Research Article

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Effect of slaughter season and muscle type on the fatty acid composition, including conjugated linoleic acid isomers, and nutritional value of intramuscular fat in organic beef

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Abstract

BACKGROUND: Consumer awareness regarding the intake of beef of organic origin is strongly associated with the beneficial outcomes to human health, the environment and animal welfare. In this paper the effects of slaughter season and muscle type on the fatty acid composition, conjugated linoleic acid (CLA) isomeric profile, total cholesterol, α-tocopherol and β-carotene contents and nutritional quality of intramuscular fat in organic beef ($n = 30$) are reported for the first time.

RESULTS: Organic beef showed a very low total lipid content, with seasonal changes in the levels of some fatty acids, CLA isomers, n-6/n-3 polyunsaturated fatty acid (PUFA) ratio, total cholesterol and β-carotene. In addition, differences between *longissimus lumborum* (relatively red) and *semitendinosus* (relatively white) muscles were found for many fatty acids, specific CLA contents, many CLA isomers and both PUFA/saturated fatty acid (SFA) and n-6/n-3 ratios. However, in spite of the seasonal and carcass variations, all organic meats analysed had values of beef similar to pasture-fed cattle.

CONCLUSION: From a nutritional perspective, organic meat from both slaughter seasons seems to have high CLA contents, PUFA/SFA and n-6/n-3 indices within the recommended values for the human diet. The data indicate that intramuscular fat in organic meat has a high nutritional value throughout the year.

Keywords: organic beef; fatty acids; CLA isomers; cholesterol; fat-soluble vitamins

INTRODUCTION

Meat fatty acid composition has been studied for many years owing to its implications for human health.[1] Currently, nutritionists recommend not only limiting fat intake but also consuming higher amounts of polyunsaturated fatty acids (PUFA), especially those of the n-3 family rather than those from the n-6 series.[2] In addition, conjugated linoleic acid (CLA) is a group of minor fatty acids occurring naturally in ruminant-derived foods and with a multitude of beneficial health effects.[3] The most common strategy to produce meat animals, particularly beef cattle, is based on the intake of high levels of concentrate diets rich in cereals, at least in the finishing period.[4] However, it is well documented that animals consuming fresh pastures have higher contents of unsaturated fatty acids in their milk and meat than those receiving a cereal-based concentrate diet.[5,6] In fact, grass is a good natural source of n-3 PUFA, mainly α-linolenic acid (ALA, 18:3n-3), although variations occur regarding its maturity and floristic diversity.[7] In addition, it is also well known that meat from pasture-fed cattle is rich in vitamin E as well as other natural antioxidants such as carotenoids.[8] Thus meat from cattle produced under grass-based systems combines potentially beneficial fatty acid profiles and lipid-soluble antioxidant vitamin contents.[9]

Consumer studies continue to show that expectations concerning health effects of organic food are the strongest motive for consumers to buy organic products.[10] There has also been an increase in consumer awareness of the environmental impact and animal welfare benefits of organic foods.[11,12] In addition, commer-

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cial crossbred bovines reared in pasture-based systems have been progressively reintroduced in Portuguese dietary habits as a result of public perception of chemical residue safety[13] and absence of animal genetic modifications.[14]

The scientific information available to support the nutritional quality of organic beef, mainly dependent on its lipid composition, is scarce. In addition, few works have been conducted to assess seasonal changes in lipid composition of organic beef. Therefore the aim of the current study was to determine the influence of slaughter season and muscle type on the lipid content, fatty acid composition, including detailed CLA isomeric profile, and total cholesterol, α-tocopherol and β-carotene contents of organic meat. Moreover, the seasonal and carcass variations in nutritional quality of intramuscular fat in organic beef were assessed.

EXPERIMENTAL

Animals, diets and meat samples

Limousin × Charolais crossbred young bulls ($n = 30$) were maintained according to a traditional pasture-based production system following the established guidelines for organic meat (EC Council Regulation No. 834/2007 of 28/07). Calves were reared with their dams until weaning at 9 months of age. After weaning, young bulls were raised in an extensive grazing system for 14 months (Alentejo, southern Portugal). Some of the young bulls were maintained on pasture lands until slaughter in June 2008 (which corresponds to late spring sampling; $n = 15$, 23.1 ± 0.9 months, 209 ± 7.5 kg cold carcass weight). The remaining animals were maintained on pasture lands and slaughtered in November 2008 (which corresponds to early autumn sampling; $n = 15$, 22 ± 0.8 months, 206 ± 9.1 kg cold carcass weight). The time on pasture was equal in both seasons. Animals were fed improved pasture, including clover, alfalfa, ryegrass and tall fescue, as well as native grasses throughout the year. The predominant species were ryegrass and clover. Naturally, in early autumn the green pastures were less abundant than in late spring. Slaughtering in both periods was carried out under commercial processing conditions (STEC Abbattoir, Montijo, Portugal). Meat samples were taken from the loin portion of *longissimus dorsi* (L4–L6 *longissimus lumborum* muscle, LL) and distal region of *semitendinosus* (ST) muscles on the left side of the carcass. All meat samples were collected 2–3 days after slaughter (1 °C), ground in a food processor (3 × 5 s), vacuum packed and stored at −80 °C until analysis.

