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Lipid and protein oxidation and colour stability during display in high oxygen modified atmosphere packaging of beef from late-maturing bulls fed rumen protected fish oil

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Abstract

Increasing the concentration of polyunsaturated fatty acids (PUFA) in beef enhances its nutritional value but may compromise its oxidative shelf life. In this experiment, the impact of inclusion of rumen protected fish oil (PFO) in the finishing diet of late-maturing bulls on muscle fatty acid profile, antioxidant content, lipid stability, colour and protein oxidation was investigated. Charolais-sired suckler bulls were offered ad libitum, for 101 d pre-slaughter, a barley-based concentrate (C) or a concentrate containing rumen PFO. Following post-mortem ageing for 14 d, M. Longissimus thoracis muscle was subjected to simulated retail display (4°C, 1,000 lux for 12 h out of 24 h) for 3, 7 and 10 d in modified atmosphere packs (O_2:CO_2; 80:20). The concentrations of C22:6n-3, n-6 PUFA and total PUFA and the n-6:n-3 PUFA ratio were higher ($P < 0.001$) in muscle of PFO bulls compared to C bulls, while the α-tocopherol concentration was lower ($P < 0.01$). The concentrations of C18:3n-6, C20:4n-6, n-3 PUFA and highly peroxidisable PUFA were lower ($P < 0.05$) on day 14 compared to day 0 of display. Lipid oxidation after 10 d of display was higher ($P < 0.05$) in muscle of PFO bulls compared to C bulls but not to an extent that would be detected by a consumer. Colour stability was not affected. It is concluded that the increase in PUFA concentration achieved had minor effects on bull beef shelf life.

Keywords

Antioxidants • bulls • carbonyls • fish oil • lipid oxidation

Introduction

The concentration and fatty acid composition of intramuscular fat make important contributions to the nutritional value of beef (Scollan *et al.*, 2006). The long-chain *n*-3 polyunsaturated fatty acids (PUFA), in particular C20:5 (EPA) and C22:6 (DHA) have been associated with long-term health benefits, including decreases in cardiovascular disease morbidity and mortality, improved visual and neurological development and improvements in inflammatory conditions such as arthritis and asthma (Calder, 2014). The nutritional quality of beef can therefore be improved by increasing the content of these fatty acids. One strategy is the dietary inclusion of fish oil and marine algae, which are the main sources of long-chain omega-3 PUFA (Woods & Fearon, 2009). While in monogastric animals the fatty acid profile of meat is a reflection of the animal's diet, in ruminants dietary PUFA undergo a degree of hydrogenation in the rumen, thereby altering the relationship between the dietary and meat fatty acid profiles (Scollan *et al.*, 2001). To counteract biohydrogenation, strategies such as conversion of fatty acids into soaps and encapsulation into a protein cross linked with formaldehyde are often used (Jenkins & Bridges, 2007). Feeding ruminants with fish oil, protected or unprotected from ruminal biohydrogenation has been employed to increase the amounts of EPA and DHA in muscle, thereby increasing the healthiness of meat products (Scollan *et al.*, 2001; Noci *et al.*, 2007; Dunne *et al.*, 2011).

Since PUFA are preferentially deposited in the muscle membranes in ruminants (Moreno *et al.*, 2008; Dunne *et al.*, 2011), their concentration in muscle is higher in leaner late-maturing animals than in fatter early-maturing animals, in which muscle membrane fatty acids are diluted by triacylglycerols in adipose tissue when comparisons are made at a common carcass weight. Young late-maturing breed bulls, which have a physiological and genetic predisposition to leanness, may therefore be suitable animals for enrichment of muscle with PUFA through supplementation with rumen protected fish oil (PFO). Muscle foods are inherently susceptible to lipid

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oxidation due to the presence of unsaturated fatty acids and pro-oxidant components, such as iron (Luciano *et al.*, 2011). Changes in the concentration and composition of fatty acids in muscle as well as in the antioxidant concentration may therefore affect the colour and lipid stability of meat during retail display (Humada *et al.*, 2014).

Moloney *et al.* (2021) showed that the fatty acid profile of the muscle of Charolais/Limousin (late-maturing breeds)-sired suckler bulls was enhanced, from a human health perspective, by dietary inclusion of rumen PFO in a high concentrate finishing ration. This benefit, however, would be negated if the oxidative stability and associated shelf life of the muscle was compromised.

The objective of this study therefore was to determine the oxidative stability of beef from Moloney *et al.* (2021) when packaging in a high oxygen atmosphere, a common display method used in the meat retail sector.

