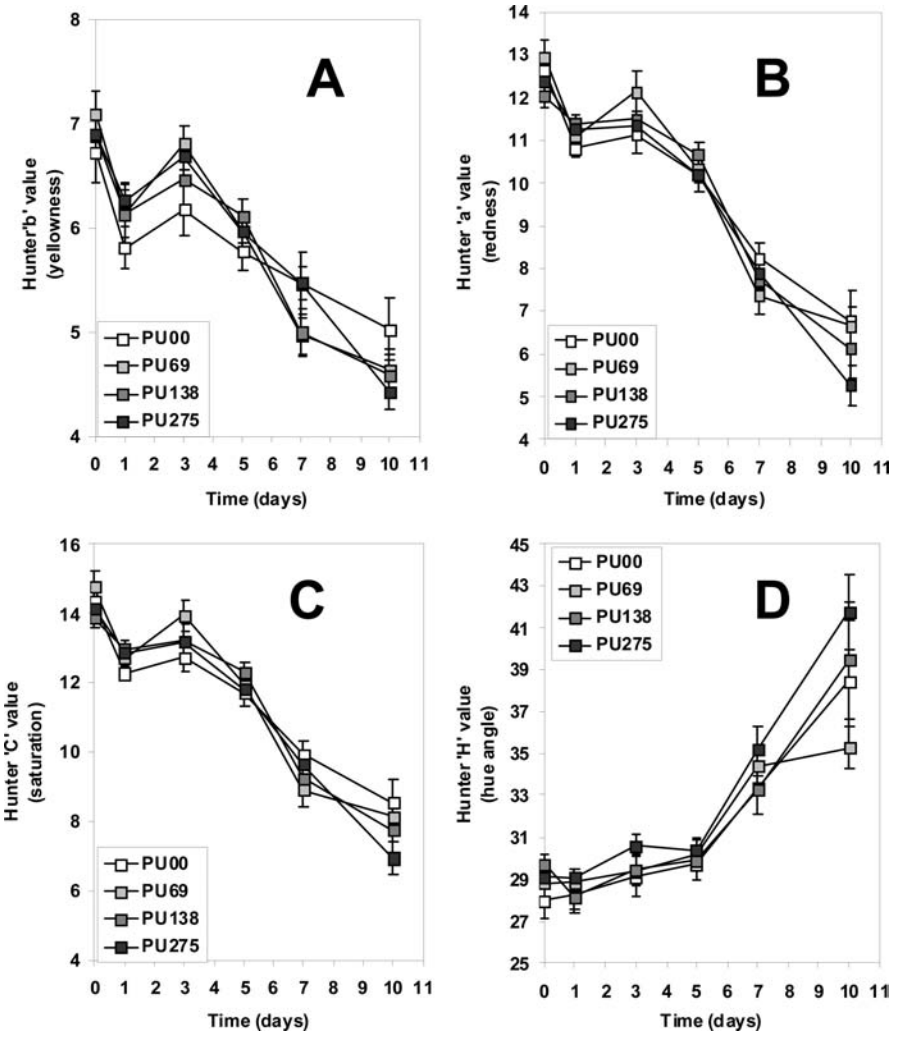

Figure 2. (A) Hunter *b* value (yellowness) of minced beef during simulated retail display (treatment × display time, $P = 0.038$; SED = 0.286) (note: *y*-axis scale commences at $b = 4$); (B) Hunter *a* value redness; time, $P < 0.001$, SED = 0.255); (C) Hunter *C* value (saturation; time, $P < 0.001$, SED = 0.266); (D) Hunter *H* (hue angle; time, $P < 0.001$, SED = 0.686) of minced beef during simulated retail display (note: scales for redness and saturation commence at 4; scale for hue angle commences at 25).

