The influence of breed and *mh*-genotype on carcass conformation, meat physico-chemical characteristics, and the fatty acid profile of muscle from yearling bulls

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Abstract

The influence of breed and *mh*-genotype on carcass conformation, meat physico-chemical characteristics and the fatty acid profile of muscle were studied. Samples from 16 yearling bulls from “Asturiana de los Valles” (AV, $n = 12$) and “Asturiana de la Montaña” (AM, $n = 4$) were collected. AV animals were classified into three groups according to the presence of the gene causing double-muscling (AV double-muscled (*mhl/mh*), $n = 4$; AV heterozygous (*mh/+*), $n = 4$; AV normal (*+/+*), $n = 4$). Double-muscled animals displayed better carcass traits, lower total fat (comprised of subcutaneous (SC), intermuscular (IT) and intramuscular (IM) deposits), higher lean, moisture and drip loss, and lighter meat than AV normal animals. Heterozygous animals showed intermediate characteristics. AM animals, being a more rustic and smaller breed, showed lower conformation, higher total fat (SC, IT and IM), lower moisture and darker meat. According to the intramuscular fatty acid profile, *mhl/mh* animals showed a lower proportion of SFA and MUFA, and a higher proportion of PUFA with an equal proportion of CLA in total fatty acid content. The P/S ratio increased with increasing number of *mh* alleles (or double-muscling character), while no differences between animal groups were found for the $n - 6/n - 3$ ratio.

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1. Introduction

There is increasing concern in industrialised countries with regard to the consumption of dietary fat, and in particular saturated fats, due to an increased association with cardiovascular-type diseases. Currently, there is greater emphasis on reducing or modifying the fat content of our diets such that saturates should be reduced and beneficial fatty acids (FA) such as long-chain polyunsaturates

(PUFA) and conjugated linoleic acids (CLA) should be increased.

Apart from reducing the saturated fat content of the diet, nutritional guidelines recommend that individuals should maintain a ratio of polyunsaturated to saturated FAs (P/S) at about 0.45 or higher and should increase the intake of $n - 3$ relative to $n - 6$ polyunsaturated FAs to achieve a $n - 6/n - 3$ ratio close to 4.0 (Department of Health, 1994; Mataix, Quiles, & Rodríguez, 2001). The ingestion of polyunsaturated FAs is important for the $n - 6/n - 3$ balance in membranes in all cells throughout the body and their positive effects in reducing coronary heart disease.

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In this sense, meat producers have responded by reducing the fat content of red meat, including beef, by selective breeding (Elías Calles et al., 2000; Huerta-Leidenz et al., 1993), and the introduction of modified feeding practices (French, O’Riordan, O’Kiely, Caffrey, & Moloney, 2001; Hornick, Van Eenaeme, Clinquart, Diez, & Istasse, 1998). Other strategies (for example some of the Fifth (EC) framework programmes, CLA in functional foods – QLK1-1999-00076, and Healthy Beef – QLRT-2000-31423), have involved the development of novel systems of producing beef and other products with improved nutritional value (in terms of FAs) and sensory quality (in terms of tenderness and flavour). The main aim of such studies is thus to provide an economically produced, healthy, and high quality product for the consumer.

Beef fat quality may be influenced by many factors including breed or genotype (Eichhorn et al., 1986; Malau-Aduli, Siebert, Bottema, & Pitchford, 1997; Nürnberg et al., 1999; Siebert, Deland, & Pitchford, 1996), age or live weight (Rule, Smith, & Romans, 1995), physiological status or gender (Zembayashi, Nishimure, Lunt, & Smith, 1995) and feeding regime (Mandell, Buchanan-Smith, Holub, & Campbell, 1997). The breed effect has been reported by several authors, mainly in cows and steers (Huerta-Leidenz et al., 1993; Itoh, Johnson, Cosgrove, Muir, & Purchas, 1999; May, Sturdivant, Lunt, Miller, & Smith, 1993), and it is one of the main factors affecting FA composition and carcass conformation because fat deposition differs between breeds, and is related to the ratio of triacylglycerols to phospholipids (Wood et al., 1999). To date, breed effects on meat quality based on genetic factors, such as the presence of mutations within the myostatin gene producing muscular hypertrophy (*mh*) have been studied in Belgian Blue and AV breeds (Clinquart et al., 1997; Fiems, De Campaneere, Bogaerts, Cottyn, & Boucqué, 1998; Oliván et al., 2004; Uytterhaegen et al., 1994) as well as in Piedmontese crossbreeds (Wheeler, Shackelford, Casas, Cundiff, & Koohmaraie, 2001). However, the effect of muscular hypertrophy on total FA composition has been mainly studied phenotypically but not genotypically. There is little information on the effect of the presence of the mutant myostatin gene causing the muscular hypertrophy, in homozygous or heterozygous states or combinations thereof (*mh/mh*, *mh/+*, *+/+*) on the FA profile (Raes, De Smet, & Demeyer, 2001).

In the present study, two local breeds from Asturias in Northern Spain “Asturiana de los Valles” (AV) and “Asturiana de la Montaña” (AM), have been compared and the differences described. The AV breed is known to produce carcasses of higher conformation and lower fat score (Piedrafita et al., 2003), meat with less intramuscular fat content and a lighter colour (Gil et al., 2001; Oliván et al., 1999a), relative to the AM breed. The aim of this study was thus to examine the differences between four biological types derived from different breeds (AV and AM) and the presence or absence of the muscular hypertrophy genotype (*mh/mh*,

mh/+, *+/+*) on carcass traits, meat composition, colour, drip loss and FA profile of *Longissimus* muscle.

