Characterisation of intramuscular, intermuscular and subcutaneous adipose tissues in yearling bulls of different genetic groups

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Abstract

Fatty acid (FA) composition of intramuscular (IM, *Longissimus thoracis* muscle), intermuscular (IT) and subcutaneous (SC) fat of one hundred intensively fed yearling bulls with different propensities to fatten were studied. Meat samples were collected from Asturiana de los Valles bulls with different genotypes with respect to the myostatin gene (*mh/mh* $n = 24$, *mh/+* $n = 26$ and *+/+* $n = 25$) and from Asturiana de la Montaña ($n = 25$) bulls lacking the mutation responsible for double muscling and characterised by small to medium-frame size adapted to less favoured mountain areas. FA profiles were expressed as percentages of total FA (g/100 g of total FA) and organised into groups (saturated (SFA), branched (BFA), monounsaturated (MUFA), C18:1*trans*, polyunsaturated (PUFA), $n - 6$, $n - 3$, conjugated linoleic acids (CLA), unsaturated (UFA)) and ratios (MUFA/SFA (M/S), PUFA/SFA (P/S), UFA/SFA (U/S), $n - 6/n - 3$).

The IT depot was the most saturated and SC depot contained the most monounsaturated FAs, while IM fat had the most polyunsaturated FAs. IM fat showed the highest P/S ratio and for the $n - 6/n - 3$ ratio there were no significant differences between adipose tissue depots.

In general, genotype effects were more pronounced in IM and SC fat profiles compared to the IT depot, for which no significant differences between genotypes were found in SFA, PUFA (including $n - 6$ and $n - 3$), UFA and most of the ratios. IM fat of *mh/mh* animals had the highest content of PUFA and thus the highest P/S ratio. Accordingly, the presence of the gene causing double muscling influenced the tendency to deposit carcass fat and its FA composition, mainly in IM fat. In general, when carcass fat decreased, SFA content decreased while PUFA and UFA contents increased due to the changes in their percentages.

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1. Introduction

Fat deposition in cattle is generally thought to occur in three phases. Greater rates of fat deposition first occur around the viscera and kidneys, stomach and adjacent organs followed by deposition in intermuscular (IT) and

subcutaneous (SC) depots and finally in the intramuscular (IM) depot (Hood, 1982). However, Wood, MacFie, Pomeroy, and Twinn (1980) found in sheep that IT fat matured earlier than SC fat and internal fat depots (kidney, knob, channel and omental fat). These authors stated that the internal depots, although present in fairly large amounts in young animals, are not early maturing as is often thought. Nevertheless, no simple chronological separation of the three phases of fat deposition is detectable as it depends highly on breed and dietary energy levels

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(Callow, 1962). Breed also influences the pattern of systemic fat depot development (Kempster, 1980–1981) with breeds noted for beef production, such as Herefords, having more SC and less abdominal fat at the same weight of total fat than breeds noted for milk production, such as Friesians (Truscott, Wood, & Denny, 1983; Wright & Russel, 1984). Furthermore, at the same body weight, late-maturing breeds have a lower concentration of fat than early-maturing breeds (Aldai et al., 2006; Martínez et al., 2003). Thus, the introduction of late-maturing breeds with a large adult size may be used to delay fat deposition and enhance lean growth in cattle populations.

Breed, therefore, affects the distribution of fat within the carcass (Wright & Russel, 1984), and together with systemic location, has been found to affect the FA composition of individual depots (Barber et al., 2000; Eichhorn, Bailey, & Blomquist, 1985; Marmer, Maxwell, & Williams, 1984; Webb, De Smet, Van Nevel, Martens, & Demeyer, 1998). The FA profile of adipose tissue can also be affected by several factors including the amount and type of diet, the rate of fat deposition and temperature (both ambient and body) (Callow, 1962). There is also preferential deposition of absorbed FAs into internal depots (Duncan & Garton, 1967; Gibney & L'Estrange, 1975), and to further complicate matters, additional variation can also be found in FA composition between similarly located muscles or SC fat depots (Callow, 1962).

The amount, location and composition of fat in cattle are important because internal, IT and SC fat are low in value and thought to be “waste fat” while premiums are paid for “taste fat” in the form of IM fat. The taste and tenderness that fat adds to beef, however, cannot be considered in isolation from its health effects and related consumer concerns (Simopoulos, 1991, 2002; Ulbricht & Southgate, 1991). The utility and value of beef fat is, however, on the rise given recent problems noted with partially hydrogenated vegetable oils as a source of solid fat due to its high *trans* monenoic acid content, and the finding that all FAs in beef fat are not detrimental to human health and some may actually be beneficial (*n* – 3 and conjugated linoleic acids (CLA)). Little is known, however, regarding the FA composition of beef fat, particularly the more beneficial FAs (specifically CLA) between early- and late-maturing cattle breeds and their fat depots. The present study was, therefore, undertaken to analyse the FA composition of IM, IT and SC fat in intensively fed asturian local cattle breeds including the Asturiana de la Montaña breed and the Asturiana de los Valles breed (with and without the double muscling (*mh*) allele) due to their different propensities to fatten (Martínez et al., 2003).