Lipid extraction and methylation

Intramuscular fat was extracted with dichloromethane/methanol (2 : 1 v/v) from lyophilised (−60 °C and 2 hPa; Edwards High Vacuum International, West Sussex, UK) meat samples (∼250 mg) for determination of total lipids and both fatty acid methyl ester (FAME) and CLA methyl ester profiles using the procedures described previously by Alfaia *et al*.[15] All extraction solvents contained 0.1 mL L^{-1} butylated hydroxytoluene (BHT) as an antioxidant. Total lipids were measured gravimetrically in duplicate by weighing the fatty residue obtained after solvent evaporation. Fatty acids and CLA isomers were converted to methyl esters by a combined transesterification procedure with NaOH in anhydrous methanol (0.5 mol L^{-1}) followed by HCl/methanol (1 : 1 v/v) at 50 °C for 30 and 10 min respectively according to Raes *et al*.[16] The same FAME solution was used for the analysis of both fatty acid composition and CLA isomeric profile, thus enabling the direct comparison of quantitative data and eliminating differences in sample preparation.

Determination of fatty acid composition

FAME were analysed by gas chromatography with flame ionisation detection (GC-FID; HP 6890 chromatograph, Hewlett-Packard, Avondale, PA, USA) using a Chrompack CP-Sil 88™ fused silica capillary column (100 m × 0.25 mm i.d., 0.2 µm film thickness; Varian Inc., Walnut Creek, CA, USA) as described by Alves and Bessa.[17] Briefly, the oven temperature was initially 100 °C (held for 1 min), then increased at 50 °C min^{-1} to 150 °C (held for 20 min), then increased at 1 °C min^{-1} to 190 °C (held for 5 min) and finally increased at 1 °C min^{-1} to 200 °C (held for 35 min). Ultrapure helium was used as carrier gas at a flow rate of 1 mL min^{-1}, with a split ratio of 1 : 50. The injector and detector temperatures were maintained at 250 and 280 °C respectively. Identification of common fatty acids was accomplished by comparison of sample peak retention times with those of FAME standard mixtures (Nu-Chek-Prep Inc., Elysian, MN, USA; Supelco Inc., Bellefonte, PA, USA) and by using published chromatograms obtained under similar analytical conditions.[18] Structural analyses of some unknown peaks were conducted by gas chromatography/mass spectrometry (GC/MS) using a Saturn 2200 system (Varian Inc.) equipped with a Chrompack CP-Sil 88™ column (100 m × 0.25 mm i.d., 0.2 µm film thickness; Varian Inc.). Quantification of total FAME was done using nonadecanoic acid (19 : 0) as internal standard. Results for each fatty acid were expressed as g kg^{-1} total fatty acids.

Determination of individual CLA isomers

Methyl esters of CLA isomers were individually separated by triple silver ion (Ag^+) columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm i.d., 5 µm particle size; Chrompack, Bridgewater, NJ, USA) using an Agilent 1100 Series high-performance liquid chromatography (HPLC) system (Agilent Technologies Inc., Palo Alto, CA, USA) according to the procedure reported previously by Alfaia *et al*.[15] Briefly, the mobile phase was 1 mL L^{-1} acetonitrile in *n*-hexane at a flow rate of 1 mL min^{-1}, the diode array detector (DAD) was adjusted to 233 nm, and volumes of 20 µL were injected by the autosampler. Identification of individual CLA isomers was achieved by comparison of their retention times with commercial and prepared standards as well as with values published in the literature.[19,20] In addition, the identity of each isomer was confirmed by typical ultraviolet (UV) spectra of CLA isomers from the DAD in the range 190–360 nm using the spectral analysis of Agilent Chemstation for LC 3D Systems Rev. A.09.01 (Agilent Technologies Inc.). Standards of CLA isomers (c9,t11, t10,c12, c11,t13, c9,c11 and t9,t11) were purchased from Matreya Inc. (Pleasant Gap, PA, USA) and Sigma Inc. (St Louis, MO, USA) or prepared (*cis/trans* and *trans,trans* from carbons 7,9 to 12,14) by the procedure reported by Destaillats and Angers.[21] Total CLA content was calculated as the sum of its main isomer c9,t11 (plus t7,c9 and t8,c10) determined by GC-FID with other minor isomers quantified by Ag^+-HPLC analysis.[22,23] Individual CLA isomers were expressed as g kg^{-1} total fatty acids.