2. Materials and methods

2.1. Animal management and diet composition

Sixteen yearling bulls from “Asturiana de los Valles” (AV, $n = 12$), a beef breed adapted to extensive production systems, and “Asturiana de la Montaña” (AM, $n = 4$), a beef breed characterised by small to medium-sized rustic animals adapted to less favoured mountain areas, were studied (Cañon, Gutierrez, Dunner, Goyache, & Vallejo, 1994). Blood samples from AV animals were analysed to determine the presence of the 11-bp deletion in the coding sequence of the myostatin gene causing double-muscling in cattle (Grobet et al., 1998) and classify animals into three groups: AV double-muscling (*mh/mh*, $n = 4$), AV heterozygous (*mh/+*, $n = 4$) and AV normal (*+/+*, $n = 4$). AM animals lack the mutation responsible for double-muscling ($n = 4$).

Calves were suckled by their mothers from birth (winter) to weaning (early autumn). After weaning, they were fattened by feeding concentrate meal (84% barley meal, 10% soya meal, 3% fat, 3% minerals, vitamins and oligoelements) and barley straw, both ad libitum, in the housing facilities of the research institute (S.E.R.I.D.A.).

2.2. Carcass measurements

Animals were slaughtered on reaching an average live weight of 556 ± 41 kg for the AV breed and 463 ± 11 kg for the AM breed (Table 1). Slaughtering was performed in a commercial abattoir according to standard procedures. Yearling bulls were weighed twice (on the day prior to slaughter and on the day of slaughter) to get the final live weight. After slaughtering and dressing, hot carcass weight was recorded before chilling the carcasses at 3 °C for 24 h.

Twenty-four hour *post-mortem*, carcasses were classified on conformation and fat cover according to the EUROP classification by a trained and experienced evaluator, with a scale ranging from 1 to 15, with 15 being the best conformation and the thickest fat cover (CEE, 1981).

The left half of each carcass was quartered between the 5th and 6th thoracic vertebrae with a circular saw. pH was measured on the *Longissimus thoracis* muscle (LT) of the 5th rib (pH 24 h) with a penetration electrode. The rib joint was excised from between the 6th and 9th ribs along the length of the bone to the limit of the *serratus dorsalis* muscle (Robelin & Geay, 1975) and transported to the laboratory.

The 6th rib was excised and total weight recorded. The LT muscle was separated and weighed and the rest of the rib was frozen at –20 °C until dissection. It was thawed overnight at 4 °C and dissected into lean, subcutaneous fat (SC), intermuscular fat (IT), bone and other tissues

Table 1
Mean values of age and live weight at slaughter, carcass quality traits, composition and pH of the 6th rib

	AV (mh/mh)	AV (mh/+)	AV(+/+)	AM	s.e.m.	P
Age (days)	463.00 ^a	440.25 ^a	435.25 ^a	535.00 ^b	8.114	.003
Live weight (kg)	566.50 ^b	554.50 ^b	548.50 ^b	463.00 ^a	9.702	.010
Cold carcass weight (kg)	354.00 ^c	327.75 ^{bc}	312.75 ^b	250.75 ^a	6.173	.001
Conformation	14.00 ^c	11.25 ^b	9.00 ^a	7.00 ^a	0.329	.000
Fat cover	1.25 ^b	4.75 ^a	4.75 ^a	5.35 ^a	0.260	.000
<i>Rib composition</i>						
% Lean	82.79 ^c	78.02 ^b	72.04 ^a	73.98 ^a	0.640	.000
% IT fat	5.61 ^a	7.70 ^a	12.47 ^b	11.02 ^b	0.425	.000
% SC fat	1.39 ^a	2.49 ^b	3.17 ^{bc}	3.37 ^c	0.122	.000
% Total fat	7.00 ^a	10.19 ^b	15.64 ^c	14.39 ^c	0.483	.000
% Bone	10.20	11.78	12.32	11.63	0.372	.269
pH (24 h)	5.42 ^b	5.53 ^{ab}	5.52 ^{ab}	5.64 ^a	0.030	.045

s.e.m., standard error of the mean; ^{a–c} means with different superscript are significantly different at $P \leq 0.05$. IT, intermuscular; SC, subcutaneous. AV(mh/mh), Asturiana de los Valles – double-muscled; AV(mh/+), Asturiana de los Valles – heterozygous; AV(+/+), Asturiana de los Valles – normal; AM, Asturiana de la Montaña.

(blood vessels, ligaments, tendons). Other tissues were recorded as bone. The 6th rib dissection was used as a predictor of the carcass composition (Oliván, Martínez, García, Noval, & Osoro, 1999b).

2.3. Analysis

The LT muscle of the 6th rib was placed in a polioxpan tray, covered with plastic film permeable to O₂ and aged at 4 °C for 7 days, then minced with an electrical chopper and myoglobin concentration assessed by absorbance measurements at 532 nm (Hornsey, 1956).

Following 24 h of blooming after cutting at 4 °C, meat colour was measured on the LT of the 7th rib at 48 h *post-mortem* with a Minolta CM-200 colorimeter using a C illuminant and a 2° standard observer in the CIE $L^*a^*b^*$ space (lightness, L^* ; redness, a^* ; yellowness, b^*).

