Animal (2011), **5:10**, pp 1643–1652 © The Animal Consortium 2011
doi:10.1017/S1751731111000607

Length of concentrate finishing affects the fatty acid composition of grass-fed and genetically lean beef: an emphasis on *trans*-18:1 and conjugated linoleic acid profiles

N. Aldai[1†], M. E. R. Dugan[1], J. K. G. Kramer[2], A. Martínez[3], O. López-Campos[1], A. R. Mantecón[4] and K. Osoro[3]

[1]*Lacombe Research Centre, AAFC, 6000 C&E Trail, Lacombe T4L 1W1, Alberta, Canada;* [2]*Guelph Food Research Centre, AAFC, Guelph N1G 5C9, Ontario, Canada;* [3]*Sistemas de Producción Animal, SERIDA, Apdo. 13 – 33300 Villaviciosa, Asturias, Spain;* [4]*Instituto de Ganadería de Montaña, CSIC-ULE, Finca Marzanas, 24346 Grulleros, León, Spain*

(Received 12 November 2010; Accepted 18 March 2011; First published online 26 April 2011)

Intensively finishing cattle on a high-grain diet is generally used to enhance marbling, whereas extensively finishing on grass is known to provide improved muscle fatty acid profiles. The objective of this study was to evaluate to what extent intensive concentrate finishing (0, 1 or 2 months) can be combined with forage feeding without negatively affecting the fatty acid profile of genetically lean animals. Bulls from the 'Asturiana de los Valles' breed were reared under grazing conditions with/without final finishing on a barley-based concentrate: 0 months (control; n *= 7), 1 month (*n *= 10) and 2 months (*n *= 7). Yearling bulls were slaughtered commercially at an average live weight of 516 ± 9.8 kg. Increasing the finishing time on concentrate significantly increased the saturated and monounsaturated fatty acids, whereas polyunsaturated fatty acids (PUFAs) tended to decrease and it was not possible to increase the long-chain PUFA content in muscle tissue of this breed. An increase was observed for total* trans*-18:1 (average 5.5% with grain* v. *3.7% for grass). The 11*t*-18:1/10*t*-18:1 ratio was significantly higher in grass-fed (average 8.1) compared with grain-finished animals (average 1.1). Grass or limited concentrate finishing reduced the n-6/n-3 ratio in muscle tissue (average 3.6 for 0 and 1 month, and 4.9 for 2 months on grain finishing). The beef was within or close to the recommended values for human consumption (i.e. polyunsaturated/saturated > 0.45, n-6/n-3 < 4.0), and total* trans*-FA content was low. However, finishing increased the content of undesirable* trans*-18:1 and conjugated linoleic acid isomers, particularly after 2 months, whereas grass finishing was judged to provide a healthier beef fatty acid profile.*

Keywords: beef, CLA, concentrate, grass, vaccenic acid

Implications

Diet is one of the most important factors influencing fat content and fatty acid composition in beef. Pasture-fed beef offers lean meat with a more desirable fatty acid composition for human consumption (n-3, conjugated linoleic acids (CLAs)). However, extensive feeding can be quite compromising in double-muscled animals due to their inherent low fat content. In order to deposit some carcass and intramuscular fat, these animals are typically finished on concentrate for few months before slaughter. However, results indicate that finishing has a major impact on the quality and the content of *trans*-18:1 and CLA isomer composition, which was negatively affected mainly with a longer period (2 months) of concentrate finishing.

Introduction

Beef fat content and composition depend primarily on breed and feeding (Rule *et al.*, 1995; Aldai *et al.*, 2006). For instance, in the 'Asturiana de los Valles' breed from northern Spain, the frequency of animals exhibiting doubled muscling has increased over the last decade to the point now where most of the animals of this breed are genetically homozygous (*mh*/*mh*) or heterozygous (*mh*/+) for the gene responsible for muscular hypertrophy (Grobet *et al.*, 1998; Pérez, 2005). Consequently, the beef obtained is very lean and the fatty acids profile can be affected (Aldai *et al.*, 2007a and 2007b). A high fat content in muscle is associated with

† Present address: Instituto de Ganadería de Montaña, CSIC-ULE, Finca Marzanas, 24346 Grulleros, León, Spain. E-mail: naldai@eae.csic.es; noeliaaldai@hotmail.com

increased neutral lipids (triacylglycerides), whereas phospholipid levels remained relatively constant (Rule *et al*., 1995).

Grain-based diets contain relatively high levels of linoleic acid, which can lead to unfavourable n-6/n-3 ratios (Aldai *et al*., 2008b) and *trans*-18:1 profiles (Aldai *et al*., 2008b; Kraft *et al*., 2008) in beef. The 10*trans* (*t*)-18:1 accumulates in intensively or concentrate-finished beef (Purchas *et al*., 2005; Leheska *et al*., 2008) and this has been associated with increased atherogenicity in animal models (Bauchart *et al*., 2007; Roy *et al*., 2007). Forages are good sources of linolenic acid that leads to an enhanced n-3 polyunsaturated fatty acid (PUFA) content in beef products with a more desirable fatty acid composition for human health (Gleissman *et al*., 2010). Moreover, rumenic acid (RA, 9*cis* (*c*), 11*t*-18:2) and its precursor vaccenic acid (VA, 11*t*-18:1) have been found to accumulate in forage-finished ruminants (Dugan *et al*., 2007; Kraft *et al*., 2008; Alfaia *et al*., 2009) and their consumption has been linked to a number of potential health benefits (Bauchart *et al*., 2007; Field *et al*., 2009; Bassett *et al*., 2010).

Designing diets and production systems for lean breeds (i.e. double-muscle animals) poses a challenge in terms of their fat content and composition. Extensive production systems (i.e. forage feeding) can be positive for beef fatty acid composition, but a low fat level can lead to a negative classification of carcasses based on the European carcass classification system (Commission Regulation, 1981; Council Regulation, 1981). Therefore, it is necessary for cattle with high lean growth potential (see review by Arthur, 1995) to be fattened on high-energy concentrate diets in order to deposit sufficient carcass and marbling fat (Nuernberg *et al*., 2005; Martínez *et al*., 2010). Thus, the objective of the present study was to evaluate the length of intensive concentrate finishing (0, 1 or 2 months) that should be combined with forage feeding to produce a carcass with sufficient fat to pass the European classification rating and still retain a fatty acid profile with a positive health image.

Material and methods

Animals and management

Twenty-four spring-born suckling male calves from 'Asturiana de los Valles' Spanish beef breed, heterozygous for the gene responsible for muscular hypertrophy (*mh*/+; Grobet *et al*., 1998), were reared with their mothers under grazing conditions until weaning in November. The average live weight of calves at weaning was 254 ± 9.5 kg and they were reared extensively until they reached 472 ± 13 kg. Pastures were mainly composed of ryegrass (*Lolium perenne*) and clover (*Trifolium repens*), and during winter when grass availability was low, animals were confined and fed hay. From mid-May, some bulls continued under extensive management (pasture grazing without concentrate; control, $n = 7$), whereas the remaining bulls were finished on a barley-based concentrate for either one (1 mo; $n = 10$) or 2 months (2 mo; $n = 7$) in the housing facilities of the SERIDA Research Institute (Asturias, northern Spain). The transition from grass to concentrate was performed over a 4 to 5-week period after which concentrate (84% barley meal, 10% soya meal, 3% vegetable oil and 3% supplement composed of a mineral/vitamin/oligoelement premix) and barley straw were fed *ad libitum.* The 4 to 5-week transition period from grass to concentrate was introduced to extend the production system *per se* as the main objective was to maximize the utilization of natural resources. During this transition period, animals were provided with 1.5 to 2 kg of concentrate per head per day. The proximate chemical and fatty acid composition of the concentrate was previously reported by Aldai *et al*. (2007b), and grass composition was indicative of its good quality (22.9% protein, 27.2% ADF, 38.3% NDF and 2.47 Mcal/kg of metabolizable energy on dry matter basis).

Yearling bulls were slaughtered commercially at an average live weight of 516 ± 9.8 kg.

Sample collection

Grass and concentrate were sampled every 2 months and 3 weeks, respectively. Samples were freeze-dried and ground through a 1 mm screen. Fatty acid methyl esters (FAMEs) were prepared according to Sukhija and Palmquist (1988) and analysed according to Dugan *et al*. (2007).

After slaughter, carcasses were chilled for 24 h at 3°C and left rib joints from the sixth to the ninth ribs were removed and transported to the laboratory. A *longissimus thoracis* steak was dissected from the eighth rib, vacuum packed and frozen at −80°C for subsequent fatty acid analyses.