2. Materials and methods

2.1. Animals and management

One hundred yearling bulls from “Asturiana de los Valles” (AV, *n* = 75), an improved beef breed, and “Asturiana

de la Montaña” (AM, *n* = 25), a beef breed characterised by small to medium-frame size adapted to less favoured mountain areas, were studied during three consecutive years (2001/2003). 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* = 24), AV heterozygous (*mh/+*, *n* = 26) and AV normal (*+/+*, *n* = 25). AM animals lack the mutation responsible for double-muscling.

Calves suckled their mothers from birth (winter) to weaning (early autumn). Weight and age at weaning were of 249 ± 6 kg and 252 ± 4 days for the AV breed and 213 ± 6 kg and 267 ± 7 days for the AM breed. After a postweaning adjustment period of about 15 days, they were fattened by feeding concentrate (84% barley, 10% soybean meal, 3% soybean oil, 3% minerals, vitamins and oligoelements) and barley straw, both *ad libitum*, in the housing facilities of the Research Institute (SERIDA).

2.2. Carcass measurements and sampling

Animals were slaughtered when they reached an average live weight of 542 ± 4 kg (462 ± 4 days at slaughter) for the AV breed and 491 ± 6 kg (520 ± 7 days at slaughter) for the AM breed. 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 average live weight. After slaughtering and dressing, carcasses were chilled at 3 °C.

Twenty-four hours *post-mortem*, the rib joint, from the 6th to the 9th ribs inclusive, of the left half carcass was removed by cutting the length of the bone at the limit of the *Serratus dorsalis* muscle (Robelin & Geay, 1975) and transported to the laboratory.

Adipose tissue (IT and SC) samples and the *Longissimus thoracis* steak of the 8th rib were dissected, vacuum packed and frozen at –80 °C at 24 h *post-mortem* for subsequent fat content and FA analysis.

2.3. Muscle fat content and fatty acid profile

For FA profile determination, duplicate 1 g muscle tissue and duplicate 30 mg adipose tissue (IT and SC independently) were saponified in 6 mL 5 M KOH in methanol/water (50:50, v/v) at 60 °C for 1 h, and the extracted FAs were methylated using trimethylsilyl-diazomethane 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 (2005) and validated in Aldai, Osoro, Barrón, and Nájera (2006).

Analyses were performed on a Varian Star CX3400 gas chromatograph (GC) with a flame ionisation detector (FID) and an automatic sample injection on a

septum-equipped programmable injector operating in “on-column” mode. Fatty acid methyl esters 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 temperature program started at 100 °C followed by ramping to 170 °C at 2 °C/min, holding for 15 min, ramping to 180 °C at 0.5 °C/min and then to 200 °C at 10 °C/min, holding for 10 min, and then ramping to 230 °C at 2 °C/min and holding 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.

Fatty acid methyl esters were identified according to peak retention times using standards (Sigma–Aldrich), and quantified using chromatographic peak area according to the internal standard method using C21:0 as the internal standard with its addition prior to saponification. The response factors of the individual FAs were previously calculated.

Selected abbreviations are SFA (saturated fatty acids) = C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0; BFA (branched fatty acids) = *iso*C15:0 + *anteiso*C15:0 + *iso*C16:0 + *iso*C17:0 + *iso*C18:0; MUFA (monounsaturated fatty acids) = C14:1*cis*9 + C16:1*cis*9 + C17:1*cis*9 + C18:1*trans* + C18:1*cis*9 + C18:1*cis*11 + C18:1*cis*12 + C18:1*cis*13 + C20:1*cis*11 + C22:1*cis*13; C18:1*trans* = coelution of several *trans* isomers; PUFA (polyunsaturated fatty acids) = $n - 6 + n - 3 + \text{CLA}$; $n - 6 = \text{C18:2}n - 6 + \text{C18:3}n - 6 + \text{C20:2}n - 6 + \text{C20:3}n - 6 + \text{C20:4}n - 6 + \text{C22:4}n - 6$; $n - 3 = \text{C18:3}n - 3 + \text{C20:3}n - 3 + \text{C20:5}n - 3 + \text{C22:5}n - 3 + \text{C22:6}n - 3$; CLA (conjugated linoleic acids) = *cis*9, *trans*11-CLA + *trans*10, *cis*12-CLA; UFA (unsaturated fatty acids) = MUFA + PUFA; M/S = MUFA/SFA; P/S = PUFA/SFA; U/S = UFA/SFA.