The LT of the 8th rib was dissected, vacuum packed and frozen at –80 °C at 24 h *post-mortem* for subsequent FA composition analysis by gas–liquid chromatography (GC) and chemical composition analysis by near infrared spectroscopy (NIRS) (Oliván, de la Roza, Mocha, & Martínez, 2002). Moisture content was calibrated by reference to an oven drying method (ISO 1442-1973), fat content by Soxhlet extraction (ISO 1443-1973) and protein content using Kjeldahl analysis (ISO 937-1978).

Drip loss was determined from the 9th rib at 48 h *post-mortem* using 50 g meat samples (Honikel, 1998).

2.3.1. Total fatty acid analysis

Tissue (1 g) was saponified in 6 mL 5 M KOH in methanol/water (50:50) at 60 °C for 1 h, and the extracted fatty acids (FA) were methylated using trimethylsilyl-diazomethane (TM-DM) in methanol:toluene (2:1 %v/v) at 40 °C for 10 min – based on a modification of the method by Elmore, Mottram, Enser, and Wood (1999) as outlined in Aldai, Murray, Nájera, Troy, and Osoro (2004). Analyses were performed on a Varian Star CX3400 GC with a flame ionisation detector (FID) and automatic sample injection

on a septum-equipped programmable injector (SPI) operating in “on-column” mode. Fatty acid methyl esters (FAME) were separated using a CP-SIL 88 for FAME (WCOT FUSED SILICA 100 m × 0.25 mm i.d., 0.2 µm film thickness) column (VARIAN). The oven temperature program was initially set at 100 °C, and then employed a temperature ramp to 170 °C at 2 °C/min where it remained for 15 min, followed by a slower ramp to 180 °C at 0.5 °C, and a subsequent one to 200 °C at 10 °C/min, where it remained for 10 min. Final ramp to 230 °C at 2 °C/min where it remained for 10 min. The injector and detector ports were set at 250 and 300 °C respectively. The carrier gas was helium with a flow rate of 2 mL/min.

FAMES were identified according to similar peak retention times using standards (Sigma–Aldrich) and quantified according to the use of an internal standard (C21:0 free FA) with its addition prior to saponification. Relative response factors were previously calculated for each FA quantified.

2.4. Statistical analysis

The statistical analysis was conducted using SPSS 11.5 for Windows. One-way ANOVA analysis was applied to study the differences between all animal groups as four independent biological types AV(mh/mh), AV(mh/+), AV(+/+) and AM. No correction (e.g., including fatness as a covariate) was made for the higher fat content of the AM breed, since this was considered to be a biological type effect. When analysis of variance gave a significant difference for the main effect, the LSD post hoc test was applied (multiple comparison of means). If the data did not achieve homogeneity of variance, the Kruskal–Wallis non-parametric test was applied, followed by the *U* Mann–Whitney post hoc test if significant differences were attained. Relationships between variables were assessed by regression analysis and determination of Pearson correlation coefficients for all animal groups.

3. Results and discussion

3.1. Carcass characteristics

Table 1 shows age, live weight at slaughter and carcass traits (weight, conformation, fat cover and composition) and pH at 24 h *post-mortem* for all animals. AM animals showed significantly higher age and lower live weight at slaughter, due to the rusticity and lower live weight gains of this breed. AM animals also showed the lowest conformation and a high fat cover, in agreement with earlier studies comparing AV and AM breeds (Piedrafita et al., 2003). The double-muscling character produced a significant increase in carcass conformation and a significant decrease of fat cover, as widely reported in Belgian Blue and AV breeds (Arthur, Makarechian, Price, & Berg, 1989; De Smet et al., 2000; Martínez et al., 2003; Nürnberg et al., 1999; Uytterhaegen et al., 1994).

The dissection of the 6th rib showed that muscular hypertrophy, both in homozygous (AV *mh/mh*) and in heterozygous (AV *mh/+*) animals, produced a significantly higher percentage of lean and a lower total, intermuscular and subcutaneous fat in agreement with earlier studies (Martínez et al., 2003). The percentage of bone was similar for all biological types.

AM animals recorded the highest ultimate pH, while the AV animals showed similar but lower pH values. The tendency of a lower pH in AV animals, particularly with the *mh/mh* genotype, could be due to the higher proportion of fast twitch fibers and higher glycolytic metabolism of the *Longissimus* muscle described in double-muscling bulls by Batjoens, Fiems, Van Hoof, Van Vooren, and Vereecke (1991), Gagnière, Picard, Jurie, and Geay (1997) and Oliván et al. (2004).

3.2. Chemical composition

Biological type significantly affected the intramuscular (IM) fat content (%) of *Longissimus thoracis* muscle (Table 2). AM animals showed the highest content of IM fat and double-muscling animals the lowest content, while other AV genotypes (*mh/+* and *+/+*) showed intermediate amounts.

This is in agreement with earlier works indicating that double-muscling decreases IM fat content (Clinquart et al., 1997; Oliván et al., 2004).

3.3. Colour

Even though there were no significant differences ($P = 0.053$) amongst the animal groups, biological type tended to affect the myoglobin content of meat, where AM animals showed higher concentrations (Table 2). AV animals exhibit a decreasing trend in myoglobin content from normal (*+/+*) to double-muscling animals (*mh/mh*). This agrees with earlier studies that describe a decrease in haem pigment content in meat of double-muscling animals with respect to normal and heterozygous animals (Bailey, Enser, Dransfield, Restall, & Avery, 1982; De Smet et al., 2000).