Determination of cholesterol, tocopherols and β-carotene

Simultaneous analysis of cholesterol, tocopherols and β-carotene in meat was performed according to Prates *et al*.[24] Briefly, saponification of homogenised fresh meat samples (∼750 mg) was carried out with 0.2 g of L-ascorbic acid and 5.5 mL of saponification solution containing 110 g L^{-1} KOH, in a mixture of absolute ethanol and distilled water (55 : 45 v/v), in a shaking water bath at 80 °C for 15 min. After cooling, 1.5 mL of distilled water and

3 mL of 25 μg mL^{-1} BHT solution in *n*-hexane were added, then the samples were vigorously vortexed for 2 min and centrifuged at 1500 × *g* for 5 min. Afterwards, an aliquot of the *n*-hexane layer was filtered through a 0.45 μm hydrophobic membrane and injected into an Agilent 1100 Series HPLC system (Agilent Technologies Inc.) using a normal phase silica column (Zorbax RX-Sil, 250 mm × 4.6 mm i.d., 5 μm particle size; Agilent Technologies Inc.), with fluorescence detection for tocopherols (excitation and emission wavelengths of 295 and 325 nm respectively) and UV–visible photodiode array detection for cholesterol (202 nm) and β-carotene (450 nm) in series. The solvent was 10 mL L^{-1} isopropanol in *n*-hexane, the flow rate was 1 mL min^{-1}, the run lasted for 17 min and the temperature of the column was maintained at 20 °C. The contents of total cholesterol, tocopherols and β-carotene in meat were calculated, in duplicate for each muscle sampled, based on the external standard technique from a standard curve of peak area *versus* concentration, using DL-α-, D-β-, D-γ- and D-δ-tocopherol (Calbiochem, Merck Biosciences, Darmstadt, Germany), all-*trans*-carotene and cholesterol (Sigma Chemical Co., St Louis, MO, USA) as standards.

Statistical analysis

Data were analysed using the MIXED procedure of SAS Version 9.1 (SAS Institute, Cary, NC, USA). The model considers the effect of slaughter season (S: late spring *versus* early Autumn), muscle type (M: LL and ST muscles) and the interaction between slaughter season and muscle type (S × M). Each animal from the slaughter season was considered as the subject and the muscle type as repeated measures. Least square means were presented and compared using the PDIFF option when the interaction effect was significant ($P < 0.05$).

RESULTS AND DISCUSSION

Intramuscular fatty acid composition

In autumn, total lipid content was higher ($P < 0.05$) in LL muscle than in ST muscle, in contrast to spring, in which both muscles had similar values ($P > 0.05$) (Table 1). This interaction between muscle type and slaughter season can be explained by modifications in muscle fibre composition throughout the year due to seasonal changes in the physical activity of animals. Compared with ST muscle, LL muscle is relatively red and differently involved in the physical activity imposed by grazing.[25] The lipid content is higher in red oxidative muscle fibres[26] and, as explained above, the LL muscle of cattle is relatively red in comparison with the ST muscle. Organic beef had lower values of total lipids (6.3–7.2 g kg^{-1}) when compared with those reported for the meat from extensively (11–14 g kg^{-1}) produced cattle.[27] In addition, higher values were also obtained by Alfaia *et al.*,[28] using the same analytical methodology as that used in the present work, for total lipids of cattle fed on pasture (10 g kg^{-1}). The levels of intramuscular fat reported here, which are in close agreement with those described above for the cold carcass weights, can be explained by the low energy content of pastures and the absence of a concentrate finishing period.[28] These values suggest low levels of muscle triacylglycerols, since the values of phospholipids, although dependent on muscle fibre composition, are fairly constant in the same muscle fibre type.[29] In fact, the contents of fatty acids from phospholipids (mainly PUFA) remain fairly constant, but those from neutral lipids, with their high proportions of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), increase markedly as total lipids increase.[30] Like the majority of beef produced in Europe, the analysed organic beef can be considered a lean meat according to the Food Advisory Committee[31] criterion (<5 g kg^{-1} fat).