the period preceding the trial, as would be customary practice on commercial beef farms in Ireland. In the present study, muscle from PU275 heifers had a concentration of EPA 3 times that of muscle from PU00 reflecting the considerable ruminal protection of dietary PUFA conferred by this technology. This finding can be compared with the similar increase in lamb muscle[25] and the 1.5-fold increase in bovine muscle[29] when the protein/formaldehyde technology was used. In contrast to the modest increase in DHA concentration observed in the present study (2-fold), a 3.5-fold increase was observed in lamb and bovine muscle in refs 25 and 29, respectively. This most likely reflects the higher ratio of DHA to EPA in the RP preparations used in the latter studies, i.e. 3:1 compared to 0.67:1 in the present study. In the present study, EPA and DHA were preferentially deposited in the PL fraction, as is generally recognized.[9,19,30] It should be noted that, in the present study, the samples came from neck muscle, chosen as a relatively inexpensive muscle that is easily sampled in the abattoir, while in the other studies the *longissimus* muscle was used: the neck muscle is likely a more oxidative muscle with more and smaller diameter fibers probably leading to a greater membrane volume and more capacity to assimilate the n-3 LC-PUFA.[12]

As animals increase in fatness, the proportion of PL decreases, due to dilution by lipid deposition in the NL fraction.[3,8] Thus, it is essential in dose−response experiments like the present study, where results could be influenced by fatness, to ensure no differences in this trait. Since there was no increase in fatness in the present study, changes in n-3 LC-PUFA reflect the dietary intake of the RP supplement. The increases in the n-3 LC-PUFA observed would suggest, in the absence of a difference in fatness, some displacement of other PUFA from membranes (in the present study, probably 20:4n-6, as is indicated by the data), a concept discussed in earlier studies.[30] The ability to make such interpretations underpinned the rationale for separating lipids into NL and PL in the present study. Apart from the n-3 LC-PUFA, changes in proportions of individual fatty acids, both in NL and in PL, were minimal in the present study, which was not unexpected since the basal diet of heifers was the same except for the RP supplement inclusion. The decrease in some individual and total MUFA proportions might be interpreted as a negative response, but the absolute changes were low.

Most of the endeavors to make beef "healthier" have focused on increasing the PUFA:SFA ratio and reducing the n-6:n-3

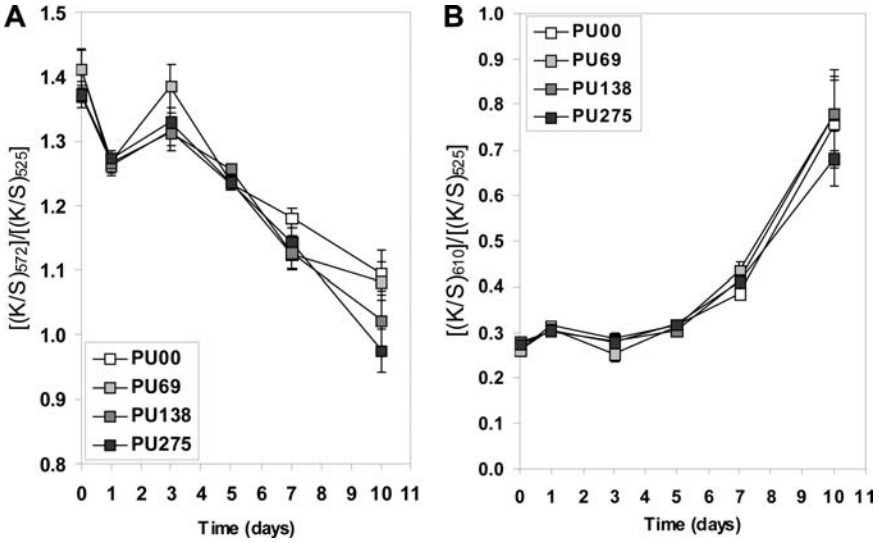

Figure 3. Indices of discoloration of minced beef during simulated retail display. (A) $[(K/S)_{572}]/[(K/S)_{525}]$ relates to metmyoglobin formation where a lower value indicates more metmyoglobin. (B) $[(K/S)_{610}]/[(K/S)_{525}]$ relates to oxymyoglobin loss where a lower value indicates more oxymyoglobin.