Colour parameters showed a significant effect of biological type on lightness (L^*) of meat (Table 2), which increased with the number of *mh* alleles. Meat from AM bulls has previously been described as one of the darkest amongst several Spanish beef breeds (Gil et al., 2001).

There were no significant differences among biological types in redness (a^* value) or in yellowness (b^* value).

3.4. Drip loss

Breed and *mh*-genotype showed a significant effect on drip loss of meat measured at 48 h *post-mortem* (Table 2). Meat from AM, AV(*+/+*) and AV(*mh/+*) animals showed lower drip loss than double-muscling animals which had the highest drip loss value. These results are in agreement with those of Uytterhaegen et al. (1994) and De Smet et al. (2000) who also observed increased drip loss in beef from double-muscling animals (*mh/mh*). Oliván et al. (2004) found that raw meat of double-muscling animals had higher drip loss and hence lower water-holding capacity than meat from heterozygous bulls. This effect could be the result of several factors including, higher glycolytic metabolism in muscle of *mh/mh* bulls (Batjoens et al., 1991; Gagnière et al., 1997; Oliván et al., 2004), differences in collagen structure (Bailey et al., 1982), or the lower IM fat content

Table 2

Effect of biological type on moisture, IM fat and protein percentages, myoglobin content, colour (L^* , a^* and b^* parameters) and drip loss of *Longissimus thoracis* muscle

	AV (<i>mh/mh</i>)	AV (<i>mh/+</i>)	AV(<i>+/+</i>)	AM	s.e.m.	<i>P</i>
Moisture (%)	75.18	74.82	75.00	74.75	0.100	.469
IM fat (%)	0.94 ^a	1.80 ^b	1.90 ^b	2.66 ^c	0.100	.002
Protein (%)	23.16	22.94	22.77	22.70	0.090	.270
Myoglobin (mg/kg)	3.41	4.14	4.49	5.06	0.227	.053
L^* (24 h)	45.68 ^b	44.43 ^{ab}	41.00 ^a	40.91 ^a	0.772	.038
a^* (24 h)	23.55	24.58	23.99	24.78	0.460	.810
b^* (24 h)	10.34	13.43	10.62	10.29	0.520	.077
Drip loss (%)	2.37 ^b	1.20 ^a	1.49 ^{ab}	0.70 ^a	0.208	.015

s.e.m., standard error of the mean. ^{a-c} Means with different superscript are significantly different at $P \leq 0.05$. IM, intramuscular; AV(*mh/mh*), Asturiana de los Valles – double-muscling; AV(*mh/+*), Asturiana de los Valles – heterozygous; AV(*+/+*), Asturiana de los Valles – normal; AM, Asturiana de la Montaña.

of *mh/mh* meat, as proposed by Oliván, Osoro, Martínez, and Guerrero (2003). The later group compared meat quality of different breeds (AV and AM) and/or genotypes (*mh/mh*, *mh/+*, *+/+*) and found that when IM fat content was high there was a concomitant lower result for juice loss from raw meat, measured as the expressible juice under pressure. In the present study, the relationship between FA content (mg/100 g muscle) and drip loss (%) was similarly confirmed ($r = -0.53$, $P \leq 0.05$) (Fig. 1(a)). As a rapid pH fall or a lower pH would tend to cause protein denaturation and greater drip loss, the relationship between pH 24 h *post-mortem* and drip loss (%) was also calculated and the observed correlation was close to significance ($r = 0.49$, $P = 0.053$) (Fig. 1(b)).

3.5. Total fatty acid composition of intramuscular fat

The total FA composition of the four biological types is shown in Tables 3 and 4, expressed as mg/100 g muscle and as % of total FAs quantified, respectively. The effect of biological type was more pronounced when comparing the content of specific FAs expressed as mg/100 g muscle; significant differences were observed in 63% of the individual FAs, in contrast, only 52% of FAs were affected when individual percentage concentrations were compared.

In general, significant differences were observed in the majority of individual and groups of FA quantities (saturated (SFA), branched (BFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids) depending on the biological type, except for individual $n - 3$ FA species. Generally, individual and total SFAs were increased significantly in AV genotypes with decreasing numbers of *mh* alleles. In contrast, AM animals showed the highest individual and total SFAs. However, when percentages were compared few significant differences were found in this group (Table 4).

BFAs are predominantly synthesised by ruminal micro-organisms and changes in their content are often correlated with changes in feeding strategies (Dewhurst, Moorby, Danielón, & Tweed, 2002). Even when diets were equal for all animals, significant differences were found between biological types in *a*-C15:0 and *i*-C16:0, and AM animals ap-

peared to have the highest individual and total BFA content. These differences in BFA quantity could be related to the higher IM fat content in AM animals. No significant differences between biological types were found for the total percentage BFA even when some individual differences were found.

Total MUFA content appeared to be similar in AV genotypes while AM animals had a significantly higher content. When comparing MUFAs individually, the general trend indicated that leaner animals (*mh/mh*, *mh/+*) were similar while fatter animals (*+/+*, AM), being similar to each other were none-the-less significantly different to the leaner animals. Trans-vaccenic acid (TVA) was the only MUFA which gave no significant quantity differences between different animal groups, even with a high variability in the data, where leaner animals (*mh/mh*, *mh/+*) showed lower contents than fatter animals (*+/+*, AM). Referring to MUFA percentages (Table 4), significant differences between biological types were observed for C14:1 and C16:1 (as for their homologous SFAs (C14:0 and C16:0)). C18:1c9 was the major MUFA and it represented a significantly higher content and percentage in fatter animals. This effect was also found by Raes et al. (2001) in Belgian Blue genotypes. No significant differences were observed for C18:1t11 proportions.

