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RESEARCH ARTICLE

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Fatty acid profile as a tool to trace the origin of beef in pasture- and grain-fed young bulls of Retinta breed

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Abstract

This research explores the possibility of using the fatty acid profiles of intramuscular fat to authenticate the origin of Retinta breed meat according to different feeding regimes based on the combined use of concentrate and grass or forage (GP, grass pasture; MC, medium concentrate; HC, high concentrate). Young bulls from GP (n=30) were reared on grass pasture and supplement with concentrate in controlled feeders; MC (n=30) and HC (n=15) were reared in farm buildings using 40 and 80% concentrate of total dry matter from diet, respectively. The stepclass function in R was used to perform a stepwise linear discriminant analysis including thirty fatty acids from intramuscular fat. Two fatty acids, 9*c*18:1 and 22:5 *n*-3 were selected as discriminators of the meat origin. Meat from the GP and MC was characterized by higher 22:5 *n*-3 ($p<0.05$), while HC meat showed higher 9*c*18:1 ($p<0.05$). The use of 9*c*18:1 and 22:5 *n*-3 fatty acids from intramuscular fat resulted in a correct assignation of 100% of beef samples to each of the feeding regimes. Therefore, in addition to serving as an effective tool for discriminating between feeding regimes in the origin of the beef, the fatty acid profile of intramuscular fat could help companies to check the authenticity of the meat origin.

Additional keywords: pasture; concentrate supplement; discriminant analysis; gas chromatography.

Abbreviations used: DHA (docosahexaenoic acid); DPA (docosapentaenoic acid); EPA (eicosapentaenoic acid); FAME (fatty acids methyl esters); GP (grass pasture); HC (high concentrate); LDF (linear discriminant function); MC (medium concentrate); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); SFA (saturated fatty acids).

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Chemical compounds used in this article: Chloroform (PubChem CID: 6212); Methanol (PubChem CID: 887); Potassium hydroxide (PubChem CID: 14797); Sodium chloride (PubChem CID: 5234); hydroxyquinone (PubChem CID: 785); Glacial acetic acid (PubChem CID: 176); Toluene (PubChem CID: 1140); (Trimethylsilyl) diazomethane (PubChem CID: 167693); Butylated hydroxytoluene (PubChem CID: 31404): n-hexane (PubChem CID: 8058); Nonadecanoic methyl ester (PubChem CID: 15610).

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Introduction

A number of security and nutritional roles exist in the production of beef for the human diet (Mapiye *et al.*, 2015). While in developing countries the system of cattle production is based on use of grass pasture in feeding animals, in developed countries the cattle with a high potential for lean beef production are frequently fattened on diets of concentrate, which may be unfavourable to the nutritional values in the meat as regards human health. While the contribution of saturated (SFA) and monounsaturated fatty (MUFA) acids to cardiovascular health is debatable (Calder, 2004), evidence regarding the role of polyunsaturated fatty acid (PUFA) in the prevention of heart disease is convincing. Clinical intervention studies and animal experiments indicate that a lower ratio *n*-6/*n*-3 PUFA in dietary fat is desirable in order to reduce the risk of chronic diseases (Simopoulos, 2001). In this sense, Wolfram (2003) and Nuernberg *et al.* (2005) reported

that the *n*-6/*n*-3 PUFA ratio could be four times higher in the meat of bulls fed on concentrates indoors than those with grass-based feeding. The influence of the feed on the fatty acid composition of the beef in calves has been widely reported (Średnicka-Tober *et al.*, 2016). Reducing concentrate in the diet of beef cattle should enhance the *n*-6 fatty acid concentration, because concentrates are a source of 18:2 *n*-6. Of the major 18:1 isomers found in beef, vaccenic acid (isomer 11*t*18:1) is the main isomer in beef from forage-based diets and has potential human health benefits (Dilzer & Park, 2012), whereas 10*t*18:1, the major *t*-18:1 isomer in beef from grain-based diets, seems to have detrimental effects (Wang *et al.*, 2012).

Changes in the diet of animals can lead to changes in the fatty acid composition of the meat and may also affect sensory eating quality (Vatansever *et al.*, 2000). In fact, consistent positive correlations between major fatty acid 9*c*18:1 (oleic) in meat and the scores given by panels for odour and flavour intensity have been reported (Sañudo *et al.*, 2000). Beef from high-concentrate fed animals is in general fatter and contains greater total proportions of unsaturated fatty acid (mainly C18:1) than that from animals finished on pasture diets, when compared at a similar age range (Daley *et al.*, 2010). Research to find dietary strategies that increase the ratios of favourable fatty acids for human health or improve sensorial properties of meat through the animal diet can, therefore, be a useful tool in the development of beef livestock production.