Not all of the FA mentioned in the aforementioned groups were quantified in all adipose tissues (IM, IT, SC) and genotypes (*mh/mh*, *mh/+*, *+/+*, AM) as some of them were not quantifiable or detectable. Within MUFA, C20:1*cis*11 and C22:1*cis*13 were quantified in all genotypes of IT and SC adipose tissues but not in IM. Within PUFA, C18:3*n* - 6 was quantified in *mh/mh* genotype of IT, and *mh/mh* and *mh/+* genotypes of SC adipose tissue. Also, it was quantifiable in muscle tissue (IM) of all genotypes. C20:5*n* - 3 and C22:6*n* - 3 were only quantified in muscle tissue (IM).

2.4. Statistical analysis

Data were analysed using Mixed Model procedures (SAS Institute Inc, 2001). The statistical model included genotype, fat depot and their interaction as main effects with animal nested within genotype as a random factor and year as a blocking factor. Factor analysis was conducted using SPSS Inc (2002) and principal components as an extraction method. FA groups and ratios were included in the test.

3. Results and discussion

3.1. Adipose tissue

The adipose tissue depots used in this study, expressed as a percentage of carcass weight, included 8.4% IT and 2.2% SC fat estimated using 6th rib dissection according to Oliván, Martínez, García, Noval, and Osoro (1999) and 1.7% IM measured using Soxhlet extraction. Wegner, Albrecht, and Ender (1998) also found that the IT depot had the greatest amount of fat which comprised 45% of total carcass fat in 24 months old Black Pied bulls. When looking at the depot composition across genotypes (Table 1), the IT depot had the greatest amount of SFA (54.4%; $P < 0.001$) and BFA (1.2%; $P < 0.001$) and IM had the lowest (46.3% SFA and 0.6% BFA) as found by Webb et al. (1998) in Belgian Blue cows. SC adipose tissue in general had intermediate values (51.2% SFA and 1.1% BFA). BFA content in IT and SC depots were similar to proportions previously observed in carcass lipids of ruminants (1.2%; Bas & Morand-Fehr, 2000; Duncan & Garton, 1978). In contrast, SC depots had the highest content of MUFA (43.5%; $P < 0.001$) while IM had the lowest content (33.3%) and IT had intermediate content (40%) similarly found by Webb et al. (1998). High MUFA content in SC adipose tissue has been previously reported and may be the result of elevated stearyl-CoA $\Delta - 9$ desaturase activity which converts myristate (C14:0), palmitate

Table 1

Overall mean values $\pm$ standard error of the fatty acid (FA) profile (main groups and ratios) for intramuscular, intermuscular and subcutaneous adipose tissues and their significance according to adipose tissue effect

	IM	IT	SC	Sign.
<i>FA (g/100 g of total FAs)</i>				
SFA	46.31 ^a $\pm$ 0.31	54.43 ^c $\pm$ 0.31	51.21 ^b $\pm$ 0.31	***
BFA	0.62 ^a $\pm$ 0.01	1.20 ^c $\pm$ 0.01	1.12 ^b $\pm$ 0.01	*
MUFA	33.31 ^a $\pm$ 0.30	39.93 ^b $\pm$ 0.30	43.53 ^c $\pm$ 0.30	*
C18:1 <i>trans</i>	5.21 ^a $\pm$ 0.28	10.70 ^c $\pm$ 0.28	8.89 ^b $\pm$ 0.28	*
PUFA	19.76 ^b $\pm$ 0.33	4.44 ^a $\pm$ 0.33	4.13 ^a $\pm$ 0.33	*
$n - 6$	17.86 ^b $\pm$ 0.32	3.71 ^a $\pm$ 0.32	3.38 ^a $\pm$ 0.32	*
$n - 3$	1.68 ^b $\pm$ 0.02	0.36 ^a $\pm$ 0.02	0.34 ^a $\pm$ 0.02	*
CLA	0.22 ^a $\pm$ 0.01	0.37 ^b $\pm$ 0.01	0.42 ^c $\pm$ 0.01	*
UFA	53.07 ^c $\pm$ 0.31	44.37 ^a $\pm$ 0.31	47.66 ^b $\pm$ 0.31	*
<i>Ratios</i>				
M/S	0.72 ^a $\pm$ 0.01	0.74 ^b $\pm$ 0.01	0.86 ^c $\pm$ 0.01	*
P/S	0.45 ^b $\pm$ 0.01	0.08 ^a $\pm$ 0.01	0.08 ^a $\pm$ 0.01	*
U/S	1.17 ^c $\pm$ 0.01	0.82 ^a $\pm$ 0.01	0.94 ^b $\pm$ 0.01	*
$n - 6/n - 3$	10.45 $\pm$ 0.14	10.37 $\pm$ 0.14	10.08 $\pm$ 0.14	NS

NS: $P > 0.05$. ^{a,b,c} Adipose tissue effect: means within a row with different superscripts are significantly different at $P < 0.05$; IM: intramuscular (*Longissimus thoracis* muscle); IT: intermuscular; SC: subcutaneous (both IT and SC from around 8th and 9th ribs); SFA: saturated fatty acids; BFA: branched fatty acids; MUFA: monounsaturated fatty acids; C18:1*trans*: coelution of several *trans* isomers; PUFA: polyunsaturated fatty acids; $n - 6$: $n - 6$ type of polyunsaturated fatty acids; $n - 3$: $n - 3$ type of polyunsaturated fatty acids; CLA: conjugated linoleic acid; UFA: unsaturated fatty acids; M/S = MUFA/SFA; P/S = PUFA/SFA; U/S = UFA/SFA.