Data on the fatty acid composition (g kg^{-1} total fatty acids) of intramuscular fat in organic beef from late spring and early autumn are presented in Table 1. In both seasons the predominant fatty acids in intramuscular fat were 16 : 0, 18 : 0, 18 : 1c9, 18 : 2n-6, 18 : 3n-3 and 20 : 4n-6. Similar results have been reported for cattle in numerous studies.[4,28,32,33] Among these, only 18 : 1c9 and 18 : 2n-6 were not affected by seasonal variations ($P > 0.05$). Nevertheless, 12 of the 41 fatty acids analysed were affected ($P < 0.05$) by this variable. Regarding minor fatty acids, organic meat from autumn had higher contents of 18 : 1c12, 18 : 1c13, 18 : 1t6+t8, 18 : 1t12, 22 : 0 and 22 : 6n-3 but lower contents of 15 : 0anteiso, 15 : 0, 17 : 0iso, 18 : 1t16+c14, 18 : 3c9,t11,c15 and 20 : 0 relative to meat from spring. It is well known that beef fatty acid composition is influenced by both diet and season.[30] The botanic diversity of pastures influences fatty acid metabolism in the rumen through the presence of secondary metabolites in plants.[34] Meat from young bulls from late spring had higher ALA contents ($P < 0.001$) when compared with that from early autumn. ALA is a very sensitive grass intake indicator owing to its presence in large amounts in grass lipids.[30] This result was expected and can be explained by the fact that young bulls raised during late spring were exposed to more abundant pastures than animals raised in early autumn. The contents of ALA in meat from spring are higher than those previously found in meat from pasture-fed cattle[32,33] but lower than those reported by Alfaia *et al.*[28] for pasture-fed bulls.

The muscle type had a high influence on the fatty acid profile, affecting 21 of the 41 fatty acids analysed. The LL muscle, relative to the ST muscle, had higher contents of 14 : 0, 15 : 0iso, 15 : 0anteiso, 15 : 0, 16 : 0, 16 : 1c9, 17 : 0iso, 17 : 0anteiso, 17 : 0, 18 : 1t12, 18 : 1t16+c14 and 18 : 2t11,c15 but lower contents of 18 : 2n-6, 18 : 3n-3, 18 : 3c9,t11,c15, 20 : 4n-6, 20 : 5n-3, 22 : 0, 22 : 4n-6, 22 : 5n-3 and 23 : 0. These differences likely result from variations in muscle fibre composition, because the lipid content, which directly affects fatty acid composition, is higher in red oxidative muscle fibres.[26] SFA and PUFA in meat were influenced by muscle type, with higher and lower values for the LL muscle respectively. The slaughter season had no influence on these fatty acid sums. Knowing that SFA are mainly located in the triacylglycerols and PUFA in the phospholipids, the muscle effect is largely explained by the higher triacylglycerol/phospholipid ratio in the relatively red LL muscle when compared with the relatively white ST muscle. Furthermore, both season and muscle type affected the values of branched-chain fatty acids (BCFA), n-6 PUFA and n-3 PUFA in meat. In agreement with our results, Rule *et al.*[35] and Alfaia *et al.*[36] reported higher proportions of PUFA, including the sums of n-3 and n-6, in the ST muscle than in the LL muscle. However, specific grass and pasture plants produce different PUFA concentrations in meat owing to distinct levels of individual PUFA and variations in the way the feed is processed in the rumen.[30]

Significant interactions between slaughter season and muscle type were observed for *trans* fatty acid (TFA) sum and for the 16 : 0, 14 : 1c9, 16 : 1t9, 17 : 0iso, 18 : 0, 18 : 1c9, 18 : 1t10, 18 : 1t11, 18 : 1t12, 18 : 1c11+t15, 18 : 3n-6 and 20 : 4n-6 fatty acids. These interactions may result from modifications of muscle metabolic type caused by adaptations to distinct season periods.[37] Grass-feeding systems promote the accumulation of 18 : 1t11 fatty acid in beef, which can be desaturated to 18 : 2c9,t11 by Δ9-desaturase

Table 1. Total lipids (g kg^{-1} muscle), fatty acid composition (g kg^{-1} total fatty acids), partial sums of fatty acids and nutritional indices (fatty acid ratios) of *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles of organic meat from late spring and early autumn