A number of discrimination procedures are used as a tool to get to know the origin of the product. For example, in the meat product industry, near infrared spectroscopy had proven to be a rapid and effective tool for determining the meat quality and the origin of the products (Fernández Cabanás *et al.*, 2011). NIR calibrations have been developed using least squared regressions models based on the use of the fatty acid profile. Besides, the effectiveness of using methodologies based on the fatty acid profile of meat in order to differentiate production systems for young bulls (Moreno *et al.*, 2006; Dias *et al.*, 2008), lambs (Juárez *et al.*, 2010) or goats (Mellado-González *et al.*, 2009) has already been reported. In fact, Martínez *et al.* (2013) proposed that a linear discriminant analysis using only 10 fatty acids of intramuscular fat is able to distinguish beef samples according to the type of finishing diet in bulls. The present work proposes a scientific initiative to improve the control of the production systems of beef and the system to authenticate the product origin. Therefore, the main objective of this work was to develop a methodology based on the fatty acid profile of intramuscular fat in beef from young bulls, in order to discriminate between feeding systems based on the combined use of different levels of concentrate and grass or forage. This methodology could be used to certified the beef origin in the market and provide the consumer with information about the nutritional values of this product before consumption.

Material and methods

Animals and sample collection

A total of 75 young bulls from the Retinta breed were selected and reared with mothers' milk until weaned at 8-9 months of age. After weaning, the young bulls were assigned to three dietary treatments (grass- and concentrate- based) as follows:

• Grass pasture (GP). For 6 months, 30 animals were fed a diet based on natural grass in 27 ha from an agrosilvopastoral system called *dehesa* that includes legumes and grass species, as described by Milán *et al.* (2006). These animals were supplemented with concentrate in controlled feeders during periods when natural resources were scarce.

• Medium concentrate (MC). For 6 months, 30 animals were fed indoors on the farm using controlled feeders with 40% concentrate and 60% forage (barley straw and grass silage) of total ration and without access to pasture.

• High concentrate (HC). For 7 months, 15 animals were housed in 10 × 10 m pens indoors on the farm and fed with 80% concentrate and barley straw *ad libitum* until slaughter. During this period, the animals had no access to pasture.

Estimated consumption rate of concentrate and forage in the three dietary treatments and composition of the diets fed to the animals is shown in Tables 1 and 2. All animals had free access to local water through waterers.

The young bulls were slaughtered according to Spanish market requirements at 14-16 months, approximately at 509.8 + 51.5, 482.2 + 51.0 and 558.3 + 51.0 kg live weight for GP, MC and HC, respectively. The young bulls were managed and slaughtered according to Spanish rules and regulations for animal care (EC, 2009).

In the slaughterhouse, at 24 h *post-mortem*, the *longissimus lumborum* muscle of left half carcass was extracted and transported, in refrigerated conditions (4ºC), to the ETSIA meat quality laboratory (University of Seville, Spain). A slice of *longissimus lumborum* muscle (~ 100 g) at 12-13th rib level was extracted for fatty acid analysis. All samples were vacuum-packed and frozen at -20ºC prior to undergoing the analytical procedure.

Fat extraction

At 24h post-mortem, a steak of *longissimus lumborum* muscle was used to determine the

Table 1. Ingredients and nutrient composition of diet in grass pasture (GP), medium concentrate (MC) and high concentrate (HC) feeding systems.

	GP	MC	HC
Estimated consumption rate (%), dry matter basis			
Concentrate mix	20[b]	40[b]	80[c]
Barley straw	-	25	20
Grass silage	-	35	-
Natural pasture	80	-	-
Composition concentrate[a], dry matter basis			
Crude protein (%)	12.2	10.5	13.1
Crude fat (%)	2.4	5.4	6.0
Ash (%)	6.9	5.9	6.2
Neutral detergent fiber (%)	62.5	32.3	23.5
Metabolisable energy (MJ/kg)	8.2	11.9	12.9

[a]Calculated from FEDNA (De Blas *et al*., 2010). [b]Ingredients (% as fed): barley grain, 36.2; oat grain, 24.5; peas, 16.6; sunflower seed cake, 19.6; minerals and vitamins, 3.1. [c]Ingredients (% as fed): maize, 34.0; barley, 33.5; corn gluten feed, 17.1; soybean meal 44, 8.4; minerals and vitamins, 3.9; palm oil, 3.1.

Table 2. Fatty acid profile (expressed as % of total fatty acids detected) in the treatments based on grass pasture (GP), and medium (MC) and high (HC) levels of concentrate in the diet.