* $P < 0.05$.

*** $P < 0.001$.

(C16:0) and stearate (C18:0) to their corresponding $n - 9$ MUFAs (Beaulieu, Drackley, & Merchen, 2002; St. John, Lunt, & Smith, 1991; Taniguchi et al., 2004). In contrast to the *cis*-monounsaturates, however, greater levels of C18:1*trans* isomers were found in IT (10.7%) than in SC (8.9%) adipose tissue ($P < 0.001$). High levels of C18:1*trans* FAs have been previously reported (up to 12% of total FA) in SC fat (Shah, Mir, Aalhus, Basarab, & Okine, 2006), however, details of *trans* composition were not presented. Recently, *trans* FAs have been a topic of interest among government, food industry, health professionals, consumers and the media as more information becomes available concerning the negative health effects of these acids (i.e. increased risk for development of heart disease) (Mensink & Zock, 1998). However, not all *trans* FAs are unhealthy. *trans*-Monenoic acid isomers are produced by rumen microorganisms as intermediates during hydrogenation of PUFAs, and it is known that C18:1*trans*11 (*trans*-vaccenic acid) can be desaturated in humans yielding the most abundant CLA isomer found in beef (*cis*9, *trans*11-CLA) (Turpeinen et al., 2002).

Poly- and unsaturated FAs percentages in IM fat were significantly greater ($P < 0.001$) than in other adipose tissues (IT, SC), and this is reflected in the relatively high contents of $n - 6$ and $n - 3$ but not for CLA. The abundance of PUFA in IM fat may be the result of smaller adipocyte sizes (Harper & Pethick, 2004; Pethick, Harper, & Oddy, 2004) and a higher phospholipid to neutral lipid ratio as indicated by Nürnberg, Wegner, and Ender (1998) and Arana et al. (2006).

Subcutaneous adipose tissue showed the highest content of CLA (0.42%; $P < 0.001$) and the IT depot had an intermediate content (0.37%) probably due to CLA's location in the neutral lipid fraction (Kazala et al., 1999; Raes, De Smet, Balcaen, Claves, & Demeyer, 2003). In contrast, muscle fat had the lowest CLA content (0.22%). In the present study *cis*9, *trans*11-CLA and *trans*10, *cis*12-CLA isomers were quantified as no other isomers could be separated. However, recently, other researchers now indicate that the major CLA isomers in beef are *cis*9, *trans*11-CLA and *trans*7, *cis*9-CLA and these co-elute during GC analysis (Cruz-Hernandez et al., 2004; Sehat et al., 1998).

When examining the FA ratios, the highest M/S ratio was observed in SC depot (0.86; $P < 0.001$). However, this value was lower than that observed by Sturdivant, Lunt, Smith, and Smith (1992) and May, Sturdivant, Lunt, Miller, and Smith (1993) in Wagyu crossbred steers (1.5) and Japanese Black Wagyu steers (2.2–2.6). The highest P/S and U/S ratios were found in IM adipose tissue (0.45 and 1.17 for P/S and U/S, respectively; $P < 0.001$), and there were no differences in $n - 6/n - 3$ ratios between the studied depots probably because $n - 6/n - 3$ ratios are much more affected by feeding as reviewed by De Smet, Raes, and Demeyer (2004) than by genetics or fat depots.

Significant ($P < 0.001$) differences between adipose tissues were found for every FA group in each genotype, with IM fat proving to be the most unique, while IT and SC

were found to be more closely related (Fig. 1). A similar pattern was observed across genotypes when studying differences between adipose tissues, but double-muscled animals presented greater differences in contrast to the other genotypes (*mh*+/+, +/+, AM). In general, these differences were approximately double in *mh*/*mh* animals in comparison to the other genotypes leading to significant adipose tissue depot per genotype interaction ($P < 0.001$ for most of FAs, groups and ratios). On the other hand, referring to SFA and UFA, no significant differences were found between IM and SC adipose tissues in AM genotype probably due to a higher marbling fat content and its higher SFA and MUFA percentages (Tume, 2004).

3.2. Genotype

Significant genotype by adipose tissue interactions ($P < 0.001$) were found for most FA groups and ratios (Table 2). In general, comparing adipose tissues, genotype effect was more pronounced in IM and SC FA profiles than in IT, for which no significant differences between genotypes were found in SFA, PUFA ($n - 6$ and $n - 3$) and UFA. In addition, the contribution of IT and SC depots to the 6th rib dissection (used as a predictor of carcass composition, Oliván et al., 1999) was dependent on genotype. Double-muscled animals were found to have the lowest IT (4.9% of total rib weight) and SC (1.1% of total rib weight) fat percentages while AM animals had the highest IT (9.8%) and SC (3.2%) fat percentages ($P < 0.001$). Heterozygous (*mh*/+) and normal (+/+) AV animals showed, in general, intermediate values. A similar pattern was observed for IM fat content. AM animals showed the highest muscle fat content (2.4%) and double-muscled animals the lowest (0.8%), while the other genotypes (*mh*/+ and +/+) showed intermediate contents (mean value of 1.8%).