Fatty acid composition of muscle

Lipids were extracted from 1 g of freeze-dried muscle using a mixture of chloroform–methanol (1:1, v/v) (Kramer *et al*., 1998). Lipid aliquots (10 mg) from each muscle sample were methylated separately using acidic (methanolic HCl) and basic (sodium methoxide) reagents (Kramer *et al*., 2008). Internal standard (1 ml of 1 mg 23:0 methyl ester per mL toluene) was added before the addition of the methylating reagent. The FAMEs were analysed using GLC and Ag^+-HPLC equipment and methods outlined by Cruz-Hernandez *et al*. (2004). *Trans*-18:1 isomers were analysed using two complementary GLC temperature programmes (Kramer *et al*., 2008). In representative samples, silver-ion solid phase extraction (Ag^+-SPE) cartridges were used to separate fractions of saturated FAMEs, mono-*trans* FAMEs plus the *t*/*t* isomers of conjugated linoleic acid (CLA), mono-*cis* FAMEs plus the *c*/*t* isomers of CLA, dienoic FAMEs, trienoic FAMEs and tetraenoic FAMEs to confirm peak identification (Kramer *et al*., 2008). Reference standards 463, 603 and U-59 from Nu-Chek Prep Inc. (Elysian, MN, USA) were used. Phytanic acid was obtained from Matreya Inc. (Pleasant Gap, PA, USA). The UC-59 standard contained all possible geometric and positional isomers of 8,10-CLA, 9,11-CLA, 10,12-CLA and 11,13-CLA. Branched-chain fatty acids (BCFA) were identified using GLC reference standard BC-Mix1, purchased from Applied Science (State College, PA, USA). The *trans*-18:1, CLA and other diene isomers not included in standard mixtures were identified by their retention times and elution

orders as reported elsewhere (Cruz-Hernandez *et al*., 2004 and 2006; Kramer *et al*., 2008; Rego *et al*., 2009). The FAMEs were quantified using chromatographic peak area and internal standard (23:0 ME)-based calculations. The contents of FAME were expressed on an mg per 100 g of fresh meat and on the % of total FAME basis.

Statistical analysis

Individual, groups and ratios of FAME from *longissimus thoracis* muscle were analysed as a one-way ANOVA using the PROC MIXED procedure of SAS (SAS Institute, 2001) with concentrate finishing (0, 1 or 2 months) as the main effect. The LSMEANS and PDIFF options were used to generate least square means and to compare treatments (*F*-test protected least significant difference). Significance was declared at $P < 0.05$.

Results

The lipid content (measured as total FAME) of the two feedstuffs utilized in the present study were 21.9 and 53.9 mg/g of dry matter for grass and concentrate, respectively (Table 1). In both, major fatty acids representing >90% of the total FAME included palmitic (16:0), stearic (18:0), oleic (9*c*-18:1), linoleic (18:2n-6) and linolenic (18:3n-3) acids. The percentage of 16:0 was similar in grass and concentrate. Grass samples were lower in 18:0, 9*c*-18:1 and 18:2n-6, but clearly higher in 18:3n-3.

Table 1 *Total FAME and fatty acid composition (mean and standard error of the mean) of grass and concentrate samples*

Feed composition	Grass ($n = 10$)		Concentrate[1] ($n = 4$)	
FAME (mg/g DM)	21.90	2.012	53.88	0.989
Fatty acid (FAME, %)				
12:0	0.070	0.004	0.012	0.003
14:0	0.252	0.010	0.176	0.003
15:0	0.107	0.010	0.047	0.002
16:0	12.81	0.923	15.32	0.081
7*c*-16:1	2.539	0.047	0.044	0.005
9*c*-16:1	0.153	0.004	0.106	0.003
17:0	0.150	0.016	0.097	0.001
9*c*-17:1	0.042	0.004	0.050	0.001
18:0	1.443	0.206	3.191	0.011
9*c*-18:1	1.875	0.167	19.82	0.032
11*c*-18:1	0.317	0.020	1.140	0.004
18:2n-6	13.80	0.367	52.58	0.174
20:0	0.324	0.053	0.334	0.002
9*c*-20:1	0.066	0.018	0.031	0.001
11*c*-20:1	0.047	0.002	0.385	0.003
18:3n-3	61.61	2.322	5.896	0.083
20:2n-6	0.048	0.004	0.061	0.001
22:0	0.487	0.098	0.358	0.004
13*c*-22:1	0.021	0.003	0.044	0.003
24:0	0.570	0.136	0.212	0.009
15*c*-24:1	0.019	0.003	0.030	0.002

FAME = fatty acid methyl ester; DM = dry matter.
FAME > 0.02% was reported.
[1]The barley-based concentrate consisted of 84% barley meal, 10% soya meal, 3% fat and 3% supplement composed of a mineral/vitamin/oligoelement premix.

Longissimus muscle from bulls fed grass only had lower total FAME contents than from bulls finished on concentrate for 2 months (0.5% and 1.0%, respectively; $P < 0.05$), whereas bulls finished on concentrate for 1 month had intermediate FAME levels (0.8%; Table 2).

Saturated and BCFA, and plasmalogenic lipids

Muscle from bulls finished on concentrate for 2 months had the highest total saturated fatty acid (SFA) content (448 mg/100 g of meat; $P < 0.05$; see summary Table 2). Bulls finished for 1 month on concentrate had an intermediate level (316 mg/100 g of meat), whereas bulls finished on grass had the lowest content (197 mg/100 g of meat). On a percentage basis, the trend was similar ($P < 0.05$) with 41% SFA after 2 months, 38% after 1 month and 36% when pasture fed (Table 3). With regard to individual SFAs, percentages of 12:0, 14:0 and 16:0 were higher ($P < 0.05$) in meat from concentrate-finished bulls, whereas longer chain SFAs (21:0, 22:0) were higher ($P < 0.05$) in grass-finished bulls. Finishing strategy did not influence the percentage of BCFA (individual or total; $P > 0.05$).

Finishing affected the percentages of plasmalogenic lipids, identified as their dimethylacetals (DMA) after methylation, and their fragmentation products produced during GLC analysis (alk-1-enyl methyl ethers, AME). Plasmalogenic

Table 2 *Total FAME content (mg/100 g meat) and summary of fatty acids of nutritional interest (mg/100 g meat) and ratios of essential fatty acids from* longissimus thoracis *of bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing*

Fatty acids (mg/100 g)	Control	1 mo	2 mo	s.e.m.	*P*-value
∑FAME	547.0^{b}	812.6^{ab}	1055^{a}	113.7	0.027
∑SFA	197.2^{b}	316.4^{ab}	447.6^{a}	56.32	0.028
∑*cis*-MUFA	126.4	211.8	309.8	25.330	0.059
∑*trans*-MUFA	24.51	55.07	62.80	6.06	0.053
10*t*-18:1	1.637^{b}	23.86^{a}	22.80^{a}	5.324	0.015
11*t*-18:1	14.20	16.26	20.54	5.344	0.713
11*t*-18:1/10*t*-18:1	8.134^{a}	0.780^{b}	1.299^{b}	0.491	<0.001
∑MUFA	156.4	273.4	355.9	52.73	0.057
∑*trans*-FA	26.75	60.95	66.10	10.21	0.053
∑PUFA	130.5	155.3	158.1	9.153	0.104
∑n-6 PUFA	99.24^{b}	123.9^{a}	130.6^{a}	7.781	0.031
18:2n-6	76.52^{b}	95.25^{ab}	103.3^{a}	6.538	0.033
∑n-3 PUFA	31.23	31.45	27.45	2.290	0.396
18:3n-3	18.20^{a}	16.32^{a}	12.49^{b}	1.348	0.026
∑n-6 HUFA	22.99	28.65	26.88	1.200	0.162
∑n-3 HUFA	13.04	15.12	15.00	0.742	0.463
n-6/n-3 PUFA	3.292^{b}	4.010^{ab}	4.872^{a}	0.331	0.014
n-6/n-3 HUFA	1.862	1.906	1.822	0.069	0.879
P/S	0.704	0.604	0.414	0.048	0.081
∑CLA	2.838	3.637	5.326	0.984	0.233
9*c*,11*t*-18:2	1.857	2.167	3.492	0.766	0.311

FAME = fatty acid methyl ester; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; HUFA = highly unsaturated fatty acid; P/S = polysaturated/saturated; CLA = conjugated linoleic acid.
Within a row, means without a common superscript differ ($P < 0.05$).
See Tables 3, 4, 5, 6 and 7 for grouping explanations.