Fatty acids	GP		MC		HC	
	Concentrate	Pasture	Concentrate	Forage[1]	Concentrate	Forage[2]
14:0	1.73	2.80	1.88	1.08	2.05	1.37
16:0	21.71	18.42	22.29	20.75	24.90	22.78
16:1	0.84	1.80	0.76	0.42	1.14	0.89
18:0	2.35	3.04	2.92	4.30	3.50	3.99
9*c*18:1	36.76	14.83	33.81	20.28	31.86	19.54
18:2	31.32	19.77	30.24	16.06	27.39	16.65
18:3	2.29	34.93	3.58	30.86	2.42	29.14
∑*n*-6	31.82	20.2	30.97	16.62	28.33	17.29
∑*n*-3	2.79	35.36	4.31	31.42	3.36	29.78
Others	2.50	4.00	3.80	5.69	5.80	5.01

[1]Composed of barley straw and grass silage; [2]Composed of barley straw.

intramuscular fat content. Total lipids were extracted from each meat product with chloroform:methanol (2:1, v/v), according to the Folch *et al.* (1957) method. The results were expressed as total intramuscular fat content per 100 g of fresh meat. Replicate analyses were carried out on the same samples.

Fatty acid analysis procedure

Intramuscular fatty acid methyl esters (FAMEs) were analysed following the method described in Horcada *et al.* (2016). This method is accurate, safe and also environmentally friendly because of the few reagents used. Two sub-samples (1 g) of the *longissimus lumborum* muscle were cut up, analysed separately and averaged to obtain a mean value. Nonadecanoic acid methyl ester (19:0 ME) at 10 mg/mL was used as an internal standard. Individual FAMEs were identified by comparing their retention times with those of authenticated standards from Sigma Chem. Co. Ltd. (Poole, UK). The separation of FAMEs was carried out using a gas chromatograph Agilent 6890 (Agilent, Santa Clara, CA, USA) equipped with a flame ionisation detector (FID) and an HP 7683 automatic sample injector fitted with an HP-88 capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness, Agilent Technologies Spain, S.L., Madrid). Hydrogen was used as a carrier gas in a constant flow of 0.8 mL/min.

Individual fatty acids from intramuscular fat were expressed as mg/100 g fresh muscle.

Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics vers. 22.0 (2013) and the statistical software environment R (R Core Team, 2015). A univariate test of equality between the group means of the three production systems was performed for each fatty acid, and a parametric test based on the usual F statistic for one-way analysis of variance was carried out when normality (Shapiro-Wilk's test) and homoscedasticity (Bartlett's test) were confirmed, while a Kruskal-Wallis test was performed when not. Post-hoc Bonferroni correction was used to compare the individual means and ratios of fatty acids, with the significance level set at $p<0.05$.

The *stepclass* function in the klaR package (Weihs *et al.*, 2005) in R was used to perform a stepwise linear discriminant analysis. The initial model is defined by the starting variables provided and at every step new models are generated by including every single variable that is not in the model, and by excluding every single variable that is in the model. The resulting performance measure for these models was estimated by cross-validation (Weihs *et al.*, 2005), whereby if the maximum value of the chosen criterion is better than 'improvement' (equal to 0.05, by default) plus the value so far, the corresponding variable is included or excluded. The procedure is stopped if the new best value is not good enough, or if the specified maximum number of variables is reached. This method provided a final classification rule whose generalization performance was estimated by the "leave-one-out" method.

Results

A total of thirty fatty acids were detected in intramuscular fat of the *longissimus lumborum* according to their relative retention times (Table 3). Palmitic acid (16:0), stearic acid (18:0), oleic acid (9*c*18:1) and linoleic acid (6*c*18:2) accounted for around 75% of the total fatty acids detected in the intramuscular fat of Retinta young bulls. The corresponding individual chromatograms of intramuscular fat of young bulls raised on GP, MC and HC feeding regimes is shown in Fig. 1. Under the conditions of our assay, the first group of fatty acids of interest in intramuscular fat (10:0 to 16:1) elutes at a retention time of between 10 and 27 min, although this group also includes 16:0 that elutes at 25 min. The group of fatty acids with 18 carbon atoms elutes between 31 and 39 min. and includes the commonest fatty acid (9*c*18:1), which elutes at 32.2 min. 9*c*18:1 acid expressed as mg/100g muscle was the greatest in all different feeding regimes. The peaks of long-chain fatty acids (carbon number >20) appear after 39 min. This group includes some *n*-6 and *n*-3 PUFA which are associated with human health. The two major long-chain peaks corresponding to 20:4 *n*-6 (arachidonic) and 22:5 *n*-3 (docosapentaenoic) fatty acids elute at 44.7 and 55.4 min, respectively.