3.2.1. Intramuscular fat

All FA groups (SFA, BFA, MUFA, C18:1*trans*, PUFA, $n - 6$, $n - 3$, CLA, UFA) and ratios (M/S, P/S, U/S, $n - 6/n - 3$) showed significant differences due to genotype (Table 2). Double-muscled animals showed the lowest SFA percentages (40.8%), as previously found by Nürnberg et al. (1999) and Raes et al. (2001) in double-muscled Belgian Blue cattle, while AM animals had the highest values (49.8%). The other two genotypes (*mh*/+, +/+) showed intermediate levels (mean value of 47.4%) as seen by Raes, De Smet, and Demeyer (2001) in Belgian Blue genotypes. Significant ($P < 0.001$) differences were found between genotypes for BFA, where it appeared to be higher in genotypes with the *mh* allele (*mh*/*mh*, *mh*/+) (0.67%) in comparison to the other two genotypes (+/+, AM) (0.57%) probably related to the muscle fat content. Similar average values were obtained by Rule, Broughton, Shellito, and Maiorano (2002) in concentrate fed cattle.

In double-muscled animals, the total MUFA percentage was significantly ($P < 0.001$) lower (26.4%) than in the other three genotypes (mean value of 35.6%) as demonstrated by

Fig. 1. Adipose tissue effect on fatty acid groups (% of total fatty acids) for each genotype studied. ^{a,b,c}Adipose tissue effect: columns with different letters within each fatty acid group are significantly different at $P < 0.05$. AV: Asturiana de los Valles breed; AM: Asturiana de la Montaña breed; *mh/mh*: double-muscled; *mh/+*: heterozygous; *+/+*: normal; IM: intramuscular; IT: intermuscular; SC: subcutaneous; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; C18:1*trans*: coelution of several *trans* isomers; PUFA: polyunsaturated fatty acids; *n* – 6: *n* – 6 type of polyunsaturated fatty acids; UFA: unsaturated fatty acids; BFA: branched fatty acids; *n* – 3: *n* – 3 type of polyunsaturated fatty acids; CLA: conjugated linoleic acids.

other researchers using Belgian Blue cattle (Nürnberg et al., 1999; Raes et al., 2001). In addition, the C18:1*trans* content found in the present study was quite high in comparison to other reports (Nürnberg et al., 2005, 2002; Raes et al., 2001; Rule et al., 2002) possibly due to the high dietary PUFA content (60% C18:2*n* – 6 in dietary lipid). Quite similar C18:1*trans* content was found within the AV genotypes (mean value of 5.6%) while its content in meat of AM animals (4.2%) was significantly lower.

A similar trend was noted for the total PUFA (including *n* – 6 and *n* – 3) and UFA percentages with double-muscled animals having the highest values (32.2% PUFA, 29.4% *n* – 6, 2.5% *n* – 3, 58.5% UFA) and AM animals the lowest (13.6% PUFA, 12.2% *n* – 6, 1.2% *n* – 3, 49.7% UFA) while the other genotypes (*mh/+*, *+/+*) showed intermediate contents. Differences in poly- and unsaturated FAs were also detected by Nürnberg et al. (1999), Raes et al. (2001) and Purchas et al. (2005) when feeding animals with concentrate, and may also be influenced by breed effects on total IM fat, the size and number of adipocytes and differences in neutral and polar lipid contents of muscle and adipose cells (Hood & Allen, 1973; Laborde, Mandell, Tosh, Wilton, & Buchanan-Smith, 2001; Webb et al., 1998).

When looking at ratios, higher M/S values (mean value of 0.74) were observed in *mh/+*, *+/+* and AM animals compared to double-muscled animals (0.65). In general, these observations were similar to those obtained by Raes et al. (2001) and lower than values obtained with fatter

breeds (Elías Calles et al., 2000; Laborde et al., 2001; Yang, Larsen, Powell, & Tume, 1999). An opposite effect was found for *n* – 6/*n* – 3 ratio where double-muscled animals showed the highest value (11.8) while in the other three genotypes lower values (mean value of 10.0) were observed. For P/S and U/S ratios, the highest values were found in double-muscled animals (0.8 and 1.5, respectively) and the lowest in AM animals (0.3 and 1.0, respectively), while intermediate values (mean values of 0.3 and 1.1, respectively) were found in the other genotypes (*mh/+*, *+/+*) (Table 2). The highest P/S ratio in *mh/mh* animals was probably related to the high PUFA and low SFA contents in these animals and these were the only ones which reached the recommended P/S values (established as 0.45 or higher as reviewed by Simopoulos, 2002). In other studies where fatter animals were employed, lower P/S values (around 0.13) were obtained (Laborde et al., 2001).