Materials and methods

Animals, diet and management

Details of animal management are provided in Moloney *et al.* (2021). In brief, 30 spring-born, late-maturing sired (Charolais) bulls from suckler herds and approximately 8 months old, were assembled in October and acclimatised to a concrete slatted floor shed for 2 weeks after arrival. They were then weighed on consecutive days, ranked in blocks of two in descending order based on the mean of the two weights and within block, assigned at random to one of two finishing rations. Bulls were offered, indoors for 140 d, high nutritive value grass silage *ad libitum* plus 2 kg/head daily of a barley-based control ration that contained per kg: 862 g barley, 60 g soyabean meal, 50 g molasses and 28 g mineral/vitamin mixture (David Taylor, Animal Nutrition Ltd., Carrick Mill, Loughbawn, Collinstown, Co. Westmeath, Ireland; 6,000 mg Vitamin E [α-tocopherol]/kg). The bulls then rotationally grazed a perennial ryegrass (*Lolium perenne* L.) dominant pasture from 7 April and were supplemented with the control ration at 50% of the expected dietary DM intake for 94 d. Thereafter, the bulls were housed in a concrete slatted floor shed and offered, together with grass silage *ad libitum*, increasing amounts of the control ration or a test ration (743 g barley, 86 g molasses, 28 g mineral/vitamin mixture and 143 g rumen protected tuna oil [supplied by The Farm Right Group, UK]) until the level of consumption was *ad libitum* (16 d). The target oil concentration of the test ration (PFO) was 50 g/kg DM and it had a similar crude protein concentration as the control ration. The grass silage offered was from a predominantly perennial ryegrass sward, cut using a rotary mower, wilted for 24 h, harvested using a precision-chop harvester and ensiled without an additive. Detailed descriptions of ration formulation, chemical composition, feeding, refusals collection and processing are given in Moloney *et al.* (2021). The duration of the pre-slaughter *ad libitum* feeding phase was 85 d after which the bulls were slaughtered at approximately 19 months of age.

Slaughter and sample collection

On the day of slaughter, the animals were transported approximately 30 km to a commercial slaughter plant (Kepak, Clonee, Co. Meath, Ireland) and slaughtered immediately after arrival by captive bolt stunning followed by exsanguination. Carcasses were not electrically stimulated. The slaughter and dressing procedures were in accordance with the European Commission Regulation Nos 1099/2009 and 853/2004. Approximately 45 min after slaughter, carcasses were placed in a chill set at 7°C, which was reduced to 0°C after 10 h, approximately. The carcasses were kept in the chill for 48 h before removal to the deboning hall (4°C). The *M. Longissimus thoracis* muscle was excised from between the 5th/6th rib interface and the 10th/11th rib interface, deboned and dissected free of adhering adipose tissue. Thereafter, the muscle was vacuum-packed and transported to Teagasc Food Research Centre (Ashtown, Dublin, Ireland). Samples from 8 of the available 15 animals per ration were randomly selected for further analysis. One 2.5 cm thick steak for proximate analysis was removed from the muscle and stored at −20°C, while the remainder of the muscle was repackaged in a vacuum pack and aged at 2°C for a further 12 d (total of 14 d post-mortem). After ageing, two 2.5 cm thick steaks were cut from the muscle and each was subdivided into two portions giving four sub-samples. The four samples were packaged in modified atmosphere packaging (MAP) (O_2: CO_2; 80:20) in polyamide-polyethylene Exovac 73 bags (McDonnells, Dublin, Ireland), using a Webomatic vacuum-packaging system equipped with a gas mixer (Witt-Gase Technik KM100-M(3) Witt Gas Techniques Ltd., Warrington, UK). The sample representing day 0 was allowed to bloom for 90 min (Wulf & Wise, 1999) before initial colour measurement, while the remaining three samples were subjected to simulated retail display (walk-in chill at 4°C, 1,000 lux for 12 h out of 24 h, samples randomly distributed across shelves) for 3, 7, and 10 d prior to colour measurement. The integrity of the MAP was confirmed by checking the gas composition at the end of each display period using a handheld gas analyser (PBI Dansensor Checkpoint, Ringsted, Denmark). Meat colour measurements following American Meat Science Association procedures (AMSA, 2012) were made through the packaging material on each day of display. A Minolta Chroma Meter (CR-400) was set at illuminant D65, 2° standard observer and colour scale “L”, “a”, “b” with “L” indicating lightness, “a” indicating redness and “b” indicating yellowness and standardised with film covered calibration tiles. Colour co-ordinates were obtained from three

different non-overlapping points on each steak and averaged. At the end of each display period, one of the four steak sub-samples from each animal was divided into four portions for subsequent fatty acid, α-tocopherol, lipid oxidation and protein oxidation analysis, vacuum-packed and stored at −20°C.