The different feeding regimes caused large differences in fatty acid profiles of intramuscular muscle fatty acids as shown in Table 3. The univariate test of equality between groups showed that 28 of the 30 fatty acids detected were affected ($p<0.05$) by the feeding regime. In fact, 20:3 *n*-3 and 24:1 content were the only fatty acids not affected by dietary treatment ($p>0.05$). Intramuscular fat of regimes based on grain feed (MC and HC) showed a higher content of 9*c*18:1, 18:0, and 6*c*18:2 than feeding regimes based on grass (GP) ($p<0.05$), while values of saturated hypercholesterolemic fatty acids (lauric, 12:0; myristic, 14:0 and palmitic, 16:0) were lower in young bulls reared on grass pasture ($p<0.05$) than young grain-fed bulls. However, the intramuscular fat of meat from GP showed higher polyunsaturated fatty acid content as 20:4 *n*-6; 20:5 *n*-3, 22:5 *n*-3 and 22:6 *n*-3 than the MC and HC feeding regimes.

In reference to the fat content in meat, the young bulls fed diets of concentrate (MC and HC) showed a higher fat content (1.86 and 1.58% of fresh meat for MC and HC, respectively) than meat from grazing young bulls (1.04% of fresh meat; $p<0.05$).

The stepwise procedure provided a final model with only two fatty acids, 9*c*18:1 and 22:5 *n*-3 (Table 4). The classification model provided two linear discriminant functions (LDF1 and LDF2) as follows: LDF1 accounted for 81.9% of the inter-group variance, while LDF2 accounted for 18.1%. The great ability of LDF1 and LDF2 to separate the three groups is shown in Fig. 2, where the discriminant scores are graphically depicted and each class is identified separately. The class means for each group in the canonical observation scores are presented in Table 4. The highest scores for the first discriminant function were associated with the GP system, while the lowest scores for discriminant function were associated with the MC and HC systems.

The three linear classification functions obtained to discriminate feeding regimes are shown in Table 5. At the modelling stage, the recognition ability of the discriminant model was evaluated by 100% correct classifications following the "leave-one-out" procedure, allowing the three production systems to be differentiated. The 9*c*18:1 and 22:5 *n*-3 fatty acid contents were selected to discriminate between groups with different feeding regimes (Fig. 3).

Table 3. Mean of fatty acids (expressed as mg/100g muscle) of intramuscular fat from young Retinta bulls in three different feeding systems: grass pasture (GP), medium concentrate (MC) and high concentrate (HC).

Peak	Fatty acid	T_{RR}	GP (n=30)	MC (n=30)	HC (n=15)	*p*-value
1	10:0	10.2	1.31[c]	2.91[a]	1.66[b]	<0.001
2	12:0	14.3	1.93[b]	4.94[a]	4.97[a]	<0.001
3	14:0	19.4	30.92[c]	66.57[a]	60.77[b]	<0.001
4	14:1	21.5	4.34[b]	10.09[a]	8.38[a]	<0.001
5	15:0	22.1	5.41[c]	13.08[a]	8.32[b]	<0.001
6	15:1	23.3	1.33[c]	4.04[a]	2.23[b]	<0.001
7	16:0	25.0	330.17[c]	611.65[a]	530.16[b]	0.002
8	16:1	26.3	33.42[b]	68.42[a]	63.20[a]	0.001
9	17:0	27.9	14.00[c]	33.32[a]	20.54[b]	<0.001
10	17:1	29.1	9.73[b]	29.58[a]	13.66[b]	<0.001
11	18:0	31.0	255.64[c]	396.8[a]	383.16[b]	<0.001
12	9*t*18:1	32.1	14.26[b]	12.64[c]	16.07[a]	<0.001
13	9*c*18:1	32.2	384.46[b]	672.21[a]	805.48[a]	<0.001
14	11*t*18:1	32.3	12.48[c]	22.73[b]	30.33[a]	<0.001
15	6*t*18:2	34.0	3.71[b]	6.95[a]	6.32[a]	<0.001
16	6*c*18:2	34.9	157.60[c]	177.02[b]	183.58[a]	<0.001
17	18:3 *n*-3	37.6	10.71[b]	11.29[a]	8.09[c]	<0.001
18	18:3 *n*-6	38.2	1.89[b]	2.83[a]	1.76[b]	<0.001
19	20:0	39.4	3.51[b]	4.61[a]	2.74[c]	<0.001
20	20:1 *n*-9	40.1	3.12[b]	5.15[a]	5.85[a]	<0.000
21	20:2 *n*-6	41.3	2.69[a]	2.57[b]	2.25[b]	<0.001
22	22:0	43.0	22.41[a]	22.09[a]	14.23[b]	<0.001
23	20:4 *n*-6	44.7	54.48[a]	52.40[b]	37.02[c]	<0.001
24	20:3 *n*-3	45.8	1.09	1.11	1.23	0.113
25	20:5 *n*-3	48.5	8.75[a]	6.05[b]	2.05[c]	<0.001
26	22:2	50.6	0.74[c]	1.42[a]	0.95[b]	<0.001
27	24:0	53.4	2.16[c]	3.94[b]	4.42[a]	<0.001
28	24:1	54.9	1.09	1.61	1.45	0.205
29	22:5 *n*-3	55.4	14.12[a]	13.56[b]	5.16[c]	<0.001
30	22:6 *n*-3	56.1	2.84[a]	2.65[b]	1.83[c]	<0.001

T_{RR}: mean relative retention time (min.); [a-c]: values in rows with different letters are significantly different ($p \leq 0.05$).