Table 3 *SFA and DMA composition (percentages) of* longissimus thoracis *from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing*

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	*P*-value
12:0	0.014[b]	0.022[a]	0.024[a]	0.003	0.046
13:0	0.015	0.012	0.008	0.002	0.131
14:0	0.604[b]	1.086[a]	1.358[a]	0.146	0.008
15:0	0.389	0.360	0.343	0.030	0.598
16:0	15.82[c]	18.37[b]	20.97[a]	0.777	0.001
17:0	0.885	0.902	0.950	0.035	0.499
18:0	15.85	14.67	15.31	0.572	0.356
19:0	0.179	0.167	0.162	0.026	0.898
20:0	0.108	0.095	0.086	0.006	0.083
21:0	0.037[a]	0.016[b]	0.015[b]	0.003	<0.001
22:0	0.251[a]	0.211[ab]	0.169[b]	0.020	0.039
24:0	0.127	0.113	0.090	0.013	0.186
*iso*14:0	0.025	0.033	0.029	0.004	0.411
*iso*15:0	0.114	0.096	0.097	0.013	0.538
*anteiso*15:0	0.179	0.183	0.171	0.019	0.899
*iso*16:0	0.209	0.183	0.181	0.015	0.376
*iso*17:0	0.460	0.438	0.367	0.015	0.059
*anteiso*17:0	0.422	0.454	0.453	0.038	0.814
*iso*18:0[U]	0.120	0.132	0.132	0.009	0.598
15:0DMA	0.100	0.077	0.052	0.007	0.053
AME1	0.095	0.119	0.094	0.015	0.359
AME2	0.105	0.083	0.064	0.013	0.105
16:0DMA	4.498	3.585	2.955	0.445	0.085
7*c*-16:1DMA	0.105[a]	0.072[b]	0.060[b]	0.006	0.030
9*c*-16:1DMA	0.222[a]	0.165[ab]	0.111[b]	0.012	0.009
17:0DMA	0.425	0.325	0.276	0.023	0.060
18:0DMA[T]	3.902[a]	2.562[b]	2.030[b]	0.340	0.004
10*t*-18:1DMA	0.080	0.166	0.152	0.016	0.077
11*t*-18:1DMA	0.369[a]	0.221[b]	0.185[b]	0.019	0.002
9*c*-18:1DMA	0.725[a]	0.522[ab]	0.380[b]	0.042	0.015
11*c*-18:1DMA	0.078[a]	0.062[ab]	0.046[b]	0.005	0.047
Pristanate (2*R*,6*R*,10*R*,14)	0.386[a]	0.288a[b]	0.186[b]	0.020	0.004
Phytanate1 (3*R*,7*R*,11*R*,15)	0.049	0.051	0.053	0.004	0.885
Phytanate2 (3*S*,7*R*,11*R*,15)	0.092[a]	0.059[b]	0.038[c]	0.004	<0.001
17:0-cyclo[V]	0.065	0.075	0.055	0.007	0.099
∑SFA	36.19[b]	37.71[ab]	41.33[a]	1.359	0.040
∑BCFA	1.526	1.514	1.432	0.101	0.811
∑AME	0.199	0.201	0.147	0.014	0.253
∑DMA	10.46[a]	7.731[b]	5.832[b]	0.840	0.012
11*t*-18:1/10*t*-18:1DMA	5.37[a]	1.43[b]	1.78[b]	0.29	<0.001

SFA = saturated fatty acid; BCFA = branched-chain fatty acid; AME = alk-1-enyl methyl ethers; DMA = dimethyl acetal.
Within a row, means without a common superscript differ ($P < 0.05$).
[T]Coelution with 13*c*-16:1.
[U]Coelution with 9*t*-18:1DMA.
[V]Cyclohexylundecanoic acid.

Table 4 Cis-*MUFA composition (percentages) of* longissimus thoracis *from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing*

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	*P*-value
9*c*-14:1	0.063[b]	0.115[ab]	0.171[a]	0.026	0.036
7*c*-16:1[W]	0.228[a]	0.213[ab]	0.194[b]	0.009	0.046
9*c*-16:1	0.804[b]	1.293[a]	1.546[a]	0.115	0.002
11*c*-16:1	0.056	0.055	0.064	0.007	0.646
12*c*-16:1	0.029	0.026	0.021	0.004	0.383
7*c*-17:1[X]	0.138[a]	0.107[b]	0.068[c]	0.010	0.001
9*c*-17:1	0.359[b]	0.507[a]	0.513[a]	0.060	0.023
9*c*-18:1[Y]	18.85	20.15	23.17	1.375	0.110
11*c*-18:1	1.435	1.340	1.281	0.059	0.232
12*c*-18:1	0.161[c]	0.421[a]	0.282[b]	0.039	<0.001
13*c*-18:1	0.083[b]	0.105[ab]	0.143[a]	0.014	0.022
14*c*-18:1	0.055	0.064	0.052	0.005	0.245
15*c*-18:1	0.058[b]	0.110[a]	0.101[a]	0.010	0.005
9*c*-20:1	0.058	0.060	0.062	0.004	0.821
11*c*-20:1	0.059[b]	0.088[a]	0.090[a]	0.008	0.033
13*c*-22:1	0.032	0.028	0.020	0.004	0.230
15*c*-24:1	0.028	0.050	0.040	0.006	0.081
∑*cis*-16:1	1.115[b]	1.531[a]	1.800[a]	0.120	0.006
∑*cis*-18:1	20.64	22.18	25.03	1.352	0.103
∑*cis*-MUFA	22.40[b]	24.59[ab]	28.43[a]	1.407	0.042
∑MUFA	27.88	31.98	34.27	1.697	0.056

MUFA = monounsaturated fatty acid; DMA = dimethyl acetal.
Within a row, means without a common superscript differ ($P < 0.05$).
[W]Coelution with 9*c*-17:1DMA.
[X]Coelution with 6*d*7*d*8*c*-18:1DMA.
[Y]Coelution with 10*c*-18:1.

lipids were greater in grass-finished beef (10.5%; $P < 0.05$) and lowest in beef finished on concentrate for 2 months (5.8%), whereas the meat from animals finished on concentrate for 1 month had intermediate values (7.7%). The two major *trans*-18:1 DMA moieties in meat lipids were 10*t*-18:1 and 11*t*-18:1DMA, with 10*t*-18:1 increasing significantly in its relative percentage with increased length of feeding concentrate, whereas 11*t*-18:1 decreased significantly. The 11*t*-18:1/10*t*-18:1DMA ratio significantly decreased from approximately 5.4 to 1.5 with the feeding of concentrate (Table 3).

Significantly higher percentages of pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) were found in meat from grass-fed bulls compared with concentrate-fed bulls and the same trend ($P < 0.001$) was observed for the phytanic acid isomer (3*S*,7*R*,11*R*,15-tetramethylhexadecanoic acid), whereas no differences were detected for the 3*R*,7*R*,11*R*,15 isomer (Table 3).

Cis-*monounsaturated fatty acids*

Total *cis*-monounsaturated fatty acids (MUFA) tended to be higher in meat from concentrate-finished than in grass-finished bulls (310 mg/100 g meat or 28% for 2 months concentrate finished; 212 mg/100 g of meat or 25% in 1 month concentrate finished; 126 mg/100 g of meat or 22% in grass finished; $P = 0.06$; Tables 2 and 4). On a percentage basis, 9*c*-16:1, 9*c*-18:1, and several other minor *cis*-MUFA isomers appeared to be higher in meat of animals fed concentrate.

Trans-*monounsaturated fatty acids*

Total *trans*-MUFA (mg/100 g meat) tended to be higher ($P = 0.053$; Table 2) in concentrate-fed bulls and this was

Table 5 Trans-*MUFA composition (percentages) of* longissimus thoracis *from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing*

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	*P*-value
6*t*/7*t*-16:1	0.032	0.024	0.023	0.009	0.131
8*t*-16:1	0.021b	0.112a	0.057b	0.008	0.001
9*t*-16:1	0.429^{b}	0.305^{a}	0.187^{a}	0.030	0.004
10*t*-16:1	0.017	0.017	0.015	0.002	0.553
11*t*/12*t*-16:1	0.035^{ab}	0.037^{a}	0.026^{b}	0.003	0.038
4*t*-18:1	0.017	0.020	0.014	0.003	0.518
5*t*-18:1	0.014^{b}	0.024^{a}	0.016^{ab}	0.003	0.036
6*t*/7*t*/8*t*-18:1	0.075^{b}	0.146^{a}	0.172^{a}	0.017	0.002
9*t*-18:1	0.148^{c}	0.208^{b}	0.275^{a}	0.015	$<$0.001
10*t*-18:1	0.291^{b}	2.824^{a}	2.280^{a}	0.422	0.001
11*t*-18:1	2.410	1.756	1.841	0.335	0.364
12*t*-18:1	0.125	0.171	0.155	0.019	0.250
13*t*/14*t*-18:1^{Z}	0.360	0.436	0.352	0.038	0.214
15*t*-18:1	0.098	0.118	0.117	0.014	0.524
16*t*-18:1	0.133	0.123	0.094	0.017	0.276
11*t*-20:1	0.051	0.045	0.026	0.008	0.095
13*t*-20:1	0.010	0.017	0.013	0.003	0.293
$\sum$*trans*-16:1	0.533^{a}	0.496^{a}	0.308^{b}	0.031	0.007
$\sum$*trans*-18:1	3.671^{b}	5.826^{a}	5.316^{a}	0.509	0.020
$\sum$*trans*-MUFA	4.249^{b}	6.363^{a}	5.747^{ab}	0.509	0.024
$\sum$*trans*-FA	5.001^{b}	7.061^{a}	6.371^{a}	0.509	0.013
11*t*-18:1/10*t*-18:1	8.134^{a}	0.780^{b}	1.299^{b}	0.491	$<$0.001