Analyses

Fatty acid methyl esters (FAME) were prepared using the rapid microwave assisted method described by Brunton *et al.* (2015). Vitamin E (α-tocopherol) in feed was measured following the method of Fratianni *et al.* (2002) with minor modifications. Thus, 2 mL ethanolic pyrogallol (6%), 0.8 mL ethanol, 0.8 mL sodium chloride (1%) and 0.8 mL KOH (60%) were added to 250 mg of sample. Samples were flushed with nitrogen, placed in a water bath at 70°C for 45 min and vortexed every 10 min. The samples were cooled in an ice bath and 2 mL of sodium chloride solution (1%) was added after which α-tocopherol was extracted three times using 2 mL of hexane/ethyl acetate (9:1 v/v). The organic layers were dried under nitrogen and the residue was dissolved in 2 mL of ethanol. Analysis was carried out on a reverse phase high-performance liquid chromatography (HPLC) system using an Agilent 1200 series instrument fitted with a fluorescence detector (λexcitation = 295 nm and λemission = 330 nm; Agilent 1260 Infinity) and a Zorbax Eclipse XDB-C18 4.6 × 150 mm 5 μm column (Agilent Technologies Ireland Limited, Dublin, Ireland). The mobile phase was methanol at a flow rate of 1 mL/min. The injection volume was 20 μL and elution time was set at 14 min with the column maintained at a temperature of 25°C. For identification and quantification of α-tocopherol, peak areas of samples were compared with external α-tocopherol standards made up to the following concentrations: 0, 0.25, 0.5, 1, 2.5, 5 and 6 μg/mL in methanol. The average percentage recoveries for grass silage, C ration and PFO ration were 98.8%, 94.9% and 96.7%, respectively. α-Tocopherol in muscle was extracted as described in Bolger *et al.* (2016). Lipid oxidation was estimated by measuring 2-thiobarbituric acid reactive substances (TBARS) using the method described by Luciano *et al.* (2011). Carbonyl concentration was determined according to the method of Vuorela *et al.* (2005). The quantification of thiols was carried out according to the method described by Jongberg *et al.* (2011) with minor modifications. Thus, 1 g of muscle was homogenised in 25 mL of 5% sodium dodecyl sulphate dissolved in 100 mM Tris buffer (pH 8.0). The homogenates were heated in a water bath set at 80°C for 30 min followed by centrifugation at 3,000 rpm for 20 min (Avanti® J-E centrifuge, rotor JLA-16.250, Beckman Coulter, USA). The supernatants were filtered using filter paper (particle retention: 5–13 μm, VWR, Dublin, Ireland). Thiols were determined by mixing 500 μL supernatant, 2.00 mL of 0.10 M Tris buffer solution (pH 8.0) and 500 μL of 10 mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) dissolved in 0.10 M Tris buffer (pH 8.0). Absorbance of the samples at 412 nm was measured prior to addition of DTNB (ABS_{PRE}) and after reaction with DTNB in the dark for 30 min (ABS_{POST}) against a reference solution of 500 μL of 5% sodium dodecyl sulphate and 2.50 mL of 0.10 M Tris buffer (pH 8.0). Another solution containing 2.00 mL 0.10 M Tris buffer (pH 8.0), 500 μL 5% sodium dodecyl sulphate and 500 μL 10 mM DTNB was used as a blank sample (ABS_{BLANK}). Thiol absorbance was calculated as:

$$ABS_{CORR} = ABS_{POST} - ABS_{PRE} - ABS_{BLANK}.$$

Calculation of thiol concentration was based on a six-point standard curve prepared using 25–100 μM cysteine. Protein concentration was determined by absorption at 280 nm. For protein quantification, a standard solution of bovine serum albumin ranging from 0 to 2 mg/mL in 20 mM sodium phosphate buffer with 6 M guanidine hydrochloride (pH 6.5) was prepared.

Statistical analysis

The experiment had a completely randomised design with animal as the experimental unit. Analysis of variance and repeated measures analysis of variance using the MIXED model procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC, USA) were used to compare the treatments. For proximate analysis, ration type was treated as a fixed effect while animal was treated as a random effect. For multiple observations per animal (fatty acid composition, α-tocopherol concentration, colour, lipid and protein oxidation), ration type, display time and their interaction were treated as fixed effects while animal was treated as a random effect. Data were checked for normality using the PROC UNIVARIATE procedure of SAS and non-normal data were transformed using the Box Cox transformation. Differences between least square means were determined at a significance level of $P < 0.05$ and considered a tendency when $P < 0.10$ but >0.05. Least square means are reported with pooled standard errors.

Results

Carcass weight averaged 400 and 402 kg for the C and PFO rations, respectively, and did not differ significantly. Carcass fat score was also similar between rations (7.3 and 7.0 for the C and PFO rations, respectively, where 1 = very lean and 15 = very fat).

Feed fatty acid and α-tocopherol concentrations

The chemical composition of the feeds used is shown in Table 1. The total feed fatty acid concentration was 2.2 times higher in the PFO ration compared to the C ration. The concentrations of C16:0, C18:0, C18:1*n*-9*c*, C18:2*n*-6*c*, C18:3*n*-3, EPA, DHA

Table 1: Fatty acid and α-tocopherol concentrations (mg/kg DM) in the control (C) and protected fish oil (PFO) rations and grass silage fed to late-maturing bulls