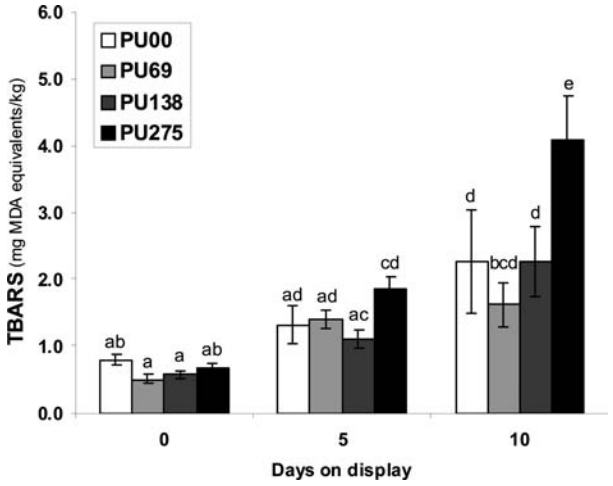

Figure 4. Thiobarbituric acid reactive substances (TBARS, expressed as mg of malondialdehyde (MDA) equivalents per kg of sample) in minced beef during simulated retail display. Mean values assigned different superscripts differ significantly ($P < 0.05$).

PUFA ratio.[31] The PUFA represent the smallest category of fatty acids in beef, almost always averaging between 5 g/100 g and 10 g/100 g of fatty acids[19,26,27] and only occasionally exceeding this value, e.g. with Belgian Blue bulls fed linseed.[32] The n-3 PUFA are typically below 2 g/100 g fatty acids in which 18:3 n-3 is the predominant n-3 PUFA. The linear increases in total PUFA, n-3 PUFA and PUFA:SFA ratio in NL and the concomitant linear decreases in n-6 PUFA and n-6:n-3 PUFA ratio as well as increases in n-3 PUFA and PUFA:SFA ratio in PL in the present study are evidence of the efficacy of RP n-3 LC-PUFA supplementation in this regard. While an increase in 18:3 n-3 consumption can lead to small increases in EPA and smaller or no increases in DHA,[3,30] Givens et al.[31] acknowledged that, to achieve "meaningful increases" specifically in n-3 LC-PUFA (i.e., EPA and DHA, not including 18:3 n-3), FO inclusion appears necessary. The concentrations of EPA and DHA achieved in the present study can be viewed in the context of recently published reference intake values for humans of 2 g and 250 mg/day for 18:3n-3, and EPA and DHA combined, respectively.[33] A 100 g serving of beef from the cattle fed PU275 would supply 27% of the recommended daily requirement for n-3 PUFA. Beef from cattle fed PU138 and PU275 could be labeled as a "source" of omega-3 fatty acids.[33]

The second and crucial objective of the present study was to examine the quality of the high n-3 LC-PUFA beef under simulated retail conditions. The retail conditions in the present study could be regarded as challenging, i.e. the beef was frozen, thawed, ground and displayed in an elevated oxygen atmosphere under retail lighting. In terms of color and lipid stability the outcomes of the present study were positive. The two principal reasons underlying this favorable outcome were likely the reasonably high mean concentrations of α-tocopherol in the muscle and the low mean cabinet temperature throughout the study (Figure 1 in the Supporting Information). Cabinet temperature has been recognized as having the largest effect on display life, as at 1 °C saturation of beef decreased by 2 units, while at 6 °C it decreased by 6 units.[34] It is generally accepted that, in food products with elevated concentrations of n-3 LC-PUFA, elevated levels of antioxidants are necessary to balance the tendency toward lipid oxidation and the problems that ensue for organoleptic and nutritional quality.[13] α-Tocopherol, the main lipid soluble antioxidant in membranes and lipoproteins, is colocated with phospholipids in the lipid bilayer of cells which provide a readily oxidizable reservoir of bis-allylic carbons. Critical to the tocopherol antioxidant activity is its capacity for close steric association via an "interdigitation" between the chromanol methyl groups of tocopherol and the *cis* double bonds of the unsaturated acyl chains of the phospholipids.[35] Thus, the efficacy of α-tocopherol as an antioxidant depends not only on its concentration and its relative location but also on other antioxidants present which likely reinforce the efficacy of tocopherols, and the PUFA concentration and type (distribution of double bonds and highly labile π electrons, substantially greater in EPA and DHA than, for example, in 18:3 n-3) in close proximity.