	Spring		Autumn			Significance level		
Component	LL	ST	LL	ST	SEM	Season	Muscle	S × M
Total lipids	6.56ab	6.92ab	7.18a	6.31b	0.410	NS	***	*
Fatty acid composition								
14:0	7.88	4.24	8.38	4.81	0.699	NS	***	NS
14:1c9	1.21a	0.55b	1.21a	0.01c	0.118	NS	***	**
15:0iso	1.23	0.84	0.95	0.83	0.072	NS	***	NS
15:0anteiso	2.06	1.55	1.49	1.03	0.100	***	***	NS
15:0	4.41	3.58	3.29	2.90	0.147	***	***	NS
16:0iso	1.42	1.21	1.22	1.21	0.073	NS	NS	NS
16:0	183a	156b	191a	177a	3.0	**	***	**
16:1c7	3.31	2.30	2.36	2.31	0.305	NS	NS	NS
16:1c9	10.9	9.21	13.3	10.5	0.757	NS	***	NS
16:1t9	4.62a	4.69a	3.17b	4.55b	0.314	NS	***	***
17:0iso	4.40a	3.90b	3.22c	3.17c	0.127	***	***	*
17:0anteiso	3.70	2.59	3.47	2.57	0.210	NS	***	NS
17:0	7.92	6.65	7.28	5.88	0.256	*	***	NS
17:1c9	6.45	6.47	6.08	5.69	0.258	NS	NS	NS
18:0	156a	153a	146a	127b	2.6	***	***	***
18:1c9	198	178	186	181	6.9	NS	***	NS
18:1c12	1.63	1.59	2.08	1.82	0.153	*	NS	NS
18:1c13	0.88	0.80	1.08	1.10	0.102	*	NS	NS
18:1c15	0.86	0.89	0.92	0.87	0.070	NS	NS	NS
18:1t6+t8	1.06	0.93	1.28	1.02	0.080	NS	**	NS
18:1t9	0.97	0.83	1.48	1.09	0.090	***	***	NS
18:1t10	0.89c	0.75c	5.75a	4.76b	0.672	***	**	*
18:1t11	16.4	13.0	17.9	10.8	1.29	NS	***	NS
18:1t12	2.38a	1.95b	2.55a	2.57a	0.139	*	*	*
18:1c11+t15	16.7c	18.0b	20.1a	19.0b	0.45	**	NS	***
18:1t16+c14	1.96	1.81	1.81	1.40	0.093	**	**	NS
18:2c9,t11[a]	5.90	5.13	5.54	5.26	0.312	NS	NS	NS
18:2t11,c15	1.96	1.41	1.98	1.14	0.159	NS	***	NS
18:2n-6	124	148	133	158	6.3	NS	***	NS
18:3n-3	52.2	61.9	35.3	42.8	2.41	***	***	NS
18:3n-6	1.15b	1.18b	0.99b	1.43a	0.078	NS	**	*
18:3c9,t11,c15	3.40	4.48	2.90	3.79	0.212	*	***	NS
20:0	1.33	1.41	1.18	1.16	0.053	**	NS	NS
20:2n-6	1.67	1.29	1.19	1.61	0.176	NS	NS	NS
20:4n-6	41.6b	47.3b	43.4b	57.7a	2.76	NS	***	*
20:5n-3	21.6	27.6	21.7	31.1	1.22	NS	***	NS
22:0	8.45	11.8	10.1	13.9	0.57	**	***	NS
22:4n-6	2.14	2.55	2.20	2.96	0.158	NS	***	NS
22:5n-3	27.8	34.8	26.7	36.2	1.34	NS	***	NS
22:6n-3	2.23	2.64	3.04	3.76	0.266	**	*	NS
23:0	2.85	3.64	3.01	3.97	0.240	NS	**	NS
Partial sums of fatty acids								
SFA	373	341	370	337	5.4	NS	***	NS
MUFA	223	198	243	203	7.8	NS	***	NS
TFA	56.2b	53.0b	64.4a	55.5b	2.13	*	***	*
PUFA	274	327	267	335	11.8	NS	***	NS
BCFA	12.8	10.1	10.4	8.81	0.445	**	***	NS
n-6 PUFA	170	200	181	222	8.1	*	***	NS
n-3 PUFA	104	127	86.7	114	4.42	**	***	NS
Unidentified	60.8	71.7	45.2	60.4	2.79	***	***	NS

Table 1. (*Continued*)

Component	Spring LL	Spring ST	Autumn LL	Autumn ST	SEM	Significance level Season	Muscle	S × M
Fatty acid ratios								
PUFA/SFA	0.74	0.96	0.74	1.00	0.044	NS	***	NS
n-6/n-3	1.65	1.59	2.10	1.96	0.052	***	***	NS

Least square means in the same row with different letters are significantly different ($P < 0.05$). SEM, standard error of mean. S × M, interaction between slaughter season and muscle type. Significance: NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. SFA, saturated fatty acids, sum of 14 : 0, 15 : 0, 16 : 0, 17 : 0, 18 : 0, 20 : 0, 22 : 0 and 23 : 0; MUFA, monounsaturated fatty acids, sum of 14 : 1c9, 16 : 1c7, 16 : 1c9, 17 : 1c9, 18 : 1c9, 18 : 1c12, 18 : 1c13 and 18 : 1c15; TFA, *trans* fatty acids, sum of 16 : 1t9, 18 : 1t6+t8, 18 : 1t9, 18 : 1t10, 18 : 1t11, 18 : 1t12, 18 : 1c11,t15, 18 : 1t16+c14, 18 : 2t11,c15, 18 : 2c9,t11 and 18 : 3c9,t11,c15; PUFA, polyunsaturated fatty acids, sum of n-6 PUFA and n-3 PUFA; BCFA, branched-chain fatty acids, sum of 15 : 0iso, 15 : 0anteiso, 16 : 0iso, 17 : 0iso and 17 : 0anteiso; n-6 PUFA, sum of 18 : 2n-6, 18 : 3n-6, 20 : 2n-6, 20 : 4n-6 and 22 : 4n-6; n-3 PUFA, sum of 18 : 3n-3, 20 : 5n-3, 22 : 5n-3 and 22 : 6n-3; PUFA/SFA, sum of PUFA/sum of SFA; n-6/n-3, sum of n-6/sum of n-3.
[a] This peak also includes minor amounts of t7,c9 and t8,c10 CLA isomers.