	C	PFO	Silage
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
	***n* = 8**	***n* = 8**	***n* = 8**
Fatty acids			
C12:0	31.0 (4.70)	162 (62.5)	62.4 (22.2)
C14:0	134 (125)	922 (403)	207 (26.6)
C15:0	30.7 (28.0)	237 (109)	25.1 (14.8)
C16:0	6,093 (689)	11,519 (2,704)	4,155 (647)
C16:1	76.8 (107)	832 (396)	84.7 (65.6)
C17:0	34.4 (29.9)	270 (123)	43.7 (7.08)
C18:0	429 (196)	2,030 (839)	445 (58.3)
C18:1*n*-9*c*	2,867 (527)	7,509 (2,390)	534 (110)
C18:1*n*-7	208 (66.2)	771 (288)	206 (58.9)
C18:2*n*-6*c*	7,366 (564)	12,368 (2,180)	3,228 (572)
C20:0	55.2 (12.2)	174 (61.4)	151 (25.3)
C18:3*n*-6	–	6.65 (4.28)	12.0 (2.38)
C20:1*n*-9	163 (58.5)	710 (283)	175 (47.4)
C18:3*n*-3	537 (29.8)	893 (156)	9,684 (1,677)
C20:2	23.8 (9.11)	61.4 (23.4)	123 (35.7)
C22:0	61.4 (9.07)	158 (51.4)	177 (205)
C22:1*n*-9	38.1 (5.78)	90.8 (29.5)	9.5 (4.63)
C20:3*n*-3	0.5 (1.38)	7.91 (3.62)	18.3 (4.34)
C22:2	–	–	37.4 (5.91)
C24:0	46.1 (5.00)	106 (32.4)	280 (47)
C20:5*n*-3	10.8 (8.60)	52.9 (40.6)	2.4 (4.8)
C24:1*n*-9	42.2 (14.3)	162 (66.6)	71.3 (24.6)
C22:5*n*-3	–	12.0 (4.88)	–
C22:6*n*-3	8.21	112 (52.7)	–
Others	27.3 (30.8)	121 (45.4)	77.1 (21.6)
Total	18,304 (2,206)	39,371 (10,235)	19,808 (356)
SFA	6,916 (962)	15,577 (4,372)	5,562 (104)
MUFA	3,396 (771)	10,074 (3,449)	1,081 (29.9)
PUFA	7,946 (596)	13,513 (2,433)	13,105 (224)
PUFA:SFA	2.39 (0.05)	1.48 (0.09)	12.38 (0.06)
n-6 PUFA	7,390 (561)	12,436 (2,204)	3,400 (562)
n-3 PUFA	557 (42.2)	1,077 (236)	9,705 (168)
α-Tocopherol (mg/kg DM)	18.4 (2.31)	34.5 (3.03)	99.2 (11.4)

SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.
n-6 PUFA = sum of C18:2*n*-6, C20:2, C20:4*n*-6, C22:2.
n-3 PUFA = sum of C18:3*n*-3, C20:3*n*-3, C20:4*n*-3, C20:5*n*-3, C22:5, C22:6*n*-3.

saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), PUFA, *n*-6 PUFA and *n*-3 PUFA were higher in the PFO ration compared to the C ration. C22:5*n*-3 was only detected in the PFO ration. The concentration of α-tocopherol was 1.9 times higher in the PFO ration compared to the C ration.

Fatty acid composition of muscle

The concentration of individual and total fatty acids in muscle is shown in Table 2. There was no interaction ($P > 0.05$) between ration type and day of display for the concentration of total fatty acids. The concentrations of C18:2*n*-6*c*, C20:0, C20:1*n*-9, C20:2, DHA, PUFA, *n*-6 PUFA and the *n*-6:*n*-3 PUFA ratio were higher ($P < 0.05$) in muscle from PFO bulls compared to C bulls. The concentration of C22:5*n*-3 was lower ($P < 0.01$) in muscle of PFO bulls compared to C bulls.
There was no difference between day 0 and day 10 of display for the concentration of total fatty acids. The concentrations of C18:3*n*-6, C20:4*n*-6, C20:1*n*-9, *n*-3 PUFA and highly peroxidisable (three or more double bonds, HP)-PUFA were lower ($P < 0.05$) on day 10 compared to day 0 of display. There was a tendency ($P < 0.1$) for the concentrations of C18:3*n*-3, EPA, C22:5*n*-3, PUFA and *n*-6 PUFA to be lower and for the *n*-6:*n*-3 PUFA ratio to be higher, on day 10 compared to day 0 of display.
The proportion of individual and total fatty acids in muscle is shown in Table 3. There was no interaction ($P > 0.05$) between ration type and day of display for the proportion of total fatty acids. The proportions of C12:0, C16:0, C18:0, C18:2*n*-6*c*, C20:0, C20:2, C20:1*n*-9, DHA, SFA and *n*-6 PUFA were higher ($P < 0.05$) in muscle from PFO bulls compared to C bulls. The proportions of C18:1*n*-7, C18:3*n*-3*c* and C22:5*n*-3 were lower ($P < 0.05$) in muscle of PFO bulls compared to C bulls.
There was no difference between day 0 and day 10 of display for the proportion of individual or total fatty acids.

Muscle α-tocopherol concentration

There was no interaction between ration type and day of display for α-tocopherol concentration (Table 4). The concentration of α-tocopherol was lower ($P < 0.05$) in muscle from PFO bulls compared to C bulls and decreased ($P < 0.05$) with display time. The α-tocopherol:PUFA and the α-tocopherol:HP-PUFA ratios were lower ($P < 0.05$) in muscle from PFO bulls compared to C bulls. The α-tocopherol:PUFA ratio was higher ($P < 0.05$) on day 0 compared to day 10.

Lipid and protein oxidation

There was an interaction ($P = 0.03$) between ration type and day of display for lipid oxidation (presented as TBARS values) (Figure 1). Thus, while TBARS values increased with display time ($P < 0.001$), TBARS values did not differ between muscle from C and PFO bulls on day 0 and day 3, but they were

Table 2: The concentration (mg/100 g muscle) of fatty acids in the *longissimus thoracis* muscle of late-maturing bulls offered a control (C) or protected fish oil (PFO) ration *ad libitum* for 100 d before slaughter