The mean concentrations of α-tocopherol in the present study were higher than in other studies in our laboratory[36,37]

but were similar to those previously reported for cattle receiving supranutritional supplementation with α-tocopherol.[38] In the present study all heifers had received grass silage during the pre-experimental period and, assuming some carryover of protection to the trial period, might have been expected to have residual antioxidant protection during the experiment. In addition, the samples came from neck muscle, which is likely a more oxidative muscle with more and smaller diameter fibers than the *longissimus* muscle used in the other studies mentioned, probably leading to the considerable tocopherol concentrations observed.[11,12] It was reported that, in pork, the muscles of the thoracic limb, neck and thorax contained greater concentrations of α-tocopherol than those of the pelvic limb and back.[39] The higher antioxidant capacity of beef from cattle fed herbage-based diets (as measured by lipophilic ORAC, 40) may reflect the diversity of antioxidant compounds (predominantly, lipophilic in nature) in the diet relative to animals fed concentrates[3,9,40] as well as possible synergism with tocopherols. It is generally accepted that oxidative stability and color and flavor quality are correlated in meat and meat products.[7,41] However, although grass feeding consistently improves oxidative stability and usually also color stability of unground beef,[42] when beef from grass-fed cattle was ground, oxidative stability decreased.[42] Grinding was done in an effort to replicate commercial practice since neck beef is usually ground. The present data can be securely interpreted since (i) there were no significant differences in α-tocopherol concentrations between treatments, (ii) due to the common diet the likely disparity in the range and diversity of dietary antioxidant compounds (and thus, in muscle) was minimal and (iii) differences in the highly oxidizable n-3 LC-PUFA were achieved. Thus, with a putatively constant "antioxidant potential" and an increased "pro-oxidant" potential, any differences in lipid and/or color stability could be attributed to the differences in pro-oxidant PUFA. In this sense, the design of the present study created in musculo conditions which could most resemble an idealized model system. The observation that color stability was not affected by increasing concentrations of n-3 LC-PUFA in PL implies that, for color, there was sufficient antioxidant protection in all treatments. A similar observation was made by Richardson et al.[29] for cattle offered either RPFO or unprotected FO.

The TBARS values were used as an index of oxidation of muscle lipids at a biochemical level. While TBARS measurement is widely used in this context, it is recognized that it is a less sensitive index of lipid oxidation than direct measurement of the end products of fatty acid oxidation. At TBARS levels above 2.28 mg/kg adverse effects on organoleptic characteristics ensue.[43] This value was measured in beef which after retail display for up to 9 days was cooked and presented for sensory assessment.[43] The increase in TBARS to values approaching or exceeding 2.3 mg/kg for all treatments (2.25, 1.62, 2.27, and 4.10 for PU00, PU69, PU138 and PU275, respectively) at day 10 indicates that, to maintain acceptable organoleptic characteristics for the highest level of supplementary RP LC n-3 PUFA beyond at least 5 days, more vitamin E would be required. Thus, while PU275 vitamin E concentration is marginally numerically greater than PU69, the TBARS value is 2.5 times greater. Vitamin E concentration also becomes more critical the longer that retail display progresses, particularly when n-3 LC-PUFA levels are elevated, since this notable increase in PU275 TBARS occurred at day 10. The increase in TBARS for the PU275 treatment was best described by the equation y (TBARS) = 0.340 (DAYS) + 0.496; $R^2 = 0.51$, $P < 0.001$. Using this equation, a critical TBARS value of 2.3 was reached at 5.26 days of simulated retail display. The corresponding time for PU00 was 10.8 days ($y = 0.177x + 0.498$, $R^2 = 0.17$, $P < 0.05$). Clearly lipid stability was less resilient than color stability under the conditions of this study. An increase in TBARS concentration in muscle due to an increase in n-3 LC-PUFA concentration is a consistent finding in the literature even when supranutritional supplementation with vitamin E is used.[29,38] The finding that consumption of n-3 LC-PUFA at the high level (PU275) led to an increase in susceptibility to lipid oxidation but not loss of redness (indicative of myoglobin oxidation) is not altogether surprising since it has been shown that significant lipid oxidation can occur without myoglobin oxidation.[44]