3.2.2. Subcutaneous depot

AM animals had the lowest level of SFA together with *mh/+* animals (50.4%). BFA were higher in leaner animals (*mh/mh*) (1.3%) than in fatter animals (AM) (1.0%). In *mh/mh*, *mh/+* and *+/+* animals the total MUFA percentage was significantly lower (mean value of 42.8%) than in AM animals (45.5%). This may be due to AV animals being leaner than AM animals and likely having smaller SC adipocytes as found by Mendizabal et al. (1999) in the AV breed. Moreover, the aforementioned researchers observed

Table 2
Genotype effect on the fatty acid (FA) profile (main groups and ratios) of intramuscular (IM), intermuscular (IT) and subcutaneous (SC) adipose tissues in yearling bulls from Asturiana de los Valles (AV) and Asturiana de la Montaña (AM) local cattle breeds

	AV (<i>mh/mh</i>)			AV (<i>mh/+</i>)			AV (+/+)			AM			Genotype
	IM	IT	SC	IM	IT	SC	IM	IT	SC	IM	IT	SC	
% Fat Φ	0.81 ^a ± 0.14	4.89 ^a ± 0.37	1.10 ^a ± 0.14	1.77 ^b ± 0.14	8.71 ^b ± 0.37	2.24 ^b ± 0.14	1.85 ^b ± 0.14	10.12 ^c ± 0.37	2.36 ^b ± 0.14	2.39 ^c ± 0.14	9.84 ^c ± 0.37	3.18 ^c ± 0.14	***
FA (<i>g/100 g of total FA</i>)													Genotype × Fat depot
SFA	40.79 ^a ± 0.62	53.48 ± 0.62	51.91 ^b ± 0.62	47.31 ^b ± 0.60	55.17 ± 0.60	51.01 ^{ab} ± 0.60	47.40 ^b ± 0.61	53.98 ± 0.61	52.07 ^b ± 0.61	49.75 ^c ± 0.61	55.08 ± 0.61	49.86 ^a ± 0.61	*
BFA	0.67 ^b ± 0.03	1.46 ^c ± 0.03	1.30 ^c ± 0.03	0.66 ^b ± 0.03	1.24 ^b ± 0.03	1.14 ^b ± 0.03	0.58 ^a ± 0.03	1.08 ^a ± 0.03	1.07 ^{ab} ± 0.03	0.56 ^a ± 0.03	1.02 ^a ± 0.03	1.00 ^a ± 0.03	*
MUFA	26.39 ^a ± 0.62	39.64 ^{ab} ± 0.62	41.90 ^a ± 0.62	35.04 ^b ± 0.60	39.11 ^a ± 0.58	43.58 ^a ± 0.60	35.74 ^b ± 0.61	40.92 ^b ± 0.61	43.09 ^a ± 0.61	36.07 ^b ± 0.61	40.05 ^{ab} ± 0.61	45.54 ^b ± 0.61	*
C18:1 <i>trans</i>	5.74 ^b ± 0.56	13.70 ^c ± 0.56	11.11 ^c ± 0.56	5.25 ^{ab} ± 0.54	10.33 ^b ± 0.54	8.08 ^{ab} ± 0.54	5.68 ^{ab} ± 0.55	10.39 ^b ± 0.55	9.30 ^b ± 0.55	4.18 ^a ± 0.55	8.40 ^a ± 0.55	7.07 ^a ± 0.55	*
PUFA	32.16 ^c ± 0.68	5.42 ± 0.68	4.88 ± 0.68	16.98 ^b ± 0.66	4.48 ± 0.66	4.27 ± 0.66	16.28 ^b ± 0.67	4.03 ± 0.67	3.77 ± 0.67	13.62 ^a ± 0.69	3.84 ± 0.67	3.60 ± 0.67	*
<i>n</i> – 6	29.45 ^c ± 0.65	4.68 ± 0.65	4.11 ± 0.65	15.21 ^b ± 0.62	3.71 ± 0.62	3.43 ± 0.62	14.57 ^b ± 0.63	3.31 ± 0.63	3.08 ± 0.63	12.22 ^a ± 0.63	3.12 ± 0.63	2.89 ± 0.63	*
<i>n</i> – 3	2.51 ^c ± 0.05	0.42 ± 0.05	0.40 ± 0.05	1.52 ^b ± 0.05	0.37 ± 0.05	0.36 ± 0.05	1.49 ^b ± 0.05	0.33 ± 0.05	0.31 ± 0.05	1.18 ^a ± 0.05	0.32 ± 0.05	0.28 ± 0.05	*
CLA	0.19 ^a ± 0.02	0.32 ^a ± 0.02	0.37 ^a ± 0.02	0.26 ^b ± 0.02	0.39 ^b ± 0.02	0.48 ^b ± 0.02	0.21 ^{ab} ± 0.02	0.38 ^b ± 0.02	0.39 ^a ± 0.02	0.22 ^{ab} ± 0.02	0.40 ^b ± 0.02	0.43 ^{ab} ± 0.02	*
UFA	58.54 ^c ± 0.63	45.06 ± 0.63	46.78 ^a ± 0.63	52.03 ^b ± 0.61	43.59 ± 0.61	47.86 ^{ab} ± 0.61	52.02 ^b ± 0.62	44.94 ± 0.62	46.86 ^a ± 0.62	49.69 ^a ± 0.62	43.89 ± 0.62	49.14 ^b ± 0.62	*
Ratios													
M/S	0.65 ^a ± 0.02	0.75 ± 0.02	0.81 ^a ± 0.02	0.74 ^b ± 0.02	0.72 ± 0.02	0.86 ^a ± 0.02	0.75 ^b ± 0.02	0.76 ± 0.02	0.83 ^a ± 0.02	0.72 ^b ± 0.02	0.73 ± 0.02	0.92 ^b ± 0.02	*
P/S	0.81 ^c ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.36 ^b ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.35 ^b ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.28 ^a ± 0.02	0.07 ± 0.02	0.07 ± 0.02	*
U/S	1.46 ^c ± 0.03	0.85 ± 0.03	0.91 ^a ± 0.03	1.10 ^b ± 0.02	0.80 ± 0.02	0.94 ^{ab} ± 0.02	1.11 ^b ± 0.02	0.84 ± 0.02	0.91 ^a ± 0.02	1.00 ^a ± 0.02	0.80 ± 0.02	0.99 ^b ± 0.02	*
<i>n</i> – 6/ <i>n</i> – 3	11.79 ^b ± 0.29	11.21 ^b ± 0.29	10.44 ± 0.29	10.12 ^a ± 0.28	10.19 ^a ± 0.28	9.69 ± 0.28	9.67 ^a ± 0.29	10.20 ^a ± 0.29	9.89 ± 0.29	10.20 ^a ± 0.29	9.89 ^a ± 0.29	10.31 ± 0.29	*