	Day 0		Day 10			Significance[1]	
	C	PFO	C	PFO	s.e.	Ration	Day
Fatty acids							
C10:0	0.79	1.26	0.68	0.77	0.10		
C12:0	0.93	1.47	0.93	1.24	0.11	†	
C14:0	37.2	55.6	33.8	37.7	3.78		
C14:1	4.59	5.47	4.95	5.20	0.62		
C15:0	6.37	8.50	5.36	5.37	0.76		
C15:1	2.84	2.58	2.61	2.58	0.09		
C16:0	395	530	349	372	33.8		
C16:1	36.3	42.9	39.7	33.4	3.28		
C17:0	15.0	24.0	13.7	12.7	2.29		
C18:0	241	325	202	210	23.2		
C18:1*n*-9*c*	487	559	427	363	40.6		
C18:1*n*-7	30.3	34.4	28.1	26.2	1.79		
C18:2*n*-6*t*	0.39	0.43	0.23	0.24	0.06		
C18:2*n*-6*c*	90.8	143.0	81.3	130.0	5.88	***	
C20:0	1.46	2.46	1.48	2.21	0.28	*	
C18:3*n*-6	0.59	0.47	0.40	0.25	0.05		*
C20:1*n*-9	1.89	5.23	1.64	2.90	0.38	**	*
C18:3*n*-3	12.1	12.6	10.6	9.91	0.59		†
C18:2*c*9*t*11	1.51	2.32	1.28	1.06	0.33		
C20:2	1.18	1.89	1.03	1.51	0.46	**	
C22:0	0.83	0.85	0.47	0.76	0.13		
C20:3*n*-6	6.50	6.34	5.66	5.86	0.21		
C20:3*n*-3	0.32	0.50	0.27	0.21	0.06		
C20:4*n*-6	23.9	22.5	19.1	21.1	0.72		*
C22:2	0.93	0.85	0.62	0.69	0.12		
C24:0	0.21	0.15	0.13	0.21	0.04		
C20:5*n*-3	6.90	7.08	6.24	6.59	0.43		†
C22:5*n*-3	12.5	9.80	11.2	8.68	0.46	**	†
C22:6*n*-3	1.33	4.20	0.85	2.90	0.31	***	
Others	8.02	6.17	3.88	2.55	1.06		
Total	1,428	1,817	1,254	1,267	114.0		
SFA	699	950	607	642	63.7		
MUFA	564	651	508	435	46.0		
PUFA	159	212	139	189	7.4	***	†
PUFA:SFA	0.27	0.25	0.24	0.32	0.01		
n-6 PUFA	124	175	108	159	6.4	***	†
n-3 PUFA	35.2	36.9	30.8	30.5	1.29		*
n-6:*n*-3 PUFA	3.52	4.80	3.56	5.28	0.15	***	†

Table 2: (continued)

	Day 0		Day 10			Significance[1]	
	C	PFO	C	PFO	s.e.	Ration	Day
HP-PUFA	64.1	63.5	54.3	55.5	1.88		*

[1]There were no interactions between ration type and day.
Samples were stored in modified atmosphere (O_2:CO_2; 80:20) and subjected to simulated retail display (4°C, 1,000 lux for 12 h out of 24 h) for 10 d.
SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.
n-6 PUFA = sum of C18:2*n*-6*t*, C18:2*n*-6*c*, C18:3*n*-6, C18:2*c*9*t*11, C20:2, C20:3*n*-6, C20:4*n*-6, C22:2.
n-3 PUFA = sum of C18:3*n*-3, C20:3*n*-3, C20:5*n*-3, C22:5, C22:6*n*-3.
HP-PUFA = highly peroxidisable PUFA, calculated as the sum of PUFA with three or more double bonds.
Others: not reported or not identified.
* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, † = $P < 0.1$ tendency.

Table 3: The proportion (% total) of fatty acids in the *longissimus thoracis* muscle of late-maturing bulls offered a control (C) or protected fish oil (PFO) ration *ad libitum* for 100 d before slaughter

	Day 0		Day 10			Significance[1]	
	C	PFO	C	PFO	s.e.	Ration	Day
Fatty acids proportion (%)							
C10:0	0.05	0.07	0.05	0.06	0.005		
C12:0	0.06	0.08	0.07	0.10	0.006	*	
C14:0	2.58	3.04	2.67	2.84	0.112	†	
C14:1	0.30	0.28	0.39	0.38	0.038		
C15:0	0.42	0.44	0.43	0.42	0.030		
C15:1	0.23	0.16	0.22	0.22	0.017		
C16:0	27.5	29.2	27.7	28.9	0.36	*	
C16:1	2.72	2.32	3.13	2.52	0.189	†	
C17:0	0.92	1.25	1.05	1.01	0.129		
C18:0	16.0	17.7	16.1	16.7	0.34	*	
C18:1*n*-9*c*	33.5	30.2	33.7	28.3	0.55	***	
C18:1*n*-7	2.25	1.91	2.29	2.10	0.071	*	
C18:2*n*-6*t*	0.04	0.03	0.02	0.02	0.008		
C18:2*n*-6*c*	7.15	8.48	6.76	10.90	0.494	**	
C20:0	0.09	0.13	0.11	0.18	2.694	*	
C18:3*n*-6	0.05	0.03	0.03	0.02	0.006		
C20:1*n*-9	0.13	0.28	0.13	0.24	0.009	***	
C18:3*n*-3	0.92	0.71	0.87	0.81	0.034	*	
C18:2*c*9*t*11	0.08	0.11	0.10	0.08	0.019		
C20:2	0.09	0.11	0.08	0.13	0.009	*	
C22:0	0.06	0.05	0.04	0.07	0.016		
C20:3*n*-6	0.52	0.38	0.48	0.50	0.038		
C20:3*n*-3	0.02	0.03	0.02	0.02	0.006		
C20:4*n*-6	1.96	1.36	1.60	1.79	0.137		
C22:2	0.06	0.04	0.06	0.06	0.012		
C24:0	0.02	0.01	0.01	0.02	0.005		