The concept that the ratio between concentrations of PUFA and tocopherols might be critical to the rate and extent of oxidative processes in both living organisms and foodstuffs is now widely accepted.[45] Not surprisingly, given the lack of a treatment effect, in the present study the ratio of EPA and DHA alone or combined in PL to vitamin E (both calculated as nanomoles per gram, data not shown) was poorly related ($P > 0.1$) to the a value at day 10. Redness was chosen to examine this relationship because it is the color variable that is most often used as the index of discoloration; redness at day 10 was specifically chosen because this represented the end of the display period when any differences between treatments were likely to be amplified due to the exposure to the high oxygen packaging atmosphere. That there was no discernible relationship between the ratio of EPA and DHA, alone or combined, in PL to vitamin E concentration and TBARS values was, however, surprising. The EPA, DHA and vitamin E concentrations were measured in muscle prior to packaging and display. The concentrations may have changed during display since Diaz et al.[46] reported a loss of 25% of EPA + DHA in the PL fraction during aerobic storage of lamb muscle enriched with n-3 LC-PUFA due to FO inclusion in the diet. It has been calculated that 15% of this loss was likely due to oxidation. The products of oxidation may therefore be less reactive than the parent species. Some loss of vitamin E may also occur during display, and measurement of both fatty acid and vitamin E concentrations at the end of the display period would be useful in future studies. Luciano et al.[47] also failed to show a clear relationship between susceptibility to lipid oxidation and PUFA to vitamin E ratio and attributed this to there being a sufficiently high level of vitamin E present to stabilize PUFA in several of the treatment groups and to a possible confounding effect of other dietary antioxidants such as β-carotene. A titration approach, whereby incremental amounts of vitamin E, in addition to RP supplements, might be offered to cattle, could perhaps address the issue of the amount of vitamin E required to attenuate the levels of oxidation at the highest RP-PUFA intake.

The present study has demonstrated that, by supplementing heifers with a RP FO source, it is possible to achieve n-3 LC-PUFA concentrations which would allow muscle to be labeled a "source" of these fatty acids in human nutrition. The sustainability of this practice in the face of declining stocks of oily fish together with the impact on the cost of producing such beef merit examination. Moreover, modification of ruminant meat requires definition of a strategy which protects these lipids from the pro-oxidative environment. Supranutritional vitamin E, as α-tocopheryl acetate, supplied in the diet as in the present study was adequate to ensure that shelf life (color) was not adversely affected. Since the beef in the present study was forequarter beef and was minced and packaged in a high oxygen atmosphere, a

substantial oxidative challenge was encountered. Thus, in a holistic context the present work demonstrates the practical utility of the chosen approach not only to improve fatty acid composition but also to achieve this without deterioration of beef appearance even when the beef is presented with such an oxidative challenge. The impact of this strategy on the sensory characteristics of beef remains to be determined and is an important issue for consumer acceptability. Previous work has shown that where n-3 LC-PUFA concentrations have been increased, lipid stability has been decreased postcooking.[48] How beef such as that produced in the present study may respond oxidatively in a postcooking environment may be another avenue for further research.

It is concluded that while supplementation effectively increased EPA and, to a lesser extent, DHA in muscle, as well as fatty acid nutritional indices by which "healthiness" is judged, there were no negative effects of increasing RP n-3 LC-PUFA inclusion on color stability based on the usual measures. n-3 LC-PUFA were preferentially deposited in PL. Further studies may be required to determine the optimum level of vitamin E required to prevent lipid oxidation during retail display when RP n-3 LC-PUFA are successfully enhanced, since in the present study adequate vitamin E was supplied to limit lipid oxidation up to 5 days of display but not to 10 days in the PU275 treatment.

■ ASSOCIATED CONTENT

Supporting Information. Temperature profiles in the display cabinet during simulated retail display and partial GC-FID chromatograms of beef intramuscular lipid extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +353 (0)46 906 1100. Fax: +353(0)46 902 6154. E-mail: aidan.moloney@teagasc.ie.

Funding Sources

J.R. was in receipt of a Marie Curie Fellowship, administered by the European Union under the EU Structuring the European Research Area—Human Resources and Mobility Programme of the Sixth Framework Programme. S.C. was funded by the Walsh Fellowship Programme of Teagasc, the Irish Agriculture & Food Development Authority.

■ ACKNOWLEDGMENT

The authors sincerely thank Paula Reid for statistical advice and analysis. Technical assistance of R. Richardson and E. Vesey is appreciated. The financial assistance of Nutreco, who also supplied the RP n-3 LC-PUFA fish oil supplement used in the study, is acknowledged.