enzyme in bovine adipose tissue and mammary gland.[38] The 18 : 1t11 fatty acid is the precursor of 18 : 2c9,t11 CLA isomer, the major CLA isomer in ruminant-derived products.[39] Alfaia *et al*.[28] reported similar values for 18 : 1t11 fatty acid in meat from bulls fed with grass, but Leheska *et al*.[40] obtained higher values in beef from grass-fed cattle. Alfaia *et al*.[28] also reported, in muscle from pasture-fed cattle, the presence of higher contents of the non-conjugated linoleic acid isomer 18 : 2t11,c15, an intermediate of ALA biohydrogenation in the rumen,[41] compared with our study.

The ratios of PUFA/SFA and n-6/n-3, indices widely used to evaluate the nutritional value of fat for human consumption, are defined and presented in Table 1. A season effect was observed for the n-6/n-3 ratio ($P < 0.001$) but not for the PUFA/SFA ratio ($P > 0.05$). According to current nutritional recommendations, the PUFA/SFA ratio in the human diet should be above 0.45 and, concerning PUFA, the n-6/n-3 ratio should not exceed 4.0.[42] However, this latter index has recently been a subject of controversy.[43] Concomitantly, Stanley *et al*.[44] have proposed that it is more important to evaluate the total amount of dietary PUFA than their respective ratio. Moreover, Brenna *et al*.[45] proposed that the n-3 PUFA status could be improved by increasing the dietary intake of n-3 PUFA or by reducing the intake of n-6 PUFA and that combining the two strategies would be the most effective nutritional measure. In the present study the PUFA/SFA and n-6/n-3 ratios in organic meat are within the recommended values for the human diet, which is favourable, for both slaughter seasons and muscle types. These desirable values clearly result from the benefits of grass feed on ruminant meat, as stated by Nuernberg *et al*.[46] Compared with available data, our values for the n-6/n-3 ratio are close to those reported for meat from grass-fed cattle.[26,28,32,40] It is well established that in meat from cattle fed with concentrates based on cereals (rich in n-6 PUFA) the n-6/n-3 ratio shifts to higher values when compared with animals produced on green pastures (rich in n-3 PUFA).[46] Our meat values for the PUFA/SFA ratio meet the recommended guidelines for the human diet throughout the year. In fact, several studies have described that the PUFA/SFA index is generally increased with pasture feeding.[7,47,48]

Intramuscular CLA isomeric profile

Data on the contents (g kg^{-1} muscle and g kg^{-1} fat) and isomeric profile (g kg^{-1} total fatty acids) of CLA in the intramuscular fat of organic meat are presented in Table 2. Total and specific CLA contents in organic beef did not show significant differences ($P > 0.05$) when slaughter seasons were compared. In contrast, the LL muscle had higher total and specific CLA contents than the ST muscle ($P < 0.001$). The results reported here for specific CLA content (2.0–3.2 g kg^{-1} fat) are lower than those reported by Realini *et al*.[48] and Alfaia *et al*.[28] in the LL muscle from grazing beef (5.3 and 5.1 g kg^{-1} fat respectively). However, a direct linear relationship between grass proportion in cattle diet and meat CLA content has been described by French *et al*.,[47] although the mechanism remains controversial.