Table 3: (continued)

	Day 0		Day 10			Significance[1]	
	C	PFO	C	PFO	s.e.	Ration	Day
C20:5*n*-3	0.58	0.44	0.54	0.57	0.064		
C22:5*n*-3	1.01	0.58	0.94	0.72	0.061	**	
C22:6*n*-3	0.11	0.25	0.07	0.23	0.028	**	
Others	0.63	0.42	0.32	0.20	0.117		
SFA	47.6	51.9	48.3	50.3	0.59	**	
MUFA	39.2	35.2	40.1	33.9	0.57	***	
PUFA	12.6	12.6	11.6	15.8	0.81	†	
n-6 PUFA	9.8	10.4	9.0	13.3	0.65	*	
n-3 PUFA	2.78	2.17	2.58	2.54	0.170		
HP-PUFA	5.15	3.79	4.55	4.67	0.329		

[1]There were no interactions between ration type and day.
Samples were stored in modified atmosphere (O_2:CO_2; 80:20) and subjected to simulated retail display (4°C, 1,000 lux for 12 h out of 24 h) for 10 d.
SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.
n-6 PUFA = sum of C18:2*n*-6*t*, C18:2*n*-6*c*, C18:3*n*-6, C18:2*c*9*t*11, C20:2, C20:3*n*-6, C20:4*n*-6, C22:2.
n-3 PUFA = sum of C18:3*n*-3, C20:3*n*-3, C20:4*n*-3, C20:5*n*-3, C22:5, C22:6*n*-3.
HP-PUFA = highly peroxidisable PUFA, calculated as the sum of PUFA with three or more double bonds.
Others = not reported or not identified.
* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, † = $P < 0.1$ tendency.

higher ($P < 0.01$) in muscle of PFO bulls compared to C bulls on day 7 and day 10 of display.

There was no interaction between ration type and day of display for either carbonyl or thiol concentrations (Table 4). There was no effect of ration type on carbonyl or thiol concentration but carbonyl concentration was lower ($P < 0.05$) while thiol concentration was higher ($P < 0.05$) on day 0 compared to day 10 of display.

Colour

There was no interaction between ration type and day of display nor was there an effect of ration type on lightness of muscle (Table 4). Lightness was similar on days 0, 3 and 7 and lower ($P < 0.05$) on day 10 compared to day 7 but similar to days 0 and 3. There was no interaction between ration type and day of display nor was there an effect of ration type on redness of muscle (Table 4). Redness was higher ($P < 0.05$) on day 7 compared to day 0 and lower ($P < 0.05$) on day 10 compared to all other days of display.

Discussion

Feed fatty acid composition and α-tocopherol concentration

The higher C18:3*n*-3, EPA, DHA and *n*-3 PUFA concentrations in the PFO compared to the C ration reflects the inclusion of the rumen PFO in the formulation of PFO. The higher α-tocopherol concentration in the PFO compared to the C ration, despite the similar amounts of α-tocopherol acetate added to the concentrates, may be due to additional α-tocopherol from fish oil. Fish oils contain α-tocopherol, with levels depending on species (McCance & Widdowson, 2014). Furthermore, the inclusion of soyabean in the PFO formulation may have contributed additional α-tocopherol to the PFO concentrate since Boschin and Arnoldi (2011) detected 14.2 mg α-tocopherol/100 g of soyabean seeds.

Muscle fatty acid and α-tocopherol concentrations

Moloney *et al.* (2021) examined the effect of inclusion of rumen PFO in the diet of suckler bulls, a subset of whom were used in the present study. They concluded that while the increase in the concentration of EPA and DHA was not enough to allow this beef to be labelled "source of omega-3 fatty acids", striploin from the bulls that consumed the PFO ration would make an important contribution to a consumer who does not eat oily fish, the main source of EPA and DHA in the human diet. In the present study, beef was aged in vacuum for 14 d, prior to retail display whereas in the study of Moloney *et al.* (2021), muscle samples were collected 48 h post-mortem. The lower concentration of total fatty acids in the present sample set prior to retail display suggests some loss of fatty acids during ageing, but as a subset of animals were used, albeit chosen

Table 4: α-Tocopherol concentration, colour and protein oxidation of *longissimus thoracis* muscle of late-maturing bulls offered a control (C) or protected fish oil (PFO) ration *ad libitum* for 100 d before slaughter