MUFA = monounsaturated fatty acids; CLA = conjugated linoleic acid.
Within a row, means without a common superscript differ ($P < 0.05$).
trans-FA: *trans*-MUFA + *c,c*-dienes/trienes and *c,t*-dienes/trienes, but not CLA.
^{Z}Coelution with 6-8*c*-18:1.

due to higher levels of several individual *trans*-18:1 isomers of which 10*t*-18:1 predominated. The second most abundant *trans*-18:1 isomer was VA, but it was not found to be different across treatments. On a percentage basis, total *trans*-MUFA and total *trans*-18:1 levels were significantly higher in meat from concentrate compared with grass-finished bulls (Table 5), although there was no apparent trend related to the length of concentrate feeding. The percentage of total *trans*-16:1 decreased with concentrate finishing.

With regard to individual *trans*-18:1 isomers, 10*t*-18:1 and 11*t*-18:1 together represented 76% of total *trans*-18:1 content. The *longissimus* muscle from bulls finished on grass had a significantly higher 11*t*-18:1/10*t*-18:1 ratio (8.13, $P < 0.001$) compared with concentrate-finished bulls (1.04). When the amounts of the individual *trans*-18:1 isomers were presented as a relative percentage of the total *trans*-18:1 content (Figure 1a), significantly higher relative proportions of 11*t*-18:1, 13t/14*t*-18:1 and 16*t*-18:1 were observed in grass-finished compared with concentrate-finished beef. In contrast, higher relative proportions of 10*t*-18:1 were consistently observed in concentrate-finished beef and this was accompanied with higher levels of 6*t*/7*t*/8*t*-18:1 and 9*t*-18:1.

PUFAs and other dienes

Linoleic acid was the major n-6 PUFA, and it was higher in concentrate-finished beef, especially in meat from animals

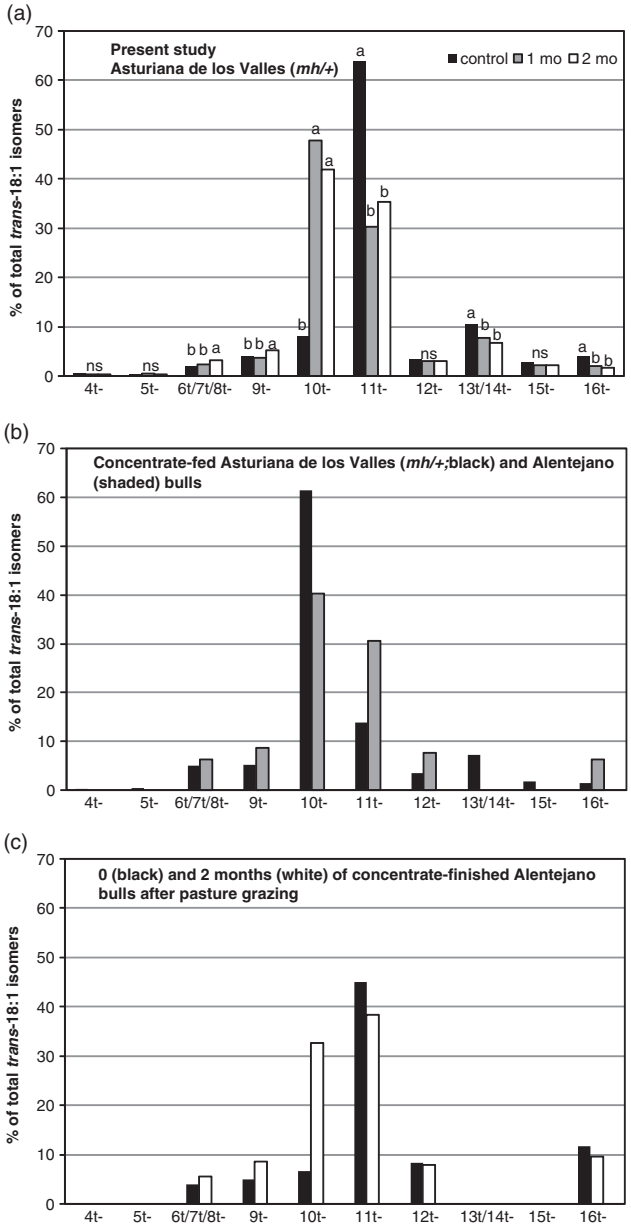

Figure 1 Relative isomeric distribution of individual *trans*-18:1 isomers of *longissimus* muscle from (a) the present study; 0 (control), 1 and 2 months of concentrate finishing after grazing, (b) concentrate-fed 'Asturiana de los Valles' (Aldai *et al.*, 2010a) and Alentejano purebred bulls (Alfaia *et al.*, 2009) and (c) 0 and 2 months of concentrate-finished Alentejano purebred bulls after pasture grazing (Alfaia *et al.*, 2009). Columns without a common superscript differ ($P < 0.05$).

fed concentrate for 2 months (103 mg/100 g of meat, $P < 0.05$; Table 2) compared with grass-finished beef (76.5 mg/100 g of meat). However, in general, the increased 18:2n-6 content did not result in the accumulation of elongation and desaturation products of 18:2n-6 (n-6 highly unsaturated fatty acid (HUFA)). When the data were expressed on a percentage basis, no significant differences were observed between these feeding strategies (Table 6).

Meat from bulls finished on grass or on 1 month of concentrate finishing showed the highest absolute contents of

Table 6 *PUFA composition (percentages) of* longissimus thoracis *from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing*

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	*P*-value
Methylene interrupted					
18:2n-6	14.55	13.05	11.30	1.261	0.239
18:3n-6	0.058	0.062	0.049	0.007	0.391
20:3n-6	0.742	0.656	0.546	0.081	0.283
20:4n-6	3.249	3.199	2.346	0.410	0.242
22:2n-6	0.025	0.020	0.016	0.004	0.246
22:4n-6	0.178	0.172	0.124	0.022	0.199
22:5n-6	0.039^{a}	0.023^{b}	0.014^{b}	0.005	0.013
18:3n-3	3.470^{a}	2.214^{b}	1.340^{c}	0.233	<0.001
20:3n-3	0.045^{a}	0.023^{b}	0.017^{b}	0.002	<0.001
20:5n-3	1.016	0.924	0.731	0.139	0.377
22:5n-3	1.304	1.146	0.887	0.163	0.235
22:6n-3	0.094	0.073	0.075	0.015	0.596
∑PUFA	24.77	21.56	17.45	2.174	0.095
∑n-6 PUFA	18.85	17.18	14.40	1.720	0.227
∑n-3 PUFA	5.929^{a}	4.381^{b}	3.051^{b}	0.524	0.005
∑n-6 HUFA	4.189	4.032	2.833	0.303	0.178
∑n-3 HUFA	2.448	2.157	1.630	0.188	0.252
P/S	0.704	0.604	0.414	0.048	0.081
n-6/n-3 PUFA	3.292^{b}	4.010^{ab}	4.872^{a}	0.331	0.014
n-6/n-3 HUFA	1.862	1.906	1.822	0.069	0.879
Other dienes					
11*t*,15*t*-18:2	0.028^{b}	0.058^{a}	0.045^{ab}	0.007	0.021
9*t*,12*t*-18:2	0.043	0.053	0.038	0.006	0.134
9*c*,13*t*-/8*t*,12*c*-18:2	0.131	0.147	0.129	0.013	0.528
8*t*,13*c*-18:2	0.084	0.082	0.074	0.007	0.563
9*c*,12*t*-18:2	0.085	0.083	0.074	0.005	0.263
c,*t*-18:2unk1	0.054^{b}	0.086^{a}	0.063^{b}	0.007	0.007
9*t*,12*c*-18:2	0.066	0.071	0.067	0.005	0.687
11*t*,15*c*-18:2	0.263	0.308	0.286	0.046	0.789
c,*c*-18:2unk2	0.081^{a}	0.045^{b}	0.043^{b}	0.005	<0.001
9*c*,15*c*-18:2	0.067	0.060	0.078	0.007	0.180
9*c*,11*t*,15*c*-18:3	0.149	0.138	0.098	0.018	0.144
∑*t*,*t*; *c*,*t* & *c*,*c*-dienes	0.738	0.803	0.745	0.052	0.604