Discussion

The fatty acid profile of intramuscular fat from Retinta young bulls in this study were in line with those reported by other authors who studied intramuscular fat from other lean young bulls of European continental breeds, such as Limousin (Aldai *et al.*, 2012) Pirenaica (Indurain *et al.*, 2006) or Serrana de Teruel (Ripoll *et al.*, 2016) slaughtered at the same live weight. Saturated fatty acids 16:0 and 18:0, monounsaturated fatty acid 9*c*18:1 and polyunsaturated fatty acid 6*c*18:2 were the most abundant fatty acids in the intramuscular fat and this concurs with results reported for meat from young bulls of different origins (*i.e.* Belgian Blue, Limousin, Irish, Argentine and Pirenaica) raised in traditional production systems (Raes *et al.*, 2003; Albertí *et al.*,

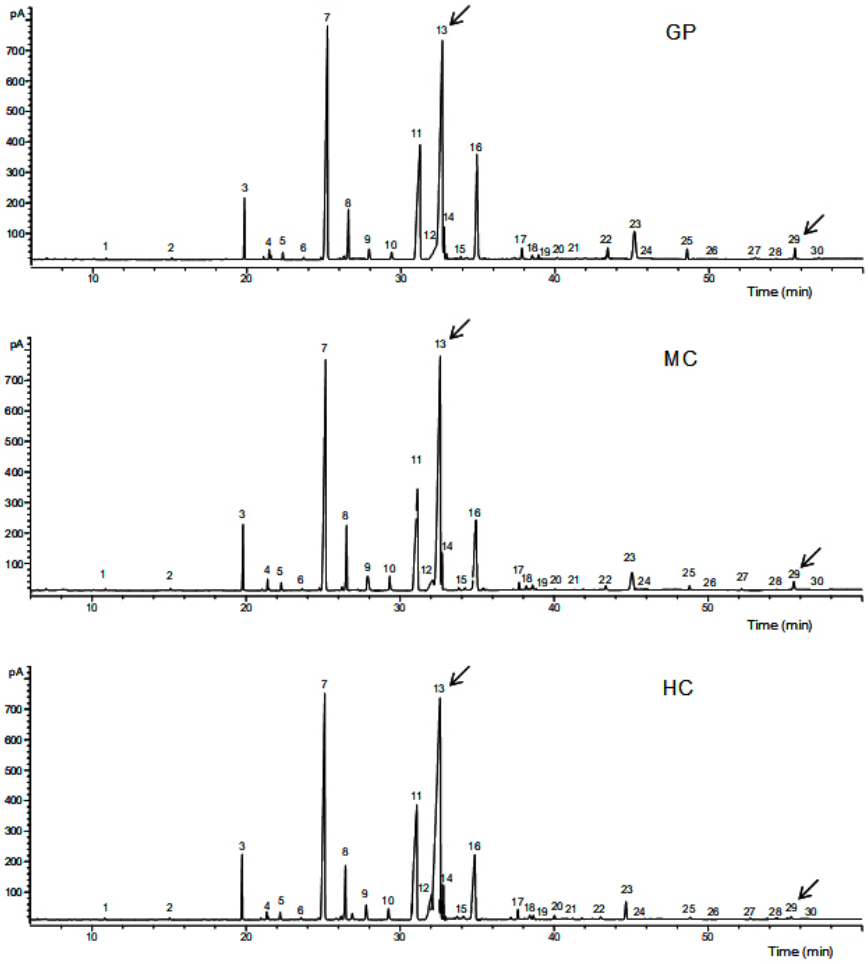

Figure 1. Gas-chromatogram of the fatty acid profile of intramuscular fat from Retinta calves in three different feeding systems: grass pasture (GP), medium concentrate (MC) and high concentrate (HC). Arrows show fatty acids included in the discriminant model.