		Production system				Significance[1]	
	Day	**C**	**PFO**	**Mean[2]**	**s.e.**	**Ration**	**Day**
Antioxidant concentration α-Tocopherol (μg/g muscle)	0	2.93	2.48	2.71[a]	0.10	**	***
	3	2.37	2.07	2.22[b]			
	7	2.13	1.65	1.89[c]			
	10	2.04	1.31	1.68[d]			
	Mean	2.37	1.88				
Antioxidant: pro-oxidant ratios α-Tocopherol:PUFA	0	19×10^{-4}	12×10^{-4}	15×10^{-4}	5×10^{-5}	***	**
	10	15×10^{-4}	7×10^{-4}	11×10^{-4}			
	Mean	17×10^{-4}	9×10^{-4}				
α-Tocopherol:HP-PUFA	0	30×10^{-4}	19×10^{-4}	25×10^{-4}	2×10^{-4}	**	
	10	27×10^{-4}	14×10^{-4}	20×10^{-4}			
	Mean	29×10^{-4}	17×10^{-4}				
Protein oxidation Carbonyls (nmol/g protein)	0	5.06	5.73	5.40	0.46		***
	10	8.27	7.97	8.12			
	Mean	6.67	6.85				
Thiols (nmol/g protein)	0	57.6	61.5	59.6	1.54		***
	10	46.2	46.8	46.5			
	Mean	51.9	54.2				
Colour							
L	0	35.9	36.9	36.4[a,b]	0.35	†	*
	3	37.1	38.2	37.7[b]			
	7	36.4	37.3	36.8[a,b]			
	10	35.1	35.7	35.4[a]			
	Mean	36.1	37.0				
a	0	13.9	14.0	13.9[a]	0.34		***
	3	15.4	15.1	15.2[a,b]			
	7	15.8	15.2	15.5[b]			
	10	11.4	11.1	11.2[c]			
	Mean	14.1	13.8				

[1]There were no interactions between ration type and day.
Samples were stored in modified atmosphere (O_2:CO_2; 80:20) and subjected to simulated retail display (4°C, 1,000 lux for 12 h out of 24 h) for 10 d.
α-Tocopherol:PUFA = ratio between the concentration of α-tocopherol and the concentration of PUFA, both expressed as mg/g of muscle.
α-Tocopherol:HP-PUFA: ratio between the concentration of α-tocopherol and the concentration of highly peroxidisable PUFA (PUFA with three or more double bonds), both expressed as mg/g of muscle.
[2]Values in "mean" column assigned different superscripts differ significantly ($P < 0.05$).
* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, † = $P < 0.1$ tendency.

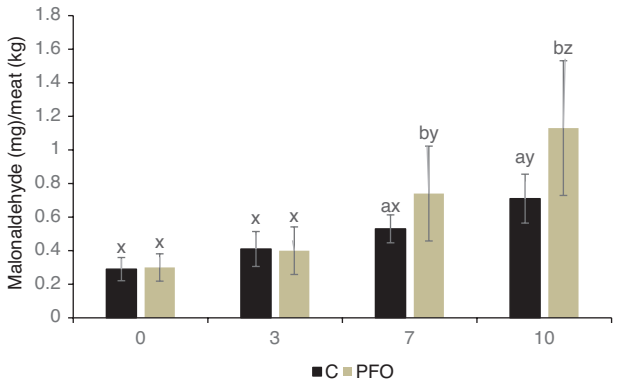

Figure 1. Lipid oxidation (thiobarbituric acid reactive substances [TBARS] values) of *longissimus thoracis* muscle of late-maturing bulls offered a control (C) or protected fish oil (PFO) ration *ad libitum* for 100 d before slaughter. Muscle was stored in modified atmosphere (O_2:CO_2; 80:20) and subjected to simulated retail display (4°C, 1,000 lux for 12 h out of 24 h). a,b = least square means assigned different superscripts differ significantly, within day, between ration ($P < 0.05$). x,y,z = least square means assigned different superscripts differ significantly, within ration, between days ($P < 0.05$).

at random, and different analytical procedures were used in different laboratories, this suggestion requires confirmation. In this regard, Mahecha *et al.* (2009) observed no change in the concentration of total fatty acids, but an increase in the proportion of SFA from 43.5% to 49.3% and a decrease in the proportion of PUFA from 13.8% to 8.4% after 14 d of vacuum storage of beef. Nevertheless, the concentrations of those fatty acids of particular interest from a human health perspective, C18:3*n*-3, EPA and DHA were largely similar in the day 0 samples in the present study and in Moloney *et al.* (2021). This response in terms of muscle fatty acid composition *per se* to consumption of the PFO ration has been comprehensively discussed by Moloney *et al.* (2021). The context for the present study was whether such an alteration in the fatty acid concentrations would impair the shelf life of beef when offered for retail sale. The packaging method was chosen as this is commonly used by industry and it is recognised that the high oxygen concentration in MAP provides a greater challenge to the stability of lipid-rich meat than alternative approaches such as aerobic or vacuum packing.

The susceptibility of fatty acids to oxidation increases with an increase in the number of double bonds (Belitz *et al.*, 2009). The decrease in the concentration of HP-PUFA after 10 d of MAP display (12% for muscle from the PFO bulls) was lower than the tendency towards a decrease in the concentration of long-chain *n*-3 PUFA in meat after 7 d of aerobic (a less oxidative environment than MAP) storage (15%) from lambs fed a fish oil supplement reported by Diaz *et al.* (2011). Moreover, the lack of effect of display in MAP on the fatty acid profile (fatty acids expressed as a proportion of total fatty acids) also indicates that preferential oxidation of long-chain PUFA was rather small.