PUFA = polyunsaturated fatty acid; HUFA = highly unsaturated fatty acids; P/s = polysaturated/saturated; unk = unknown position of double bonds.
Within a row, means without a common superscript differ ($P < 0.05$).
n-6 HUFA: sum of 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6.
n-3 HUFA: sum of 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3.

linolenic acid (17.3 mg/100 g of meat, $P < 0.05$) compared with bulls finished on concentrate for 2 months (12.5 mg/100 g of meat; Table 2). But again, the increased dietary content of 18:3n-3 did not result in increased levels of n-3 HUFA metabolites. On a percentage basis, 18:3n-3 ($P < 0.001$) and total n-3 PUFA ($P < 0.01$) were significantly higher in grass-finished beef, but did not result in increased levels of n-3 HUFA metabolites (Table 6). As expected, concentrate finishing resulted in a higher n-6/n-3 ratio ($P < 0.05$) compared with grass-finished beef, but this difference was mainly due to the diet differences in the precursor essential fatty acids, 18:2n-6 and 18:3n-3. When these were excluded the n-6 to n-3 HUFA ratio showed no differences among the feeding strategies.

Table 7 *CLA composition (percentages) of* longissimus thoracis *from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing*

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	*P*-value
9*c*,11*t*-18:2	0.306	0.249	0.322	0.050	0.532
7*t*,9*c*-18:2	0.018^{c}	0.029^{b}	0.038^{a}	0.003	<0.001
8*t*,10*c*-18:2	0.013	0.012	0.013	0.002	0.849
9*t*,11*c*-18:2	0.035	0.026	0.030	0.004	0.308
10*t*,12*c*-18:2	0.002^{b}	0.028^{a}	0.019^{a}	0.005	0.009
11*t*,13*c*-18:2	0.037^{a}	0.016^{b}	0.022^{ab}	0.005	0.041
12*t*,14*c*-18:2	0.002	0.002	0.002	0.000	0.853
12*t*,14*t*-18:2	0.008	0.007	0.006	0.001	0.492
11*t*,13*t*-18:2	0.026	0.023	0.017	0.004	0.279
10*t*,12*t*-18:2	0.008	0.007	0.005	0.002	0.708
9*t*,11*t*-18:2	0.009	0.010	0.010	0.001	0.657
8*t*,10*t*-18:2	0.004	0.004	0.003	0.001	0.224
7*t*,9*t*-18:2	0.002	0.002	0.002	0.000	0.754
12*c*,14*t*-18:2	0.001	0.001	0.001	0.000	0.660
11*c*,13*t*-18:2	0.007	0.006	0.006	0.001	0.791
9*c*,11*c*-18:2	0.001^{b}	0.004^{a}	0.004^{ab}	0.001	0.045
∑*t*,*t*-CLA	0.056	0.053	0.044	0.006	0.375
∑*c*,*t*-CLA	0.421	0.369	0.454	0.055	0.530
∑CLA	0.478	0.426	0.501	0.060	0.642

CLA = conjugated linoleic acid.
Within a row, means without a common superscript differ ($P < 0.05$).

A number of *t*/*t*-18:2, *c*/*t*-18:2 and *c*/*c*-18:2 isomers derived either from 18:3n-3 (11*t*,15*t*-18:2, 11*t*,15*c*-18:2, 9*c*,13*t*-18:2 and 9*c*,15*c*-18:2) or from 18:2n-6 (9*t*,12*t*-18:2, 9*c*,12*t*-18:2 and 9*t*,12*c*-18:2), which elute between 19:0 and 20:0 during GLC analysis, were observed but very few differences were observed on a percentage basis (Table 6).

CLAs

RA (9*c*,11*t*-18:2) was the major CLA isomer with an average content of 2.5 mg/100 g of meat across treatments (Table 2) and represented 60% of the total CLA. Most of the remaining 40% was made up of 7*t*,9*c*-18:2, 9*t*,11*c*-18:2 and 11*t*,13*c*-18:2 (Table 7).

Concentrate-finished beef had significantly higher percentages of 7*t*,9*c*-18:2 ($P < 0.001$), 10*t*,12*c*-18:2 ($P < 0.01$) and 9*c*,11*c*-18:2 ($P < 0.05$), whereas grass-finished beef had higher percentages of 11*t*,13*c*-18:2 ($P < 0.05$). When individual CLA isomers were presented on a relative percent basis, the total CLA content of 7*t*,9*c*-18:2, 10*t*,12*c*-18:2 and 9*c*,11*c*-18:2 represented significantly higher proportions in concentrate-finished beef, whereas 11*t*,13*c*-18:2, 12*t*,14*t*-18:2 and 11*t*,13*t*-18:2 represented significantly higher proportions in grass-finished beef (data not shown).

Discussion

The type of animals used in the present study ('Asturiana de los Valles') were very lean as previously reported (Aldai *et al*., 2007a; Martínez *et al*., 2010). This cattle breed has a mutation in the bovine myostatin gene that is responsible for

the double-muscling phenotype (Grobet *et al*., 1998), and animals used in this study were all heterozygous for the presence of the *mh* allele (*mh*/+). The double-muscled syndrome is an inherited condition, and is found in many breeds of cattle. It is also associated with many physical, physiological and histological characteristics, and meat obtained from these animals is reported to be leaner (see review by Arthur, 1995).

The intramuscular fat content was shown to be directly related to the extent of the concentrate-finishing period as reviewed by Wood *et al*. (2008). The accretion of fat in beef animals was reported to be mainly due to increased triacylglycerol, not phospholipids, which would result in higher percentages of SFA and MUFA relative to PUFA in the finished beef (Rule *et al*., 1995). The higher percentages of long-chain SFA observed in grass-finished beef (Table 3) are consistent with previous reports of ruminants (Dugan *et al*., 2007) suggesting that long-chain SFA are associated with higher intake of grass/pasture that is known to contain waxy cuticle rich in long-chain esters compared with grain-based concentrates (Post-Beittenmiller, 1996). Concentrate diets provide, in general, increased availability of oleic and linoleic acids that are major fatty acids in cereal grains (Table 1), which results in more linoleic acid on an absolute basis in the meat from concentrate-finished bulls. In addition, higher contents of metabolites derived from 18:2n-6, such as the methylene-interrupted 18:2 (9*t*,12*t*-18:2, 9*t*,12*c*-18:2), CLA (10*t*,12*c*-18:2) and 18:1 (10*t*-18:1) isomers, were also observed. On the other hand, as noted by Enser *et al*. (1998), pastures are a good source of linolenic acid (18:3n-3; Table 1), which explains the inverse relationship between concentrate-finishing time and muscle levels of 18:3n-3. Recognized metabolites of 18:3n-3, including trienes (9*c*,11*t*,15*c*-18:3), dienes (11*t*,15*c*-18:2, 9*c*,13*t*-18:2 and 9*c*,15*c*-18:2), CLA (11*t*,13*c*-18:2, 11*t*,13*t*-18:2 and 12*t*,14*t*-18:2) and monoenes (13*t*- to 16*t*-18:1), were not different across finishing strategies. These rumen metabolites of PUFA were previously identified in a number of studies (Kraft *et al*., 2003; Cruz-Hernandez *et al*., 2004 and 2006; Destaillats *et al*., 2005; Bessa *et al*., 2007; Gomez-Cortes *et al*., 2009).

Trans-MUFA, and particularly the *trans*-18:1 isomers, are the major intermediates which accumulate during biohydrogenation of PUFA (i.e. 18:2n-6, 18:3n-3; Bessa *et al*., 2000). The significantly higher absolute and relative contents of *trans*-18:1 found in concentrate-finished beef compared with grass-finished beef were mainly due to higher contents of 6*t*/7*t*/8*t*-18:1, 9*t*-18:1 and 10*t*-18:1 as observed by Alfaia *et al*. (2009) and Leheska *et al*. (2008). High contents of 10*t*-18:1 have been observed in tissues of concentrate-fed ruminants (Aldai *et al*., 2008b and 2010b), whereas 11*t*-18:1 has been consistently associated with forage feeding in beef (Bessa *et al*., 2006; Dugan *et al*., 2008; Kraft *et al*., 2008; Alfaia *et al*., 2009; Figure 1). In Figure 1b, the *trans*-18:1 profile of concentrate-fed bulls from two studies (Alfaia *et al*., 2009; Aldai *et al*., 2010a) using different breeds are presented for comparison purposes. In both studies, 10*t*-18:1 was clearly the major isomer. In Figure 1c, the *trans*-18:1 profile of beef from bulls concentrate-finished for 0 and 2 months after pasture grazing is presented (Alfaia *et al*., 2009).