2014). In fact, 9*c*18:1 and 16:0 fatty acids were the commonest fatty acids in the intramuscular fat of the young bulls (around 30 and 25% of the total of fatty acid detected, respectively), as was described by other authors in beef (Alfaia *et al.*, 2009; Martínez *et al.*, 2013). The group of long-chain fatty acids detected in intramuscular fat of Retinta young bulls includes some *n*-6 (18:3 *n*-6; 20:2 *n*-6; 20:4 *n*-6) PUFA and *n*-3 (18:3 *n*-3; 20:3 *n*-3; 20:5 *n*-3; 22:5 *n*-3; 22:6 *n*-3) PUFA, which are regarded as being beneficial for human health (Wood *et al.*, 2004). In fact, nutritionists recommend a higher intake of long-chain PUFA, especially *n*-3 PUFA rather than *n*-6 PUFA (Kamihiro *et al.*, 2015) to prevent coronary diseases.

The feeding regime had a major impact on the individual fatty acids of the intramuscular fat. These results are in accordance with Alfaia *et al.* (2009), who reported the influence of the feeding system in 27 of the 36 fatty acids analysed in intramuscular fat of the *longissimus lumborum* muscle of young bulls of the Alentejana breed. Walshe *et al.* (2006) and Średnicka-Tober *et al.* (2016) reported that greater differences in the fatty acid profiles of intramuscular fat of beef obtained from pasture- and grain-fed were mainly due to the PUFA content, while the concentration of SFA or MUFA was similar or slightly lower, respectively, in pasture compared with meat from a diet of concentrate. However, in our study, the 9*c*18:1 content in the intramuscular fat of Retinta breed young bulls fed on grain-based regimes was approximatively twice that of pasture-raised young bulls. An increase in 9*c*18:1 in meat from concentrate-feeding is expected because the content of 9*c*18:1 in cereal grains was high (Table 2). On the other hand, according to Urrutia *et al.* (2015) accumulation of oleic fatty acid in meat from concentrate-feeding could be linked to increased stearoyl-CoA desaturase activity in grain feeding. Stearoyl-CoA desaturase (SCD1 gene) catalyzes the

Table 4. Total-sample standardized canonical coefficients, pooled within canonical structure from grass pasture (GP), medium concentrate (MC) and high concentrate (HC) feeding systems.

	Canonical structure		Standardized canonical coefficients	
	Function 1	**Function 2**	**Function 1**	**Function 2**
9*c*18:1	-0.853	0.521	-0.509	1.085
22:5 *n*-3	0.905	0.425	0.625	1.023
Class means				
GP	2.940	-0.323		
MC	-1.554	1.307		
HC	-3.164	-1.924		

main reaction to synthesize oleic acid (C18:1*c*9) from stearic acid (C18:0). SCD1 inserts the cis-9 double bond in stearic acid to make oleic acid. In fact, Hiller *et al.* (2011) reported an increase in SCD1 gene expression in bovine muscle after feeding bulls a grain-based diet, while Dervishi *et al.* (2011) and Corazzin *et al.* (2013) reported that SCD gene expression could be reduced by grazing.

The percentages of *n*-6 and *n*-3 fatty acids were different between the feeding regimes ($p<0.001$). Some studies reported that increases in *n*-3 PUFA are matched by reductions in *n*-6 PUFA, because competition occurs between these fatty acids for the same set of elongation and desaturation enzymes (Lorenz, 2004). However, Nuernberg *et al.* (2005) in beef muscle from German Simmental and German Holstein showed no evidence of this inverse relationship. In our experiment, evidence of this inverse relationship was not found and the highest percentages of *n*-6 PUFA and *n*-3 PUFA were observed in the GP system ($p<0.05$). The percentages of long-chain fatty acids beneficial to human health *n*-6 (20:4 *n*-6) and *n*-3 (20:5 *n*-3; 22:5 *n*-3 and 22:6 *n*-3) were higher in young bulls fed with grass pasture compared to concentrate-fed animals ($p<0.05$). As regards the *n*-6/*n*-3 ratio calculated from the content of individual fatty acid *n*-6 and *n*-3, in this experiment we have calculated the ratio *n*-6/*n*-3 from the percentage of *n*-6 and *n*-3 to total fatty acid detected and we could observe a less favourable nutritional value in meat from the highest concentrate diet (HC; 12.70) than meat from the lowest concentrate diet (6.38 and 5.71 for MC and GP, respectively). Several studies using young bulls (French *et al.*, 2000; Razminowicz *et al.*, 2006) have demonstrated that increasing the proportion of grass in the diet and decreasing the concentrate intake caused a decrease in the *n*-6/*n*-3 ratio. As reported by Nuernberg *et al.* (2005), the availability of 18:3 fatty acid in the grass-based or reduced concentrate diet resulted in an increase in the synthesis of the C20:5 *n*-3, C22:5 *n*-3 and C22:6 *n*-3 fatty acid concentration in the animals' muscle.