The mean absolute amount of PUFA was significantly higher for AM, lower for *mh/mh* and *mh/+* animals, and intermediate for *+/+* animals (Table 3). Due to the larger differences in total FA content between biological types, the relative proportion of PUFA in total FAs significantly increased with increasing *mh* alleles (Table 4). In other words, the higher IM fat content of bulls of AM and *+/+* compared with the *mh/+* and *mh/mh* is mainly due to a two to three times higher content of SFA and MUFA as found by Raes et al. (2001) in concentrate fed Belgian Blue bulls. This could be related to the heterogeneous composition of IM fat, containing triglycerides in adipocytes, and polar lipids, mainly phospholipids, in the membrane structures of both adipocytes and muscle fibres. A lower fat content reflects fewer and smaller adipocytes, containing fewer triglycerides, accompanied by a relative increase in the proportion of phospholipids and an increased PUFA

Fig. 1. Correlation between drip loss (%) and sum of total FAs (mg/100 g of muscle) (a), and pH at 24 h *postmortem* (b). Data taken from all the animal types: ●*mh/mh*, ■*mh/+*, ■*+/+*, ●AM.

Table 3

Effect of biological type on intramuscular total fatty acid content (mg/100 g muscle) of *Longissimus thoracis* muscle

	Fatty acid	AV(mh/mh)	AV(mh/+)	AV(+/+)	AM	s.e.m.	P
C14:0 <i>np</i>	Myristic acid	16.25 ^a	30.67 ^{ab}	44.81 ^b	80.87 ^c	7.40	.018
C15:0 <i>np</i>	Pentadecanoic acid	3.25 ^a	5.69 ^{ab}	6.35 ^b	10.80 ^c	0.92	.026
C16:0 <i>np</i>	Palmitic acid	253.36 ^a	363.93 ^{ab}	486.65 ^b	775.68 ^c	61.76	.024
C17:0	Margaric acid	9.38 ^a	14.42 ^a	17.20 ^a	26.29 ^b	2.06	.011
C18:0	Stearic acid	144.94 ^a	190.90 ^a	210.78 ^a	338.99 ^b	24.48	.014
C20:0	Arachidic acid	1.33	1.07	1.22	2.04	0.59	.075
<i>i</i> -C15:0 <i>np</i>	13-Methyltetradecanoic acid	0.70	1.06	0.98	1.89	0.17	.131
<i>a</i> -C15:0 <i>np</i>	12-Methyltetradecanoic acid	1.30 ^a	2.13 ^{bc}	1.87 ^b	3.72 ^c	0.32	.035
<i>i</i> -C16:0	14-Methylpentadecanoic acid	1.67 ^a	1.80 ^a	2.15 ^a	3.77 ^b	0.26	.003
<i>i</i> -C17:0	15-Methylhexadecanoic acid	3.13	3.43	3.80	5.19	0.34	.146
C14:1 <i>cis</i> 9	Myristoleic acid	1.97 ^a	4.29 ^{ab}	7.43 ^b	8.89 ^b	0.98	.036
C16:1 <i>cis</i> 9 <i>np</i>	Palmitoleic acid	15.61 ^a	30.97 ^{ab}	43.26 ^b	72.72 ^b	6.87	.014
C17:1 <i>cis</i> 10	<i>cis</i> -10-Heptadecenoic acid	5.14 ^a	7.23 ^a	10.46 ^{ab}	15.15 ^b	1.33	.021
C18:1 <i>trans</i> 11	Trans-vaccenic acid (TVA)	58.62	51.65	94.22	93.92	9.46	.239
C18:1 <i>cis</i> 9 <i>np</i>	Oleic acid	157.13 ^a	273.58 ^{ab}	374.69 ^b	624.82 ^b	58.29	.024
C18:1 <i>cis</i>	Other <i>cis</i> isomers	44.92 ^a	63.65 ^a	63.78 ^a	109.46 ^b	34.74	.034
C18:2 <i>n</i> – 6	Linoleic acid (LA)	170.87 ^a	188.93 ^a	194.68 ^{ab}	235.57 ^b	8.91	.048
C18:3 <i>n</i> – 6	γ -Linolenic acid	3.26	1.70	2.73	3.15	0.27	.147
C20:2 <i>n</i> – 6	<i>cis</i> -11,14-Eicosadienoic acid	1.38 ^a	1.44 ^a	2.01 ^b	2.04 ^b	0.11	.028
C20:3 <i>n</i> – 6	Dihomo- γ -linolenic acid	7.69	8.11	9.13	8.76	0.31	.380
C20:4 <i>n</i> – 6	Arachidonic acid	25.17 ^a	27.17 ^a	28.34 ^a	35.34 ^b	1.16	.001
C22:4 <i>n</i> – 6	<i>cis</i> -7,10,13,16-Docosatetraenoic acid	3.86 ^a	4.49 ^a	4.60 ^a	6.05 ^b	0.28	.016
C18:3 <i>n</i> – 3	α -Linolenic acid (ALA)	9.85	12.50	13.27	16.47	0.92	.064
C20:5 <i>n</i> – 3	Eicosapentaenoic acid (EPA)	3.50	3.49	3.54	1.95	0.29	.136
C22:5 <i>n</i> – 3	Docosapentaenoic acid (DPA)	20.91	21.85	23.94	18.90	21.40	.181
C22:6 <i>n</i> – 3	Docosahexaenoic acid (DHA)	0.55	0.56	0.63	0.40	0.04	.271
<i>cis</i> 9, <i>trans</i> 11-CLA <i>np</i>		1.28 ^a	2.47 ^{ab}	3.38 ^b	5.29 ^b	0.59	.035
Σ SFA <i>np</i>		427.17 ^a	605.61 ^{ab}	765.80 ^b	1232.65 ^c	95.48	.024
Σ BFA		6.80 ^a	8.42 ^a	8.80 ^a	14.56 ^b	1.06	.029
Σ MUFA		283.40 ^a	431.36 ^a	593.84 ^a	924.96 ^b	79.29	.008
Σ PUFA		248.33 ^a	272.72 ^a	286.29 ^{ab}	339.92 ^b	10.67	.015
P/S <i>np</i>		0.44 ^b	0.39 ^{ab}	0.28 ^a	0.22 ^a	0.03	.036
<i>n</i> – 6/ <i>n</i> – 3		6.09	6.15	5.87	7.76	0.27	.053
Desaturation index (C16)		8.07 ^b	7.48 ^{ab}	8.45 ^b	5.78 ^a	0.38	.050
Desaturation index (C18)		63.95 ^b	56.86 ^{ab}	63.98 ^b	51.64 ^a	1.79	.017
Desaturation index (CLA) <i>np</i>		2.20	5.11	5.77	6.37	1.10	.229