■ ABBREVIATIONS USED

BH, biohydrogenation; CP, crude protein; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; DHA-me, docosahexaenoic acid methyl ester, methyl docosahexaenoate; DM, dry matter; EPA, eicosapentaenoic acid; EPA-me, eicosapentaenoic acid methyl ester, methyl eicosapentaenoate; FAME, fatty acid methyl ester; FO, fish oil; LC-PUFA, long chain polyunsaturated fatty acids; MDA, malondialdehyde; MUFA, monounsaturated fatty acid; NL, neutral lipid; n-3 PUFA, n-3 polyunsaturated fatty acid; n-3 LC-PUFA, n-3 long chain polyunsaturated fatty acid; n-6 PUFA, n-6 polyunsaturated fatty acid; PL, polar lipids; RP, ruminally protected; RPFO, ruminally protected fish oil; SFA, saturated fatty acid; TAG, triacylglycerol; TBA, 2-thiobarbituric acid;TBARS, 2-thiobarbituric acid reactive substances; UFA, unsaturated fatty acids.

■ REFERENCES

(1) Kris-Etherton, P. M.; Harris, W. S.; Appel, L. J. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. *Circulation* **2002**, *106*, 2747–2757.

(2) Roche, H. M. Unsaturated fatty acids. *Proc. Nutr. Soc.* **1999**, *58*, 397–401.

(3) Wood, J. D.; Richardson, R. I.; Nute, G. R.; Fisher, A. V.; Campo, M. M.; Kasapidou, E.; Sheard, P. R.; Enser, M. Effects of fatty acids on meat quality: a review. *Meat Sci.* **2003**, *66*, 21–32.

(4) Alfaia, C. P. M.; Alves, S. P.; Martins, S. I. V.; Costa, A. S. H.; Fontes, C. M. G. A.; Lemos, J. P. C.; Bessa, R. J. B.; Prates, J. A. M. Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chem.* **2009**, *114*, 939–946.

(5) Dannenberger, D.; Nuernberg, G.; Scollan, N.; Ender, K.; Nuernberg, K. Diet alters the fatty acid composition of individual phospholipids classes in beef muscle. *J. Agric. Food Chem.* **2007**, *55*, 452–460.

(6) Jenkins, T. C.; Bridges, W. C., Jr. Protection of fatty acids against ruminal biohydrogenation in cattle. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 778–789.

(7) Monahan, F. J. Oxidation of lipids in muscle foods: fundamental and applied concerns. In *Antioxidants in muscle foods—Nutritional strategies to improve quality*; Decker, E. A., Faustman, C., Lopez-Bote, C. J., Eds.; John Wiley & Sons, Inc.: New York, 2000; pp 3−24.

(8) DeSmet, S.; Raes, K.; Demeyer, D. Meat fatty acid composition as affected by fatness and genetic factors: a review. *Anim. Res.* **2004**, *53*, 81–98.

(9) Dannenberger, D.; Nuernberg, G.; Scollan, N. D.; Schabbel, W.; Steinhart, H.; Ender, K.; Nuernberg, K. Effect of diet on the deposition of n-3 fatty acids, conjugated linoleic and C18:1 trans fatty acids isomers in muscle lipids of German Holstein bulls. *J. Agric. Food Chem.* **2004**, *52*, 6607–6615.

(10) Igene, J. O.; Pearson, A. M.; Dugan, A. M.; Price, J. F. Role of triglycerides and phospholipids on development of rancidity in meat model systems during frozen storage. *Food Chem.* **1980**, *5*, 263–276.

(11) Liu, Q.; Lanari, M. C.; Schaefer, D. M. A review of dietary vitamin E supplementation for improvement of beef quality. *J. Anim. Sci.* **1995**, *73*, 3131–3140.

(12) Faustman, C.; Chan, W. K.; Schaefer, D. M.; Havens, A. Beef colour update: the role for vitamin E. *J. Anim. Sci.* **1998**, *76*, 1019–1026.

(13) Jacobsen, C.; Let, M. B.; Nielsen, N. S.; Meyer, A. S. Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated lipids: a comparative evaluation. *Trends Food Sci. Technol.* **2008**, *19*, 76–93.

(14) Childs, S.; Hennessy, A. A.; Sreenan, J. M.; Wathes, D. C.; Cheng, Z.; Stanton, C.; Diskin, M. G.; Kenny, D. A. Effect of level of dietary n-3 polyunsaturated fatty acid supplementation on systemic and tissue fatty acid concentrations and on selected reproductive variables in cattle. *Theriogenology* **2008**, *70*, 595–611.