In order to discriminate the fatty acids involved in the identification of feeding regimes, a stepwise linear discriminant analysis with individual fatty acids of the intramuscular fat was proposed, which offers both a classification rule and a way to explain the differences between groups. The stepwise linear discriminant analysis previously described in the Statistical Analysis section was applied on the set of the thirty fatty acids of intramuscular fat (Table 3). This procedure identified two fatty acids (9*c*18:1 and 22:5 *n*-3), and therefore a

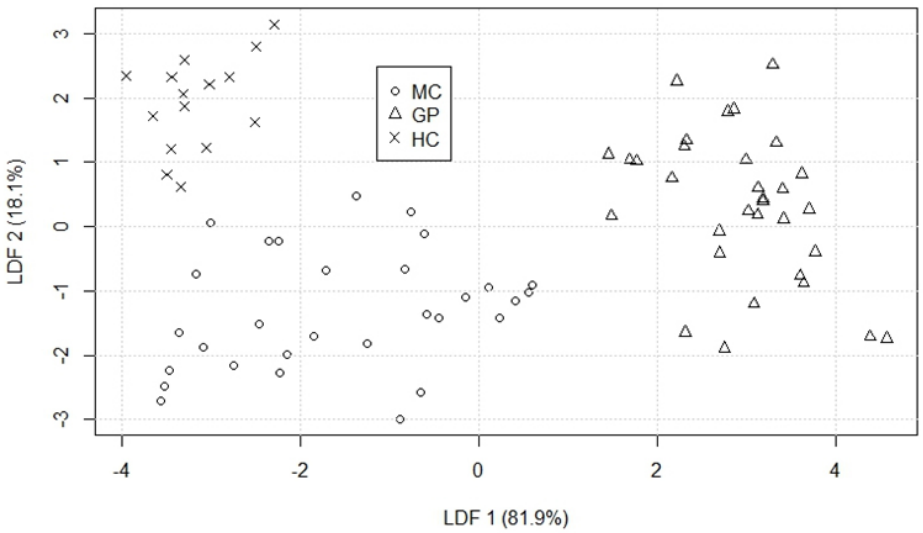

Figure 2. Scatter plot of the linear discriminant functions (LDF1 and LDF2) for all the meat samples from grass pasture (GP), medium concentrate (MC) and high concentrate (HC) feeding systems.

Table 5. Coefficients of Fisher's linear discriminant functions for classifying beef samples from grass pasture (GP), medium concentrate (MC) and high concentrate (HC) feeding systems.

	Production system		
	GP	**MC**	**HC**
Constant	-662.263	-767.203	-673.805
9*c*18:1	38.908	42.617	40.160
22:5 *n*-3	215.817	207.961	178.271

linear discriminant analysis based on these two variables was built to discriminate among the feeding regimes from Retinta young bulls (Fig. 1). The ability of 9*c*18:1 to discriminate between beef meat from animals raised under different feeding regimes has been suggested previously. In fact, the discriminant capacity of this fatty acid has been reported by Moreno *et al.* (2006) to separate young bulls weaned and not weaned before slaughter or to classify bulls according to the feeding system based on the use of concentrate for two or four months during the finishing period (Alfaia *et al.*, 2009). However, there are no reported references to 22:5 *n*-3 regarding its discriminant capacity to differentiate between production systems based mainly on different dietary treatments. Other PUFA (*i.e.* 18:3 *n*-3; 20:3 *n*-3; 22:6 *n*-3) have been proposed as predictors of feeding systems based on grass feeding or concentrate feed in young bulls (Moreno *et al.*, 2006), but this is not relevant to the present study.

When pooled in a canonical structure (Table 4), they showed that both 9*c*18:1 and 22:5 *n*-3 were closely correlated with LDF1, the former negatively and the latter positively, whereas moderate and positive correlations where found for LDF2. The greater discriminating ability of 9*c*18:1 and 22:5 *n*-3 ties in with the findings of Walshe *et al.* (2006) in relation to young bulls feeding, given that the MUFA content is negatively related to the grazing system, while the PUFA content is positively related to that system. By contrast, the MC system based on forage intake (60% of the diet) provides higher 18:3 *n*-3 and its long chain derivatives (including 22:5 *n*-3) than the HC system. Similar results were reported by Kamihiro *et al.* (2015), who showed an increase of 12% of 22:5 *n*-3 in meat from grass pasture-fed compared to grain-fed lean beef.

The great ability of LDF1 and LDF2 to separate the three groups suggests that the feeding regime associated with grazing or different levels of concentrated feeding can be considered as a main factor for discriminating the beef fatty acid profile. One fatty acid selected as a predictor was of primarily dietary origin (9*c*18:1) and was deposited with minimal changes in intramuscular fat, while a significant amount of 22:5 *n*-3 could be of microbial origin from 18:3 *n*-3 fatty acid (French *et al.*, 2000). The increased concentration of 22:5 *n*-3 in meat from young bulls fed on grass pasture suggests that the high availability of 18:3 *n*-3 in the pasture diet has resulted in an enhanced synthesis of these *n*-3 long-chain PUFAs in intramuscular fat (Nuernberg *et al.*, 2002).