Using the intake data reported by Moloney *et al.* (2021) and the α-tocopherol concentration of feedstuffs (Table 1), we calculated the daily intake of α-tocopherol to be 280 and 508 mg for bulls offered the C and PFO rations, respectively. Despite the higher intake of α-tocopherol by the PFO bulls, the α-tocopherol concentration was higher overall in muscle of C bulls, which may reflect higher *in vivo* utilisation of α-tocopherol in the muscle of PFO bulls, possibly to counteract increased oxidation. A similar trend was seen in Vatansever *et al.* (2000). The decrease in muscle α-tocopherol concentration during simulated retail display was expected, since α-tocopherol quenches the peroxyl radicals formed from PUFA during lipid oxidation and is subsequently converted into its oxidation products (Liebler *et al.*, 1996). The lower α-tocopherol:PUFA and α-tocopherol:HP-PUFA ratios in the muscle of PFO bulls reflect the higher PUFA and HP-PUFA concentrations in their muscle. The lower ratios on day 10 reflect the lower α-tocopherol concentration on day 10. Nevertheless, the relatively minor loss of long-chain PUFA in the present study likely reflects the relatively high α-tocopherol concentration in muscle since Alvarez *et al.* (2009) reported that when lamb muscle contained 0.9 mg/100 g, the fatty acid profile was substantially changed due to display in MAP, but when the muscle α-tocopherol concentration was increased to 2.67 mg/100 g due to dietary supplementation, there was little effect of MAP display on the fatty acid profile. In the present study, the decrease in HP-PUFA after display in MAP reflected a tendency towards a small decrease in the nutritional value of the muscle from the bulls that consumed the PFO ration, for example, EPA + DHA decreased from 11.3 mg/100 g muscle on day 0 of display to 9.5 mg/100 g muscle after 10 d of display. A protective effect of an increase in α-tocopherol concentration in the PFO ration would likely be observed based on Alvarez *et al.* (2009).

Lipid and protein oxidation

Dietary supplementation with fish oil generally increases lipid oxidation during retail display of beef when compared with beef from un-supplemented cattle. This has been reported for non-rumen PFO (Vatansever *et al.*, 2000; Scollan *et al.*, 2005; Bahnamiri *et al.*, 2019) or rumen PFO (Richardson *et al.*, 2004; Dunne *et al.*, 2011). A similar effect was more recently observed in beef from cattle supplemented with DHA-rich microalgae (Phelps *et al.*, 2016). The scale of this effect is influenced by the concentration of long carbon chain PUFA, especially EPA, C22:5 and DHA, the concentration of α-tocopherol (and other antioxidants) and whether display is aerobic or MAP. The higher concentration of TBARS in muscle from PFO bulls on day 10 of display in the present study is consistent with the above literature and reflected the lower α-tocopherol and

higher PUFA concentration on day 0 than in muscle from the C bulls (Ponnampalam *et al.*, 2014). Nevertheless, the TBARS values for muscle from the PFO bulls remained below the 2 mg malonaldehyde/kg threshold value for the detection of rancidity in meat by consumers reported by Campo *et al.* (2006). Indices of protein oxidation were measured as an increase in protein oxidation has been suggested to result in a decrease in meat tenderness (Lagerstedt *et al.*, 2011). The concentration of carbonyl compounds in oxidised muscle ranges from 2 to about 14 nmol/mg protein depending on the actual initiator of oxidation, the level of oxidation, the muscle type, and protein solubility (Lund *et al.*, 2007) and the values in the present study are within this range. The free thiol values were similar to those reported by Gatellier *et al.* (2010) for vacuum-packaged beef from cattle fed a linseed/rapeseed oil supplemented ration. Notwithstanding the higher lipid oxidation, during retail display, muscle from PFO bulls had similar amounts of free thiols and carbonyls to muscle from C bulls. The lag in initiation of protein oxidation after the initiation of lipid oxidation as well as the slower rate of protein oxidation compared to lipid oxidation (Insani *et al.*, 2008) may explain this observation.

Colour stability

Colour is an important influence on the purchasing decision of the consumer and bright red is preferred (Faustman *et al.*, 1998). When exposed to air, the bright red, myoglobin colour of beef slowly changes to brown because of conversion of myoglobin to metmyoglobin. This most likely occurs as a result of the combined effects of lipid oxidation and muscle pigment oxidation, particularly the oxidation of myoglobin (Monahan *et al.*, 2005). The observation that colour stability was not affected by feeding rumen PFO is in agreement with Dunne *et al.* (2011) (measured as redness) and Richardson *et al.* (2004) (measured as saturation). For non-rumen PFO, Scollan *et al.* (2001) reported no effect on colour stability measured as redness and saturation, respectively, but Vatansever *et al.* (2000) reported a decrease in saturation in minced beef. A decrease in redness was also observed in beef from cattle supplemented with DHA-rich microalgae (Phelps *et al.*, 2016), but this was avoided when additional antioxidants were included with the microalgae (Phelps *et al.*, 2020). As with lipid oxidation, loss of colour stability due to elevation of long carbon chain PUFA can be mitigated by increasing the concentration of antioxidants in the diet and ultimately in muscle (Burnett *et al.*, 2020).

Conclusion

Dietary inclusion of rumen PFO in the diet of late-maturing breed-sired suckler bulls enhanced the fatty acid profile of beef from a human health perspective, and had minor effects on beef shelf life. There is potential for a higher level of inclusion and greater enhancement of the fatty acid profile of beef without excessive oxidation of lipid and protein during retail display if the antioxidant supply is also increased. The willingness of the consumer to accept the increase in the cost of producing nutritionally enhanced beef merits examination.

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