^{a,b,c} Genotype effect for each adipose tissue: means with different superscript letters within a row and within an adipose tissue depot are significantly different at $P < 0.05$; *mh/mh*: double-muscled; *mh/+*: heterozygous; *+/+*: normal; SFA: saturated fatty acids; BFA: branched fatty acids; MUFA: monounsaturated fatty acids; C18:1*trans*: coelution of several *trans* isomers; PUFA: polyunsaturated fatty acids; *n* – 6: *n* – 6 type of polyunsaturated fatty acids; *n* – 3: *n* – 3 type of polyunsaturated fatty acids; CLA: conjugated linoleic acid; UFA: unsaturated fatty acids; M/S = MUFA/SFA; P/S = PUFA/SFA; U/S = UFA/SFA. Φ IM fat% was determined by NIRS calibrated by reference to a Soxhlet fat extraction method (ISO 1443-1973) (Oliván et al. (2002)), while IT fat% and SC fat% were recorded according to the 6th rib dissection into lean, IT fat, SC fat and bone (Oliván et al. (1999)).

* $P < 0.05$.

*** $P < 0.001$.

that FA esterification and *de novo* FA biosynthesis were also low in small adipocytes from AV cattle. Genetic variation in fatness can thus be accompanied by quantitative and qualitative differences in the pattern of fat metabolism (Robelin, 1986; Sinnott-Smith & Woolliams, 1988). Moreover, this may in part be due to a higher $\Delta 9$ -desaturase enzyme activity in fatter animals (AM) as found by Siebert et al. (2003) in SC fat when comparing Jersey-sired and Limousin-sired cattle. Consequently, greater potential SC adipocytes volumes together with the AM genotype (early-maturing with higher fat deposition) could have influenced the MUFA content. Interestingly, however, the opposite effect was found for C18:1*trans* where AM animals had the lowest percentage (7.1%) and the double-muscled animals the highest (11.1%) while the other two genotypes showed intermediate values (8.8%) indicating differential dilution effects related to differences in endogenous fat synthesis.

No significant differences in PUFA (including $n-6$ and $n-3$) were detected between genotypes. However, AM animals, together with *mh/+*, showed the highest content of UFA (mean value of 48.5%) in comparison to the other genotypes (mean value of 46.8%). Some small differences in the percentage of CLA were noted between genotypes but explanations for these are not immediately apparent.

When looking at FA ratios, AM animals were found to have the highest M/S (0.92) and U/S ratios (0.99) compared to the other three genotypes (mean value of 0.83 and 0.92, respectively) probably due to their higher content of MUFA. However, M/S values obtained in the present study were lower than values obtained by Sturdivant et al. (1992) with Wagyu cattle which show a high propensity to fatten. No significant differences were found between the genotypes for P/S and $n-6/n-3$ ratios.