As observed in Table 5, with the exception of 9*t*-18:1, there were no significant differences in *trans* isomers in the meat lipids when comparing concentrate-finished animals (i.e. 1 month compared with 2 months). These results show that certain changes in meat lipid composition were evidently complete after 1 month of feeding. The reason for the increased content of 10*t*-18:1 could be related to a decrease in rumen pH and associated bacteria changes (Harfoot and Hazlewood, 1997), whereas ruminal vitamin E content associated with the grass intake could have also been a potential reason for the improvement of the 11*t*-18:1/10*t*-18:1 ratio (Pottier *et al*., 2006; Juárez *et al*., 2010).

The n-3 content in the meat of grass-fed animals in this study was high in comparison with others (Ponnampalam *et al*., 2006; Kraft *et al*., 2008), whereas the n-6/n-3 was also higher (3.3) than reported by Enser *et al*. (1998; 2.0 to 2.3 for British cattle), Nuernberg *et al*. (2002; 1.3 for German Simmental bulls and Holstein steers) and Alfaia *et al*. (2009; 1.8 for Alentejano purebred). These differences could be in part explained by differences in breed (Raes *et al*., 2003; Aldai *et al*., 2007b; Kraft *et al*., 2008), forage species (Collomb *et al*., 2002; Fraser *et al*., 2009) and/or stage of pasture maturity (Dewhurst *et al*., 2001; Vanhatalo *et al*., 2007), or the relatively high content of 18:2n-6 in the concentrate. The lack of increased desaturation and elongation metabolites from 18:2n-6 (substrate) and 18:3n-3 (competition) in this breed of cattle was surprising. However, it has been recently demonstrated that the expression of some of the genes involved in lipid metabolism is inhibited in the *mh*/*mh* genotype of 'Asturiana de los Valles' (Perez *et al*., 2010), which might also apply to the *mh*/+ genotype. The lack of response could also be due to the limited length of feeding (60 days in this study). Duckett *et al*. (1993) started to observe changes in meat lipids of Angus × Hereford steers after 56 days of concentrate feeding. Furthermore, an alternate explanation could reflect low enzyme activity (Δ^9-desaturase and elongase enzymes) in this particular genotype supported by previous observations of Aldai *et al*. (2008a). Similar results of decreased desaturation and elongation of the essential fatty acids were observed in the Limousine breed fed concentrate diets for a much longer period (Kraft *et al*., 2008). The overall low CLA content is also evidence of low Δ^9-desaturase activity within the muscle. Across treatments, there were slight differences in some of the individual CLA isomers, similar to those observed by Dannenberger *et al*. (2005) in beef and Dugan *et al*. (2007) in muskox, where 11*t*,13*c*-18:2 was the second most abundant isomer in grass-finished, and 9*t*,11*c*-18:2 and 10*t*,12*c*-18:2 in concentrate-fed animals. The 11*t*,13*c*-18:2 isomer is a metabolite of 18:3n-3, which has been linked to the isomerization of 11*t*,15*c*-18:2 (Fukuda *et al*., 2009).

The plasmalogens in the beef intramuscular fat are seldom discussed, mainly because total beef lipids are methylated either using base catalysts to avoid isomerization of CLA (Kramer *et al*., 1997) that does not break the alk-1-enyl bond

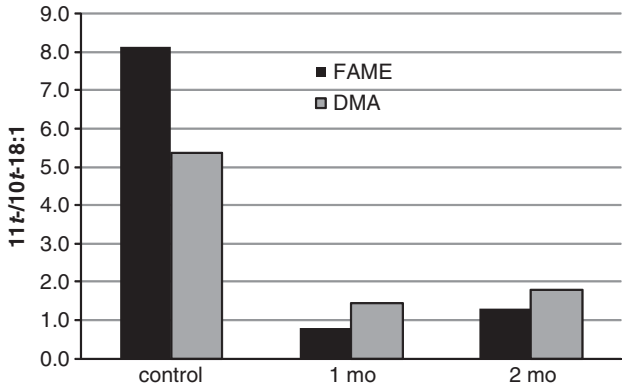

Figure 2 Fatty acid methyl ester and dimethylacetal 11*t*-18:1/10*t*-18:1 ratio of *longissimus thoracis* from bulls with 0 (control), 1 or 2 months (mo) of concentrate finishing after pasture grazing.

or using acid catalysts, but the DMAs formed are generally not reported (Cruz-Hernandez *et al*., 2006). The current results show an increased content of plasmalogens in pasture-fed compared with concentrate-fed beef, which is in agreement with two other studies showing that pasture feeding results in increased amounts of plasmalogens in muscle lipids of beef (Dannenberger *et al*., 2005; Kraft *et al*., 2008). The fact that the plasmalogenic content can also be affected by breed (Kraft *et al*., 2008) makes it difficult to compare results between studies and the question of which factors affect the plasmalogenic content in muscle is difficult to assess. Concentrate finishing, which increases the dietary fat content, did not affect the absolute amount of plasmalogenic lipids in muscle, but did significantly reduce the relative abundance ($P < 0.05$). This is understandable as increased levels of fat in muscle tissue are mainly associated with triacylglycerols and not phospholipids, and plasmalogens being phospholipids would thus not be affected. We also considered whether different dietary fatty acids, such as 18:2n-6 and 18:3n-3, could affect the plasmalogenic content in muscle tissue, even though these unsaturated fatty acids are not incorporated into plasmalogenic lipids (Horrocks, 1972; Wolff, 2002). It would appear, however, that increased dietary 18:3n-3 is generally associated with higher levels of plasmalogens, but this should be confirmed by comparing appropriate diets using the same breed. The results of this study clearly showed that the *trans* fatty acid metabolites formed by rumen bacteria were incorporated into plasmalogenic lipids (Table 3), as was previously demonstrated by Wolff (2002). The ratio of the 11*t*-18:1/10*t*-18:1 FAME and DMA moieties are shown in Tables 2 and 3, respectively, and are presented for comparison in Figure 2. VA (11*t*-18:1) predominated over 10*t*-18:1 in muscle tissue from pasture-fed beef when expressed as mg/100 g or as relative %; the ratio was higher for FAMEs (about 8) than for DMAs (about 5). On the other hand, the 11*t*-18:1/10*t*-18:1 ratio was similar (about 1) for both FAMEs and DMAs whether expressed as mg/100 g or relative %, and were not significantly different between 1 and 2 months of feeding the concentrate.

Phytanic acid is a multibranched-chain fatty acid (MBCFA) originating from the phytol side-chain of chlorophyll, which is hydrolized and oxidized by ruminal bacteria (Patton and Benson, 1966). It is a normal constituent of the tissues, milk and plasma of ruminants (Lough, 1977). In cattle, the proportion of phytanic acid has been found to vary widely depending on the composition of the feed ingested (Lough, 1977). Pristanic acid is also an MBCFA derived from the α-oxidation of phytanic acid and it also accumulates in tissues (Ferdinandusse *et al*., 2002). Even though MBCFAs are related to higher grass intake (i.e. leafy chlorophyll-rich material), and has been associated with prostate cancer risk (Xu *et al*., 2005), we did not find any differences in MBCFA on an absolute basis.

Overall, the beef intramuscular fat content was very low and it could in fact be classified as 'lean meat' (<5%). If we compare the profile against nutritional recommendations, all finishing strategies were close to or above 0.45 for the polysaturated/saturated (P/S) ratio, whereas meat only from grass-finished or concentrate-finished bulls for 1 month achieved the recommended n-6/n-3 ratios of less than 4. In several countries such as Denmark, Canada and the United States, recommendations for human health have now come to include reduced *trans* fat (*trans*-FA) intake, as their consumption has been linked to health issues (Mensink *et al*., 2003; Odegaard and Pereira, 2006). On the other hand, ruminant fats are exempt from *trans* labelling requirements because these sources are considered to be 'natural' and therefore assumed to be 'healthy' (mainly VA and RA; Ratnayake and Zehaluk, 2005). For regulatory purposes, *trans*-FAs are defined as *trans* monoenes plus other fatty acids containing isolated *trans* double bonds, except *trans*-containing CLA. Any food containing less than 0.2 g *trans*-FA per serving (or 2% of total fat content) in Canada, and less than 0.5 g *trans*-FA per serving in the USA are considered free of *trans*-FA. The beef obtained in this study had a maximum content of 0.14 g *trans*-FA/100 g of meat that was well below the limit set either in Canada or in the USA.