The slaughter season influenced the content of t11,t13, t11,c13, t10,c12 and t7,c9 CLA isomers in organic beef. In addition, the muscle type had an impact on t11,t13, t11,c13, c9,t11 and t7,c9 CLA isomers. Only three of the 14 CLA isomers analysed were affected by the interaction between slaughter season and muscle type, t9,t11, t8,t10 and c11,t13, as well as the sum of total *trans,trans* CLA isomers. The higher c9,t11 CLA content in the LL muscle of organic beef may be explained by the higher content of 18 : 1t11 in this muscle, which is the substrate of Δ9-desaturase, relative to the ST muscle, as proposed by Daniel *et al*.[49] The CLA isomeric profile showed a clear predominance of the bioactive c9,t11 isomer in both muscle types and slaughter seasons (68–73%). Several authors reported that diets with proportionally high levels of ALA, such as fresh grass, grass silage and concentrate containing linseed, resulted in an increased deposition of the c9,t11 CLA isomer in the muscle.[47,50] The t11,c13 isomer, the second most prevalent CLA isomer in the organic meat reported here, was higher in the meat from spring than in that from autumn. In addition, the t11,t13 (the third most prevalent isomer) and t12,t14 CLA isomers are also sensitive grass intake indicators.[46,50] In line with this, the high content of these isomers in our meat reflects the exposition to abundant availability and quality of pasture from spring-slaughtered animals, relative to that of autumn-slaughtered animals. According to Dannenberger *et al*.,[50] the variations found in CLA isomeric profile might be explained by differences in grass intake, because pasture feeding increases some CLA isomers (the sensitive grass intake indicators) and decreases the t7,c9 CLA isomer in beef lipids. The LL muscle in early autumn had higher contents of the t7,c9 CLA isomer, relative to the other meats, which originates from 18 : 1t7[51] and is frequently recognised as the second most predominant isomer in ruminant fat from concentrate-fed animals.[52] All other CLA isomers are presumed to arise from ruminal microflora by biohydrogenation.[51] The other bioactive CLA isomer, t10,c12, appeared in residual amounts in all meats (<2.6%). This isomer was influenced by the slaughter season, displaying higher proportions in meat from early autumn

Table 2. Total (g kg^{-1} muscle) and specific (g kg^{-1} fat) conjugated linoleic acid (CLA) contents and its individual isomers (g kg^{-1} total fatty acids) in *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles of organic meat from late spring and early autumn

	Spring		Autumn			Significance level		
Component	LL	ST	LL	ST	SEM	Season	Muscle	S × M
Total CLA	0.02	0.01	0.02	0.01	0.002	NS	***	NS
Specific CLA	3.18	1.97	2.77	2.09	0.333	NS	***	NS
CLA isomers								
t12,t14	0.10	0.06	0.06	0.04	0.018	NS	NS	NS
t11,t13	0.30	0.25	0.20	0.12	0.028	**	*	NS
t10,t12	0.04	0.04	0.05	0.06	0.008	NS	NS	NS
t9,t11	0.24b	0.29a	0.19b	0.07c	0.016	***	NS	***
t8,t10	0.05b	0.04b	0.11a	0.03b	0.009	*	***	***
t7,t9	0.05	0.04	0.04	0.04	0.008	NS	NS	NS
t6,t8	0.04	0.03	0.02	0.02	0.006	NS	NS	NS
Total *trans,trans*	0.81a	0.74a	0.66a	0.37b	0.049	***	NS	*
c/t12,14	0.03	0.02	0.04	0.05	0.009	NS	NS	NS
t11,c13	0.38	0.30	0.29	0.21	0.025	**	***	NS
c11,t13	0.08a	0.07a	0.04b	0.07a	0.006	NS	NS	**
t10,c12	0.07	0.06	0.11	0.10	0.011	***	NS	NS
c9,t11	3.61	2.81	3.32	2.81	0.221	NS	***	NS
t7,c9	0.13	0.10	0.26	0.17	0.021	***	**	NS
Total *cis/trans*	4.29	3.36	4.07	3.41	0.251	NS	**	NS
Total *cis,cis* (c9,c11)	0.03	0.04	0.03	0.05	0.005	NS	NS	NS

Least square means in the same row with different letters are significantly different ($P < 0.05$). SEM, standard error of mean. S × M, interaction between slaughter season and muscle type. Significance: NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

relative to that from late spring, which is in agreement with the results described by Alfaia *et al*.[15]

Contents of cholesterol, tocopherols and β-carotene in meat

Data on the contents of total cholesterol, α-tocopherol and β-carotene in organic meat are presented in Table 3. Total cholesterol content was higher in meat from spring-slaughtered young bulls than in that from autumn-slaughtered animals ($P < 0.001$). This effect is in line with results reported by Alfaia *et al*.[53] In contrast, the muscle type had no influence on total cholesterol contents. The levels of total cholesterol in organic beef (0.36–0.42 g kg^{-1} muscle) were similar to those found by Muchenje *et al*.[33] in beef raised on natural pasture (0.37–0.42 g kg^{-1} muscle). However, Leheska *et al*.[40] found a higher value of cholesterol (0.57 g kg^{-1} muscle) in strip steak from grass-fed animals, in contrast to Padre *et al*.,[54] who reported lower levels (0.46–0.48 g kg^{-1} muscle) in beef from pasture-based production systems.