np, non-parametric test (data not achieving homogeneity of variance condition); s.e.m., standard error of the mean.^{a–c} means within a row with different superscripts are significantly different at $P \leq 0.05$.

Σ SFA = sum of all saturated FAs; Σ BFA = sum of all branched FAs; Σ MUFA = sum of all monounsaturated FAs; Σ PUFA = sum of all polyunsaturated FAs; $n - 6$ = sum of C18:2, C18:3, C20:2, C20:3, C20:4, C22:4; $n - 3$ = sum of C18:3, C20:5, C22:5, C22:6; P/S = (C18:2*n* – 6 + C18:3*n* – 3)/(C14:0 + C16:0 + C18:0); Desaturation index (C16) = 100*[(C16:1 *cis*9)/(C16:0 + C16:1 *cis*9)]; Desaturation index (C18) = 100*[(C18:1 *cis*9)/(C18:0 + C18:1 *cis*9)]; Desaturation index (CLA) = 100*[(*cis*9*trans*11CLA)/(C18:1*trans*11 + *cis*9*trans*11CLA)].

AV(mh/mh), Asturiana de los Valles – double-muscled; AV(mh/+), Asturiana de los Valles – heterozygous; AV(+/+), Asturiana de los Valles – normal; AM, Asturiana de la Montaña.

content (De Smet et al., 2000; Laborde, Mandell, Tosh, Wilton, & Buchanan-Smith, 2001; Nürnberg et al., 1999). Breed affects the composition of fat tissue mainly through its effect on total fat content (Eichhorn, Bailey, & Blomquist, 1985). The quantities of the $n - 6$ series of FAs showed significant differences, except for C18:3 and C20:3. Looking at proportionate amounts, double-muscled animals showed significantly higher proportions of C18:2*n* – 6, C18:3*n* – 6, C20:2*n* – 6, and C20:3*n* – 6. $N - 3$ FAs did not show any content differences between biological types but in comparing percentages, they appeared in higher proportions in leaner animals (mh/mh, mh/+).

In the present study, the *c*9,*t*11CLA isomer of conjugated linoleic acid is the only isomer reported. Many studies have indicated that this is the isomer which represents the major proportion (>85%) of CLA in beef (Chin, Storkson, Ha, & Pariza, 1992; Mossoba et al., 1999). The absolute *c*9,*t*11CLA content (mg/100g muscle) was about twice as high in AM and AV(+/+) animals than in the other AV genotypes, probably due to the much higher fat content of the AM and AV(+/+) animals. This effect was also found in other studies where *c*9,*t*11CLA content variation was influenced by the total lipid content, and hence with variation in the neutral lipid fraction (Noci, Moloney, & Monahan, 2004; Raes et al., 2001; <http://>

Table 4

Effect of biological type on intramuscular total fatty acid percentage (% of total fatty acids quantified) of *Longissimus thoracis* muscle