Conclusions

The beef obtained from the studied finishing strategies was within or close to the recommended values for human consumption (i.e. P/S > 0.45, n-6/n-3 < 4.0), and the total *trans*-FA content was low. However, the results indicated that finishing had a major impact on *trans*-18:1 and CLA isomeric profile, with grass finishing judged to provide a healthier beef fatty acid profile, whereas 2 months of concentrate finishing negatively affected the *trans*-18:1 and CLA isomer composition. Overall, these results reinforce evidence that beef from pasture-fed animals had the highest nutritional quality, whereas 2 months of concentrate feeding produced a significant reduction in the desirable fatty acids such as VA and RA and gave rise to many undesirable *trans*-18:1, 18:2 and CLA isomers. However, when working with lean breeds an increased forage-to-concentrate ratio will likely be necessary to maintain high levels of desirable

and low levels of undesirable fatty acids while achieving good production efficiencies (e.g. carcass fatness) and consumer acceptance (e.g. juiciness) of the final meat. It remains a challenge to sufficiently increase intramuscular fat in this extremely lean genotype to achieve consumer acceptance and to meet beef grade standards, and at the same time maintain or preferably increase the content of healthful fatty acids. The lack of response of increased levels of 18:2n-6 and 18:3n-3 to produce their long-chain PUFA metabolites in this breed is unique and indicates that a strategy to increase their content cannot be achieved through dietary increase of these essential fatty acids.

Acknowledgements

Funding for this research was provided by the Spanish National Institute for Agricultural Research (INIA). The authors thank the valuable assistance of the staff from Livestock Production area (SERIDA) on animal management, and D. Rolland from Lacombe Research Centre for assisting on sample analysis. This research was supported by a Marie Curie International Outgoing Fellowship (N. Aldai) within the 7th European community Framework Program (SPACANBEEF, PIOF-GA-2008-220730).

References

Aldai N, Nájera AI, Dugan MER, Celaya R and Osoro K 2007a. Characterisation of intramuscular, intermuscular and subcutaneous adipose tissues in yearling bulls of different genetic groups. Meat Science 76, 682–691.

Aldai N, Nájera AI, Martínez A, Celaya R and Osoro K 2007b. Correlation between carcass conformation and fat cover degree, and muscle fatty acid profile of yearling bulls depending on breed and mh-genotype. Livestock Science 107, 199–212.

Aldai N, Dugan MER, Nájera AI and Osoro K 2008a. N-6 and n-3 fatty acids in different beef adipose tissues depending on the presence or absence of the gene responsible for double-muscling. Czech Journal of Animal Science 53, 515–522.

Aldai N, Dugan MER, Kramer JKG, Mir PS and McAllister TA 2008b. Nonionophore antibiotics do not affect the *trans*-18:1 and conjugated linoleic acid composition in beef adipose tissue. Journal of Animal Science 86, 3522–3532.

Aldai N, Dugan MER, Juárez M, Martínez A and Osoro K 2010a. Double-muscling character influences the *trans*-18:1 and conjugated linoleic acid profiles in concentrate-fed yearling bulls. Meat Science 85, 59–65.

Aldai N, Dugan MER, Kramer JKG, Robertson WM, Juárez M and Aalhus JL 2010b. *Trans*-18:1 and conjugated linoleic acid profiles after the inclusion of buffer, sodium sesquicarbonate, in the concentrate of finishing steers. Meat Science 84, 735–741.

Aldai N, Murray BE, Oliván M, Martínez A, Troy DJ, Osoro K and Nájera AI 2006. The influence of breed and mh-genotype on carcass conformation, meat physico-chemical characteristics, and the fatty acid profile of muscle from yearling bulls. Meat Science 72, 486–495.

Alfaia CPM, Alves SP, Martins SIV, Costa ASH, Fontes CMGA, Lemos JPC, Bessa RJB and Prates JAM 2009. 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 Chemistry 114, 939–946.

Arthur PF 1995. Double muscling in cattle: a review. Australian Journal of Agricultural Research 46, 1493–1516.

Bassett CMC, Edel AL, Patenaude AF, McCullough RS, Blackwood DP, Chouinard PY, Paquin P, Lamarche B and Pierce GN 2010. Dietary vaccenic acid has antiatherogenic effects in $LDLr^{-/-}$ mice. Journal of Nutrition 140, 18–24.

Bauchart D, Roy A, Lorenz S, Chardigny JM, Ferlay A, Gruffat D, Sébédio JL, Chilliard Y and Durand D 2007. Butters varying in *trans* 18: 1 and *cis*-9, *trans*-11 conjugated linoleic acid modify plasma lipoproteins in the hypercholesterolemic rabbit. Lipids 42, 123–133.

Bessa RJB, Santos-Silva J, Ribeiro JMR and Portugal AV 2000. Reticulo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. Livestock Production Science 63, 201–211.

Bessa RJB, Alves SP, Jerónimo E, Alfaia CM, Prates JAM and Santos-Silva J 2007. Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acids in lambs. European Journal of Lipid Science and Technology 109, 868–878.

Bessa RJB, Alves SP, Figueiredo R, Teixeira AJR, Rodrigues AIP, Janeiro A, Costa M, Santos-Silva J and Prates JAM 2006. Discrimination of production system and origin of animal products using chemical markers. In Animal products from the Mediterranean area (ed. JMC Ramalho Ribeiro, AEM Horta, C Mosconi and A Rosati), EAAP Publication No. 119, pp. 231–240. Wageningen Academic Publishers, Wageningen, The Netherlands.

Collomb M, Bütikofer U, Sieber R, Jeangros B and Bosset JO 2002. Correlation between fatty acids in cows' milk fat produced in the Lowlands, Mountains and Highlands of Switzerland and botanical composition of the fodder. International Dairy Journal 12, 661–666.

Commission Regulation 1981. No 2930/81 of 12 October 1981 adopting additional provisions for the application of the Community scale for the classification of carcasses of adult bovine animals. Official Journal L293, 6–7.

Council Regulation 1981. No 1208/81 of 28 April 1981 determining the Community scale for the classification of carcasses of adult bovine animals. Official Journal L123, 3–6.

Cruz-Hernandez C, Deng Z, Zhou J, Hill AR, Yurawecz MP, Delmonte P, Mossoba MM, Dugan MER and Kramer JKG 2004. Methods for analysis of conjugated linoleic acids and *trans*-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. Journal of AOAC International 87, 545–562.

Cruz-Hernandez C, Kramer JKG, Kraft J, Santercole V, Or-Rashid M, Deng Z, Dugan MER, Delmonte P and Yurawecz MP 2006. Systematic analysis of *trans* and conjugated linoleic acids in the milk and meat of ruminants. In Advances in conjugated linoleic acid research, volume 3 (ed. MP Yurawecz, JKG Kramer, O Gudmundsen, MW Pariza and S Banni), pp. 45–93. AOCS Press, Champaign, IL, USA.

Dannenberger D, Nuernberg K, Nuernberg G, Scollan N, Steinhart H and Ender K 2005. Effect of pasture vs. concentrate diet on CLA isomer distribution in different tissue lipids of beef cattle. Lipids 40, 589–598.

Destaillats F, Trottier JP, Galvez JMG and Angers P 2005. Analysis of α-linolenic acid biohydrogenation intermediates in milk fat with emphasis on conjugated linolenic acids. Journal of Dairy Science 88, 3231–3239.

Dewhurst RJ, Scollan ND, Youell SJ, Tweed JKS and Humphreys MO 2001. Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. Grass and Forage Science 56, 68–74.

Dugan MER, Rolland DC, Aalhus JL, Aldai N and Kramer JKG 2008. Subcutaneous fat composition of youthful and mature Canadian beef: emphasis on individual conjugated linoleic acid and *trans*-18:1 isomers. Canadian Journal of Animal Science 88, 591–599.

Dugan MER, Kramer JKG, Robertson WM, Meadus WJ, Aldai N and Rolland DC 2007. Comparing subcutaneous adipose tissue in beef and muskox with emphasis on *trans* 18:1 and conjugated linoleic acids. Lipids 42, 509–518.

Duckett SK, Wagner DG, Yates LD, Dolezal HG and May SG 1993. Effects of time on feed on beef nutrient composition. Journal of Animal Science 71, 2079–2088.

Enser M, Hallett KG, Hewett B, Fursey GAJ, Wood JD and Harrington G 1998. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. Meat Science 49, 329–341.