3.2.3. Intermuscular depot

This depot constituted the most homogeneous adipose tissue between genotypes with no differences found for SFA (mean value of 54.4%), PUFA (including $n-6$ and $n-3$) (mean value of 4.4%), UFA (mean value of 44.4%) or any of the ratios except for $n-6/n-3$ (Table 2). Callow (1962) also reported a low and a quite invariable iodine number (closely linked to the FA composition) of the IT fatty tissue while the iodine number of SC fatty tissue has been found to vary with the total fat percentage. Nevertheless, some differences were found in BFA, MUFA and C18:1*trans*. In general, BFA and C18:1*trans* increased significantly with an increase in the number of *mh* alleles, with *mh/mh* animals having the highest contents (1.5% BFA, 13.7% C18:1*trans*) and AM animals having the lowest contents (1.0% BFA, 8.4% C18:1*trans*). In general, quite similar MUFA percentages were found across the genotypes (mean value of 39.9%). For CLA, however, double-muscled animals had a significantly lower percentage (0.32%; $P < 0.01$) in comparison to the other three genotypes (mean value of 0.39%).

No significant differences were found for M/S, P/S and U/S ratios while double-muscled animals showed the highest $n-6/n-3$ value (11.21) whilst other genotypes showed the lowest values (mean value of 10.10).

3.3. Principal component analysis

Principal component analysis (PCA) was carried out including FA groups (SFA, BFA, MUFA, C18:1*trans*, PUFA, $n-6$, $n-3$, UFA and CLA) and ratios (M/S, P/S, U/S, $n-6/n-3$) from all adipose tissue depots of genotypes as study variables. Three principal components were obtained which explained 87.8% of the variability found among samples. Fig. 2 shows the loading plot, using the first two components, where aforementioned variables are represented. Component 1 explained the 60.7% of the variability and it was positively defined by polyunsaturated groups (PUFA ($n-6$ and $n-3$) and UFA) and ratios (P/S, U/S), but negatively defined by more saturated groups (SFA, MUFA, BFA). Component 2 explained 16.6% of the variance, showing high and positive values for M/S ratio. In Fig. 3, sample representation on the first two components obtained in the PCA is shown, and thus, the reliability of using FA profiles to allocate different adipose tissues. IM fat was well differentiated from IT and SC adipose tissues, and was related to highly unsaturated FAs (groups and ratios). However, high dispersion was observed in IM fat probably relating to differences between genotypes (*mh/mh*, *mh/+*, *+/+*, AM), in contrast to IT and SC fat depots where FA profiles were more similar. In this sense, IT and SC adipose tissues appeared to form homogeneous groups and they overlapped at the point related

Fig. 2. Loading plot of the variables (fatty acid groups and ratios) in the Principal Component Analysis including intramuscular, intermuscular and subcutaneous adipose tissue depots from all genotypes studied. SFA: saturated fatty acids; BFA: branched fatty acids; MUFA: monounsaturated fatty acids; C18:1*trans*: coelution of several *trans* isomers; PUFA: polyunsaturated fatty acids; $n-6$: $n-6$ type of polyunsaturated fatty acids; $n-3$: $n-3$ type of polyunsaturated fatty acids; UFA: unsaturated fatty acids; CLA: conjugated linoleic acids; M/S = MUFA/SFA; P/S = PUFA/SFA; U/S = UFA/SFA.

Fig. 3. Plot of different adipose tissue depot samples, from all studied genotypes, in the Principal Component Analysis according to their fatty acid composition. Each point represents an individual animal for each adipose tissue depot. IM: *Longissimus thoracis* muscle intramuscular fat; IT: intermuscular fat; SC: subcutaneous fat.

to BFA, C18:1*trans* and CLA contents. IT fat was mainly related to SFA, while SC fat was mainly related to MUFA and M/S ratio.

4. Conclusions

In general, genotype effects were more pronounced in IM and SC FA profiles compared to the IT depot, for which no significant differences between genotypes were found in SFA, PUFA (including $n-6$ and $n-3$), UFA and most of the ratios. General patterns of the FA profiles, however, remained consistent within depot across genotype but double-muscled animals, as the leanest, were the most different from the other genotypes as highlighted by their high IM PUFA content (nearly two-fold greater than the other genotypes) and consequently the highest P/S ratio.

The results of this study show that IT depot is the most saturated and SC depot contains the most monounsaturated FAs, while IM fat, which cannot be considered in isolation and which represents a low proportion in the muscle, contains the highest percentage of PUFAs and the highest P/S values. No differences between adipose tissues were found for $n-6/n-3$ ratio. In general, IM fat was the most different while IT and SC depots showed quite similar FA profiles with high contents on SFA, MUFA (including C18:1*trans*), BFA and CLA in comparison to IM fat. This was also corroborated in the PCA analysis.

Present results confirm that FA composition differ between adipose tissue depots and highlight the exciting potential for using adipose tissue depot and genotypes differences as tools for producing designer fat. In order for research to advance in this area, however, further analyses to determine the CLA and C18:1*trans* isomeric composition and how these will be influenced by diet will be needed.

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