(15) Siu, G. M.; Draper, H. H. A survey of the malonaldehyde content of retail meat and fish. *J. Food Sci.* **1978**, *43*, 1147–1149.

(16) O'Keefe, S. F.; Wang, H Effects of peanut skin extract on quality and storage stability of beef products. *Meat Sci.* **2006**, *73*, 278–286.

(17) Buttriss, J. L.; Diplock, A. T. High performance liquid chromatography methods for the determination of vitamin E in tissues. *Methods Enzymol.* **1984**, *105*, 131–138.

(18) Bostian, M. L.; Fish, D. L.; Webb, N. L.; Arey, J. J. Automated methods for determination of fat in meat and poultry products: Collaborative study. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 876–881.

(19) Noci, F.; Monahan, F. J.; French, P.; Moloney, A. P. The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture-fed beef heifers: influence of duration of grazing. *J. Anim. Sci.* **2005**, *83*, 1167–1178.

(20) Golay, P. A.; Dionsis, F.; Hug, B.; Giuffrida, F.; Destaillats, F. Direct quantification of fatty acids in dairy powders with special emphasis on trans fatty acid content. *Food Chem.* **2006**, *101*, 1115–1120.

(21) Demeyer, D.; Doreau, M. Targets and procedures for altering ruminant meat and milk lipids. *Proc. Nutr. Soc.* **1999**, *58*, 593–607.

(22) Kim, E. J.; Huws, S. A.; Lee, M. R. F.; Wood, J. D.; Muetzel, S. M.; Wallace, R. J.; Scollan, N. D. Fish oil increases the duodenal flow of long chain polyunsaturated fatty acids and trans-11 18:1 and decreases 18:0 in steers via changes in the rumen bacterial community. *J. Nutr.* **2008**, *138*, 889–896.

(23) Wachira, A. M.; Sinclair, L. A.; Wilkinson, R. G.; Hallett, K.; Enser, M.; Wood, J. D. Rumen biohydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. *J. Agric. Sci.* **2000**, *135*, 419–428.

(24) Scollan, N. D.; Dhanoa, M. S.; Choi, N. J.; Maeng, W. J.; Enser, M.; Wood, J. D. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipids. *J. Agric. Sci.* **2001**, *136*, 345–355.

(25) Kitessa, S. M.; Gulati, S. K.; Ashes, J. R.; Scott, T. W.; Fleck, E. Effect of feeding tuna oil supplement protected against hydrogenation in the rumen on growth and n-3 fatty acid content of lamb fat and muscle. *Aust. J. Agric. Res.* **2001**, *52*, 433–437.

(26) French, P.; Stanton, C.; Lawless, F.; O'Riordan, E. G.; Monahan, F. J.; Caffrey, P. J.; Moloney, A. P. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *J. Anim. Sci.* **2000**, *78*, 2849–2855.

(27) García, P. T.; Pensel, N. A.; Sancho, A. M.; Latimori, N. J.; Kloster, A. M.; Amigone, M. A.; Casal, J. J. Beef lipids in relation to animal breed and nutrition in Argentina. *Meat Sci.* **2008**, *79*, 500–508.

(28) Duckett, S. K.; Neel, J. P. S.; Fontenot, J. P.; Clapham, W. M. Effects of winter stocker growth rate and finishing system on: III. Tissue proximate, fatty acid, vitamin, and cholesterol content. *J. Anim Sci.* **2009**, *87*, 2961–2970.

(29) Richardson, R. I.; Hallett, K. G.; Ball, R.; Robinson, A. M.; Nute, G. R.; Enser, M.; Wood, J. P.; Scollan, N. D. Effect of free and ruminally-protected fishoils on fatty acid composition, sensory and oxidative characteristics of beef loin muscle. In *Proceedings 50th ICoMST, Helsinki, Finland*, 2005.

(30) Scollan, N. D.; Enser, M.; Gulati, S. K.; Richardson, I.; Wood, J. D. Effects of including a ruminally-protected lipid supplement in the diet on the fatty acid composition of beef muscle. *Br. J. Nutr.* **2003**, *90*, 709–716.

(31) Givens, D. I.; Kliem, K. E.; Gibbs, R. A. The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. *Meat Sci.* **2006**, *74*, 209–218.