Ferdinandusse S, Rusch H, van Lint AE, Dacremont G, Wanders RJ and Vreken P 2002. Stereochemistry of the peroxisomal branched-chain fatty acid alpha- and betaoxidation systems in patients suffering from different peroxisomal disorders. Journal of Lipid Research 43, 438–444.

Field CJ, Blewett HH, Proctor S and Vine D 2009. Human health benefits of vaccenic acid. Applied Physiology, Nutrition, and Metabolism 34, 979–991.

Fraser MD, Davies DA, Vale JE, Nute GR, Hallett KG, Richardson RI and Wright IA 2009. Performance and meat quality of native and continental cross steers grazing improved upland pasture or semi-natural rough grazing. Livestock Science 123, 70–82.

Fukuda S, Nakanishi Y, Chikayama E, Ohno H, Hino T and Kikuchi J 2009. Evaluation and characterization of bacterial metabolic dynamics with a novel profiling technique, real-time metabolotyping. PloS One 4 (e4893), 1–10.

Gleissman H, Yang R, Martinod K, Lindskog M, Serhan CN, Johnsen JI and Kogner P 2010. Docosahexaenoic acid metabolome in neural tumors: identification of cytotoxic intermediates. The FASEB Journal 24, 906–915.

Gomez-Cortes P, Tyburczy C, Brenna JT, Juarez M and De La Fuente MA 2009. Characterization of *cis*-9 *trans*-11 *trans*-15 C18:3 in milk fat by gas chromatography and covalent adduct chemical ionization tandem mass spectrometry. The Journal of Lipid Research 50, 2412–2420.

Grobet L, Poncelet D, Royo JL, Brouwers B, Pirottin D, Michaux C, Ménissier F, Zanotti M, Dunner S and Georges M 1998. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. Mammalian Genome 9, 210–213.

Harfoot CG and Hazlewood GP 1997. Lipid metabolism in the rumen. In The rumen microbial ecosystem, second edition (ed. PN Hobson and CS Stewart), pp. 382–426. Blackie Academic & Professional, New York, NY, USA.

Horrocks LA 1972. Content, composition, and metabolism of mammalian and avian lipids that contain ether groups. In Ether lipids. Chemistry and biology (ed. F Snyder), pp. 177–272. Academic Press, New York, NY, USA.

Juárez M, Dugan MER, Aalhus JL, Aldai N, Basarab JA, Baron VS and McAllister TA 2010. Dietary vitamin E inhibits the *trans*10-18:1 shift in beef backfat. Canadian Journal of Animal Science 90, 9–12.

Kraft J, Collomb M, Möckel P, Sieber R and Jahreis G 2003. Differences in CLA isomer distribution of cow's milk lipids. Lipids 38, 657–664.

Kraft J, Kramer JKG, Schoene F, Chambers JR and Jahreis G 2008. Extensive analysis of long-chain polyunsaturated fatty acids, CLA, *trans*-18: 1 isomers, and plasmalogenic lipids in different retail beef types. Journal of Agricultural Food Chemistry 56, 4775–4782.

Kramer JKG, Hernandez M, Cruz-Hernandez C, Kraft J and Dugan MER 2008. Combining results of two GC separations partly achieves determination of all *cis* and *trans* 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using ag-ion SPE fractionation. Lipids 43, 259–273.

Kramer JKG, Fellner V, Dugan MER, Sauer FD, Mossoba MM and Yurawecz MP 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. Lipids 32, 1219–1228.

Kramer JKG, Sehat N, Dugan MER, Mossoba MM, Yurawecz MP, Roach JAG, Eulitz K, Aalhus JL, Schaefer AL and Ku Y 1998. Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high-performance liquid chromatography. Lipids 33, 549–558.

Leheska JM, Thompson LD, Howe JC, Hentges E, Boyce J, Brooks JC, Shriver B, Hoover L and Miller MF 2008. Effects of conventional and grass feeding systems on the nutrient composition of beef. Journal of Animal Science 86, 3575–3585.

Lough AK 1977. The phytanic acid content of the lipids of bovine tissues and milk. Lipids 12, 115–119.

Martínez A, Aldai N, Celaya R and Osoro K 2010. Effect of breed body size and the muscular hypertrophy gene in the production and carcass traits of concentrate-finished yearling bulls. Journal of Animal Science 88, 1229–1239.

Mensink RP, Zock PL, Kester ADM and Katan MB 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. American Journal of Clinical Nutrition 77, 1146–1155.

Nuernberg K, Nuernberg G, Ender K, Lorenz S, Winkler K, Rickert R and Steinhart H 2002. N-3 fatty acids and conjugated linoleic acids of longissimus muscle in beef cattle. European Journal of Lipid Science and Technology 104, 463–471.

Nuernberg K, Dannenberger D, Nuernberg G, Ender K, Voigt J, Scollan ND, Wood JD, Nute GR and Richardson RI 2005. Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. Livestock Production Science 94, 137–147.

Odegaard A and Pereira MA 2006. *Trans* fatty acids, insulin resistance, and type 2 diabetes. Nutrition Reviews 64, 364–372.

Patton S and Benson AA 1966. Phytol metabolism in the bovine. Biochimica et Biophysica Acta 125, 22–32.

Pérez A 2005. Meat production in Asturias: beef cattle and other species (2003–2004). Statistical Report of the Rural and Marine Council of the Principality of Asturias, Spain.

Perez R, Cañón J and Dunner S 2010. Genes associated with long-chain omega-3 fatty acids in bovine skeletal muscle. Journal of Applied Genetics 51, 479–487.

Ponnampalam EN, Mann NJ and Sinclair AJ 2006. Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and *trans* fatty acids in Australian beef cuts: potential impact on human health. Asia Pacific Journal of Clinical Nutrition 15, 21–29.

Post-Beittenmiller D 1996. Biochemistry and molecular biology of wax production in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47, 405–430.

Pottier J, Focant M, Debier C, De Buysser G, Goffe C, Mignolet E, Froidmont E and Larondelle Y 2006. Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. Journal of Dairy Science 89, 685–692.

Purchas RW, Knight TW and Busboom JR 2005. The effect of production system and age on concentrations of fatty acids in intramuscular fat of the longissimus and triceps brachii muscles of Angus-cross heifers. Meat Science 70, 597–603.

Raes K, De Smet S, Balcaen A, Claeys E and Demeyer D 2003. Effect of diets rich in N-3 polyunsatured fatty acids on muscle lipids and fatty acids in Belgian Blue double-muscled young bulls. Reproduction Nutrition Development 43, 331–345.

Ratnayake WMN and Zehaluk C 2005. *Trans* fatty acids in foods and their labeling regulations. In Healthful lipids (ed. CC Akoh and OM Lai), pp. 1–32. AOCS Press, Champaign, IL, USA.

Rego OA, Alves SP, Antunes LMS, Rosa HJD, Alfaia CFM, Prates JAM, Cabrita ARJ, Fonseca AJM and Bessa RJB 2009. Rumen biohydrogenation-derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils. Journal of Dairy Science 92, 4530–4540.

Roy A, Chardigny JM, Bauchart D, Ferlay A, Lorenz S, Durand D, Gruffat D, Faulconnier Y, Sébédio JL and Chilliard Y 2007. Butter rich in *trans*10-C18:1 plus *cis*9, *trans*11-CLA differentially affects plasma lipids and aortic streak in experimental atherosclerosis in rabbits. Animal 1, 467–476.

Rule DC, Smith SB and Romans JR 1995. Fatty acid composition of muscle and adipose tissue of meat animals. In Biology of fat in meat animals (ed. SB Smith and DR Smith), pp. 144–165. American Society of Animal Science, Champaign, IL, USA.

SAS Institute 2001. SAS user's guide: statistics. SAS for Windows, release 9.2. SAS Institute Inc., Cary, NC, USA.

Sukhija PS and Palmquist DL 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. Journal of Agricultural and Food Chemistry 36, 1202–1206.

Vanhatalo A, Kuoppala K, Toivonen V and Shingfield KJ 2007. Effects of forage species and stage of maturity on bovine milk fatty acid composition. European Journal of Lipid Science and Technology 109, 856–867.

Wolff RL 2002. Characterization of *trans*-monounsaturated alkenyl chains in total plasmalogens (1-*O*-alk-1'-enyl-2-acyl glycerophospholipids) from sheep heart. Lipids 37, 811–816.

Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI and Whittington FM 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Science 78, 343–358.

Xu J, Thornburg T, Turner AR, Vitolins M, Case D, Shadle J, Hinson L, Sun J, Liu W, Chang B, Adams TS, Zheng SL and Torti FM 2005. Serum levels of phytanic acid are associated with prostate cancer risk. The Prostate 63, 209–214.