The slaughter season and muscle type did not affect ($P > 0.05$) α-tocopherol contents in organic meat. The levels of α-tocopherol in beef, ranging from 5.28 to 5.48 mg kg^{-1} muscle, were higher than the values reported by Prates *et al*.[24] (3.30–3.90 mg kg^{-1} muscle), Costa *et al*.[55] (1.30–2.80 mg kg^{-1} muscle) and, more recently, Röhrle *et al*.[56] (2.43–2.63 mg kg^{-1} muscle). It was reported by some authors that pasture feeding increases the accumulation of α-tocopherol in the muscle tissue owing to the higher levels of this lipid-soluble antioxidant vitamin in grass relative to concentrate feeds.[57,58]

β-Carotene content was only influenced by the slaughter season, with higher levels in organic beef from late spring than in that from early autumn. The contents of β-carotene, depending on the muscle and slaughter season considered (0.06–0.10 mg kg^{-1}), reached the lower limit described for meat from cattle grazed on green pastures (0.09–0.22 mg kg^{-1}), which are naturally rich in tocopherols and carotenoids.[58]

Table 3. Total cholesterol (g kg^{-1} muscle) and lipid-soluble antioxidant vitamins α-tocopherol and β-carotene (mg kg^{-1} muscle) in *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles of organic beef from late spring and early autumn

	Spring		Autumn			Significance level		
Component	LL	ST	LL	ST	SEM	Season	Muscle	S × M
Total cholesterol	0.37	0.36	0.41	0.42	0.013	***	NS	NS
α-Tocopherol	5.28	5.43	5.45	5.48	0.530	NS	NS	NS
β-Carotene	0.10	0.09	0.09	0.06	0.010	**	NS	NS

SEM, standard error of mean. S × M, interaction between slaughter season and muscle type. Significance: NS, not significant ($P > 0.05$); ** $P < 0.01$; *** $P < 0.001$.

Nutritional quality of intramuscular fat

A daily consumption of 150 g of steak from the organic meat reported here, trimmed of all visible fat with the exception of intramuscular fat, provides 54–62 mg of cholesterol, 0.8 mg of α-tocopherol and 8.5–14.5 µg of β-carotene. These values represent 18–21% of the daily maximum recommended for cholesterol ($<$300 mg day^{-1}),[59] 5.3–5.5% of the recommended dietary allowance for α-tocopherol (15 mg day^{-1}) and only 1.2–2.1%/1.0–1.6% (women/men) of the recommended dietary allowance for β-carotene (700/900 µg day^{-1} for women/men).[60]

The European Food Safety Authority (EFSA)[61] proposed not to set any dietary value of reference or range for CLA intake. However, some extrapolations based on animal studies suggest that 0.8–3.0 g day^{-1} is needed to promote human health benefits.[62] Facing this range, 150 g of the organic beef described here provides only 2.1–3.0 mg of total CLA, i.e. less than 0.4% of the minimum required to reach the beneficial effects. Notwithstanding, the range of values proposed by Ip *et al*.[62] represents only approximate values with potential beneficial effects to humans, because it was based on animal data extrapolation. Hence it should be interpreted with caution until experimental human consistent findings are available.[63]

Regarding the n-3 PUFA, the EFSA[60] proposed an intake of 250 mg day^{-1} of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) for primary prevention of cardiovascular disease. This organic meat (150 g of steak) provides 21.1–29.7 mg of EPA plus DHA, thus representing from 8.5 to 11.9% of the recommended daily intake for EPA plus DHA.

CONCLUSION

The data indicate that organic beef is a lean meat with seasonal differences in the levels of some fatty acids (12 in a total of 41), CLA isomers (four in a total of 14), n-6/n-3 PUFA ratio, total cholesterol and β-carotene. In addition, significant differences were obtained between LL (relatively red) and ST (relatively white) muscles for 21 fatty acids, specific CLA content, four CLA isomers and both PUFA/SFA and n-6/n-3 ratios. In spite of the seasonal and carcass variations, organic beef seems to have values of meat similar to pasture-fed cattle, owing to the contents of some individual fatty acids and CLA isomers (sensitive grass intake indicators) and to the contents of lipid-soluble antioxidant vitamins (α-tocopherol and β-carotene).

From a nutritional point of view, organic meat from both slaughter seasons and muscles has health-related parameters, since the content of the c9,t11 CLA isomer is high and the PUFA/SFA and n-6/n-3 indices are within the recommended values for the human diet. Based on the synergistic antioxidant effect between α-tocopherol and β-carotene, these findings suggest that organic beef seems to have high stability towards lipid oxidation. Taken together, the data indicate that organic meat intramuscular fat, as a result of the beneficial effects of grass feeding on the characteristics of the meat lipids, has high nutritional quality throughout the year.

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