(32) Raes, K.; Haak, L.; Balcaen, A.; Claeys, E.; Demeyer, D.; DeSmet, S. Effect of linseed feeding at similar linoleic acid levels on the fatty acid composition of double-muscled Belgian Blue young bulls. *Meat Sci.* **2004**, *66*, 307–315.

(33) EFSA. Scientific opinion: Labeling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *EFSA J.* **2009**, *1176*, 1-11.

(34) MacDougall, D. B.; Powell, V. H. The relative importance of temperature, wavelength and intensity of light on the colour display life of fresh and aged beef cuts. In *Proc. 43rd ICoMST, Auckland, New Zealand*, 1997, pp 668−669.

(35) Atkinson, J.; Epand, R. F.; Epand, R. M. Tocopherols and tocotrienols in membranes: a critical review. *Free Radical Biol. Med.* **2008**, *44*, 739–764.

(36) Dunne, P. G.; Monahan, F. J.; O'Mara, F. P.; Moloney, A. P. Colour stability, under simulated retail display conditions, of *M. longissimus dorsi* and *M. semimembranosus* from steers given long-term daily exercise and supplemented with vitamin E. *Meat Sci.* **2005**, *71*, 480–489.

(37) Daly, C. M.; Moloney, A. P.; Monahan, F. J. Lipid and colour stability of beef from grazing heifers supplemented with sunflower oil alone or with fish oil. *Meat Sci.* **2007**, *77*, 634–642.

(38) Vatansever, L.; Kurt, E.; Enser, M.; Nute, G. R.; Scollan, N. D.; Wood, J. D.; Richardson, R. I. Shelf-life and eating quality of beef from cattle of different breeds given diets differing in n-3 polyunsaturated fatty acid composition. *Anim. Sci.* **2000**, *71*, 471–482.

(39) O'Sullivan, M. G.; Kerry, J. P.; Buckley, D. J.; Lynch, P. B.; Morrissey, P. A. The distribution of dietary vitamin E in the muscles of the porcine carcass. *Meat Sci.* **1997**, *45*, 297–305.

(40) Wu, C.; Duckett, S. K.; Neel, J. P. S.; Fontenot, J. P.; Clapham, W. M. Influence of finishing systems on hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC) in beef. *Meat Sci.* **2008**, *80*, 662–667.

(41) Kanner, J. Oxidative processes in meat and meat products: quality implications. *Meat Sci.* **1994**, *36*, 169–189.

(42) Realini, C. E.; Duckett, S. K.; Brito, G. W.; Dalla Rizza, M.; Mattos, D. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Sci.* **2004**, *66*, 567–577.

(43) Campo, M. M.; Nute, G. R.; Hughes, S. I.; Enser, M; Wood, J. D.; Richardson, R. I. Flavour perception of oxidation in beef. *Meat Sci.* **2006**, *72*, 303–311.

(44) Monahan, F. J.; Skibsted, L. H.; Andersen, M. L. Mechanism of oxymyoglobin oxidation in the presence of oxidising lipids in bovine muscle. *J. Agric. Food Chem.* **2005**, *53*, 5734–5738.

(45) Valk, E. E. J.; Hornstra, G. Relationship between vitamin E requirement and polyunsaturated fatty acid intake in man: a review. *Int. J. Vitam. Nutr. Res.* **2000**, *70*, 31–42.

(46) Diaz, M. T.; Cañeque, V.; Sánchez, C. I.; Lauzurica, S.; Pérez, C.; Fernández, C.; Álvarez, I.; De La Fuente, J. Nutritional and sensory aspects of light lamb meat enriched in n-3 fatty acids during refrigerated storage. *Food Chem.* **2011**, *124*, 147–155.

(47) Luciano, G.; Moloney, A. P.; Priolo, A.; Röhrle, F. T.; Vasta, V.; Biondi, L.; López-Andrés, P.; Grasso, S.; Monahan, F. J. Pro-oxidant and antioxidant components in muscle and the oxidative stability of beef from cattle grown on forage or concentrate-based rations. *J. Anim. Sci.* **2011**submitted.

(48) Kenny, D. A.; Kelly, J. P.; Monahan, F. J.; Moloney, A. P. Effect of dietary fish and soyoil supplementation on muscle fatty acid concentrations and oxidative lipid stability in beef cattle. *J. Anim. Sci.* **2007**, *85* (Suppl. 1), 657.