	Fatty acid	AV(mh/mh)	AV(mh/+)	AV(+/+)	AM	s.e.m.	P
C14:0 <i>np</i>	Myristic acid	1.66 ^a	2.11 ^{ab}	2.69 ^{bc}	3.21 ^c	0.18	.015
C15:0	Pentadecanoic acid	0.34	0.40	0.38	0.43	0.02	.273
C16:0 <i>np</i>	Palmitic acid	26.13 ^a	26.77 ^{ab}	29.42 ^{bc}	30.85 ^c	0.64	.022
C17:0	Margaric acid	0.97	1.09	1.04	1.04	0.03	.663
C18:0	Stearic acid	15.05	14.89	12.87	13.38	0.49	.324
C20:0 <i>np</i>	Arachidic acid	0.14	0.08	0.07	0.08	0.01	.298
<i>i</i> -C15:0	13-Methyltetradecanoic acid	0.07	0.07	0.06	0.07	0.00	.542
<i>a</i> -C15:0	12-Methyltetradecanoic acid	0.14	0.15	0.11	0.15	0.01	.190
<i>i</i> -C16:0	14-Methylpentadecanoic acid	0.17 ^b	0.13 ^a	0.13 ^a	0.15 ^{ab}	0.01	.012
<i>i</i> -C17:0	15-Methylhexadecanoic acid	0.33 ^b	0.26 ^{ab}	0.23 ^a	0.21 ^a	0.02	.029
C14:1 <i>cis</i> 9	Myristoleic acid	0.20 ^a	0.29 ^a	0.44 ^b	0.34 ^{ab}	0.03	.027
C16:1 <i>cis</i> 9	Palmitoleic acid	1.60 ^a	2.18 ^{ab}	2.59 ^b	2.85 ^b	0.16	.014
C17:1 <i>cis</i> 10 <i>np</i>	<i>cis</i> -10-Heptadecenoic acid	0.53	0.52	0.63	0.60	0.03	.446
C18:1 <i>trans</i> 11	Trans-vaccenic acid (TVA)	6.00	3.57	5.54	4.26	0.49	.248
C18:1 <i>cis</i> 9	Oleic acid	16.08 ^a	19.80 ^{ab}	22.73 ^b	24.01 ^b	1.04	.013
C18:1 <i>cis</i> 11	<i>cis</i> -Vaccenic acid	4.60	4.76	3.79	4.33	0.16	.126
C18:2 n – 6 <i>np</i>	Linoleic acid (LA)	17.85 ^b	15.74 ^{ab}	11.67 ^a	9.91 ^a	1.04	.042
C18:3 n – 6	γ -Linolenic acid	0.35 ^b	0.15 ^a	0.17 ^a	0.14 ^a	0.03	.040
C20:2 n – 6	<i>cis</i> -11,14-Eicosadienoic acid	0.14 ^b	0.12 ^{ab}	0.12 ^{ab}	0.09 ^a	0.01	.050
C20:3 n – 6	Dihomo- γ -linolenic acid	0.81 ^b	0.70 ^b	0.56 ^{ab}	0.34 ^a	0.06	.032
C20:4 n – 6 <i>np</i>	Arachidonic acid	2.64	2.38	1.73	1.49	0.18	.051
C22:4 n – 6 <i>np</i>	<i>cis</i> -7,10,13,16-Docosatetraenoic acid	0.40	0.40	0.28	0.25	0.03	.222
C18:3 n – 3	α -Linolenic acid (ALA)	1.02 ^c	0.98 ^c	0.80 ^b	0.66 ^a	0.04	.000
C20:5 n – 3	Eicosapentaenoic acid (EPA)	0.37 ^b	0.31 ^b	0.22 ^{ab}	0.09 ^a	0.35	.037
C22:5 n – 3	Docosapentaenoic acid (DPA)	2.20 ^b	1.91 ^b	1.48 ^{ab}	0.82 ^a	0.18	.021
C22:6 n – 3 <i>np</i>	Docosahexaenoic acid (DHA)	0.06 ^b	0.05 ^b	0.04 ^b	0.02 ^a	0.01	.020
<i>cis</i> 9, <i>trans</i> 11-CLA <i>np</i>		0.13	0.18	0.21	0.20	0.02	.272
Σ SFA		44.28	45.34	46.48	49.00	0.70	.072
Σ BFA		0.71	0.62	0.53	0.58	0.02	.058
Σ MUFA		29.02 ^a	31.12 ^{ab}	35.72 ^b	36.38 ^b	1.12	.033
Σ PUFA <i>np</i>		25.99 ^b	22.92 ^{ab}	17.28 ^a	14.03 ^a	1.55	.028

np, non-parametric test (data not achieving homogeneity of variance condition); s.e.m., standard error of the mean.^{a–c} Means within a row with different superscripts are significantly different at $P \leq 0.05$. Σ SFA = sum of all saturated FAs; Σ BFA = sum of all branched FAs; Σ MUFA = sum of all monounsaturated FAs; Σ PUFA = sum of all polyunsaturated FAs.

AV(mh/mh), Asturiana de los Valles – double-muscled; AV(mh/+), Asturiana de los Valles – heterozygous; AV(+/+), Asturiana de los Valles – normal; AM, Asturiana de la Montaña.

www.healthybeef.iger.bbsrc.ac.uk). Hence, the content of *c9t11CLA* was positively correlated with the total FA content ($r = 0.85$, $P \leq 0.001$). On the other hand, when comparing on a proportionate basis, *c9t11CLA* content did not vary much between biological types (approximately 0.18 g per 100 g FAs). Regarding the relationship between TVA and *c9t11CLA*, there are some studies where linear correlation between these two FAs was found (Enser et al., 1999; Lawless et al., 1999), and other studies where no significant correlation was found, which was explained by the interference of *trans* isomers other than C18:1 t 11 (Raes et al., 2001). It appears endogenous formation of *c9t11CLA* in animal tissues can also be important (Baumgard, Corl, & Griinari, 1999) compared to its formation in the rumen, as a result of biohydrogenation of PUFA. Breed or genotype effects could act by enhancing or inhibiting the $\Delta 9$ -desaturase activity, the enzyme responsible for the conversion of C18:1 t 11 into

c9t11CLA. Differences in fat content and a possible higher desaturase activity in fatter animals, as found by Siebert et al. (2003), could help explain the higher *c9t11CLA* content in fatter animals in comparison to leaner animals. We could neither find any relationship between TVA and *c9t11CLA* content, nor significant differences in the CLA desaturation index between the different animal groups (Table 3). A high variability in animal-to-animal TVA content was observed in this study and may have masked any significant differences if present.