A number of studies on the influence of diet on the fatty acid profiles of meat have concluded that beef from animals fed on pasture has greater amounts of *n*-3 PUFA compared to beef from animals fed a concentrate-based diet (French *et al.*, 2003; Ponnampalam *et al.*, 2006). In

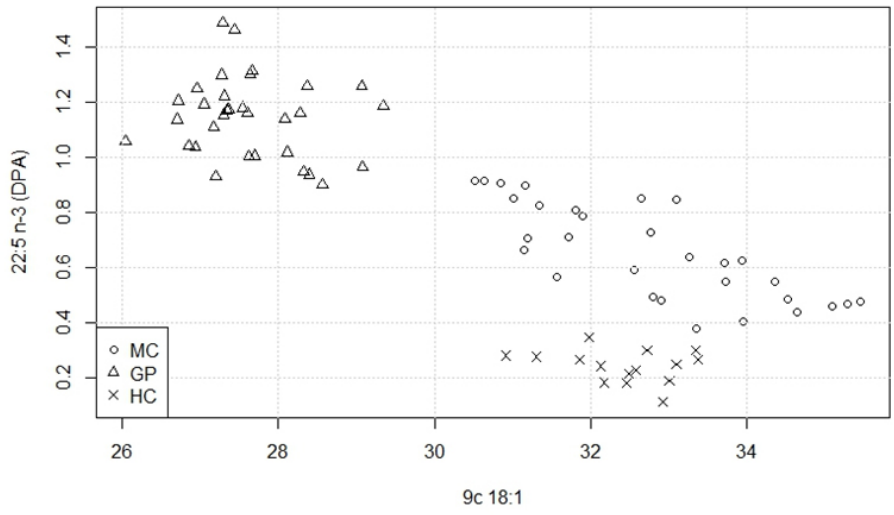

Figure 3. Scatter plot of the two fatty acids selected (9c 18:1 and 22:5 n-3) in stepwise discriminant analysis for the meat samples from grass pasture (GP), medium concentrate (MC) and high concentrate (HC) feeding systems.

fact, Turner *et al.* (2015) reported a greater proportion of 22:5 *n*-3 and other long chain PUFAs (20:5 *n*-3 and 22:6 *n*-3) in meat from pasture regime than beef from high concentrate feed. The results show that it is possible to use 22:5 *n*-3 to discriminate between the three feeding regimes considered in this study and, indeed, the two selected variables in the stepwise linear discriminant analysis (including 22:5 *n*-3 and 9*c*18:1) show a clear separation between the three production systems (Fig. 3). The GP group is located at a distance from the MC and HC groups, while the MC and HC groups are close to each other. The GP group is located at the highest point, which is indicative of the highest level of 22:5 *n*-3, whereas the MC and HC groups are located on the right-hand side (Fig. 3), indicating the higher level of 9*c*18:1 in concentrate regimes. The proximity between MC and HC can be observed because both production systems include a significant amount of 9*c*18:1 in the animals' diet, whereas the GP system is associated with a high percentage of 22:5 *n*-3 from the diet of grass. The MC group was also found to have a higher percentage of 22:5 *n*-3 than the HC group. Thus, evidence can be found to support the classification of origin of meat according to the 9*c*18:1 and 22:5 *n*-3 fatty acid content.

According to the "leave-one-out" procedure, the final model offered a success rate of 100% for all the three groups studied. Our results are in agreement with other authors who obtained 77.8-100% (García *et al.*, 2008) or 100% (Alfaia *et al.*, 2009) of cases correctly classified using the fatty acid profiles of intramuscular fat when different diets (pasture only, pasture plus concentrate and concentrate only) were tested. The results indicate that only two fatty acids found in meat (9*c*18:1 and 22:5 *n*-3) are needed to assign meat very accurately to either of these feeding regimes for young bulls.

In summary, the results obtained from this study show that the fatty acid profile of intramuscular fat could be proposed as a way of differentiating meat from young bulls according to distinctive feeding regimes based on use of grain or grass. In addition to the usual practices carried out by companies to guarantee their labelling, the levels of two fatty acids (9*c*18:1 and 22:5 *n*-3) in the meat could be proposed as an effective tool for authenticating the origin of the beef and contribute to the traceability of the meat production system. These results provide consumers, the medical profession and meat producers with more information on the characteristics of beef and a scientific method for authenticating this product in the market. This approach would help not only to assure consumers of the origin of the meat they purchase but also to reinforce their confidence in this type of product.

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