The ratio of PUFA to SFA (P/S) was different between biological types (Table 3). The P/S ratio increased significantly as the number of *mh* alleles increased. This higher P/S ratio of the *mh/mh* and *mh/+* animals was mainly due to the higher relative amount of PUFA to the lower content of SFA and MUFA. The content of SFA and MUFA increases faster with increasing animal fatness than does the content of PUFA, leading to a decrease in the

relative proportion of PUFA and consequently in the P/S ratio. Only double-muscled animals approached the recommendations of nutritional guidelines for a P/S of 0.45 or higher. According to some reports, the P/S ratio of beef can drop to a value of 0.05 in fat breeds and can rise to >0.5 in very lean breeds (e.g., double-muscled animals) as reviewed by De Smet, Raes, and Demeyer (2004). This variation is much larger than what can actually be achieved in beef by alterations of the diet. Apart from using very lean animals, the only way to improve the P/S ratio in ruminant meats is by preventing ruminal biohydrogenation or by feeding protected PUFA supplements (Scollan, Enser, Gulati, Richardson, & Wood, 2003). P/S ratios obtained in this study were similar to the values obtained by Raes et al. (2001) in Belgian Blue bulls, and in general, higher than values reported in other studies for animals fed grain diets (Enser et al., 1998; Rule, Broughton, Shellito, & Maiorano, 2002), and found in commercial steaks (Enser, Hallett, Hewitt, Fursey, & Wood, 1996). Hence, as suggested by Raes et al. (2001) for the Belgian Blue breed, the genetically determined lean nature of the AV breed, resulting in a larger proportion of PUFA, could partly have the same effect as replacing concentrates with forage in the fattening diets of fat breeds (i.e., Hereford, Angus, AM).

Referring to the $n-6/n-3$ ratio, even though no significant differences were found in AM animals, they did tend to show a higher value than AV genotypes. In general, none of the animal groups ratio was as low as the nutritionally recommended value (4.0). As seen in the P/S ratio, the $n-6/n-3$ ratio of the total lipid fraction may vary depending on the $n-6/n-3$ ratio of the phospholipid and triacylglycerol fractions though the $n-6/n-3$ ratios are much more affected by feeding regimes than by genetics (Enser et al., 1996; Itoh et al., 1999).

For a further improvement of the intramuscular FA composition, there should be greater focus on the possibility of feeding these genetically lean animals with forage-based diets.

3.5.1. Fatty acid metabolism

The relative proportions of the individual $n-3$ and $n-6$ FAs in their corresponding series is shown in Fig. 2(a) and (b), respectively. Differences in the metabo-

lism could be observed when comparing $n-3$ and $n-6$ type of FAs.

Concerning $n-3$ FAs, while the C18:3 $n-3$ was significantly lower in the AV genotypes compared with the AM animals, the $n-3$ derivative C20:5 $n-3$ was significantly higher, but not so for the final products of this biosynthetic pathway (C22:5 $n-3$ and C22:6 $n-3$), i.e., the conversion of C18:3 $n-3$ to C20:5 $n-3$ occurred faster in AV animals than in AM. However, proportionally, a lower conversion of C20:5 $n-3$ to C22:5 $n-3$ occurred in AV animals. The last conversion, C22:5 $n-3$ to C22:6 $n-3$, appeared to occur to the same extent in all biological types. It should be mentioned that the intermediates C18:4 $n-3$ and C20:4 $n-3$ were not detected. These findings could be a reflection of differences in enzyme (desaturase and elongase) activities between breeds (AV and AM) as suggested by Raes et al. (2001).

No differences in the metabolism of the $n-6$ series could be seen (Fig. 2(b)) as the conversion of C18:2 $n-6$ to C22:4 $n-6$ took place at the same rate in all biological types. The metabolites C18:3 $n-6$, C20:2 $n-6$ and C20:3 $n-6$ did not accumulate and were rapidly converted to C20:4 $n-6$. Moreover, C20:4 $n-6$ tended to resist further conversion into C22:4 $n-6$ (Rosenthal, Garcia, Jones, & Sprecher, 1991), explaining the low concentration of C22:4 $n-6$ found generally in animal muscles. Comparing the conversion of C20:5 $n-3$ to C22:5 $n-3$ and C20:4 $n-6$ to C22:4 $n-6$, both governed by the $\Delta 5$ -desaturase, it seems that this enzyme preferentially acts on the $n-3$ type of FAs.

The results of this study suggest that breed effect is more visible in $n-3$ FA metabolism than in $n-6$ FA metabolism. As observed in other studies, it is difficult to postulate a clear mechanism about FA competition for deposition in muscle tissue.

4. Conclusions

Double-muscling (*mh/mh*) in AV beef is associated with better carcass traits, lower fat (SC, IT and IM), higher drip loss, and lighter meat than normal (+/+) animals. In general, heterozygous animals (*mh/+*) showed intermediate characteristics. In contrast, AM animals had lower conformation, higher fat (SC, IT and IM) and darker meat. Double-muscled animals showed a lower proportion of SFA

Fig. 2. Influence of biological type on the metabolism of (a) $n-3$ and (b) $n-6$ fatty acids; ^{a,b} significantly different with $P \leq 0.05$.

and MUFA, and a higher proportion of PUFA with an equal proportion of CLA in total fatty acid content. The P/S ratio increased with increasing number of *mh* alleles (or double-muscling character), while no differences between animal groups were found for the $n - 6/n - 3$ ratio.

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