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Effects of castration and slaughter age on the fatty acid composition of ovine muscle and adipose tissue from two breeds

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Highlights

- Castrates have higher intramuscular fat (IMF) compared to rams
- Scottish Blackface have higher IMF than Texel×Scottish Blackface lambs
- Branched chain fatty acids are detectable in subcutaneous fat of all lambs
- 4-Methylnonanoic acid is higher in rams than castrates in older animals

Abstract

Fatty acids (g/100g total fatty acids) in *M. longissimus thoracis et lumborum* (LTL) and total branched chain fatty acids (µg/g fat) in subcutaneous adipose tissue (SAT) of rams and castrates from Scottish Blackface (SB) or Texel × Scottish Blackface (T×SB) lambs, slaughtered at mean ages of 196, 242, 293, 344 or 385 days were determined. Lambs were fed pasture prior to a 36-day finishing period on a barley/maize-based concentrate ration. The intramuscular fat content (IMF; %) was higher ($P < 0.001$) in castrates than in rams and in SB compared to T×SB lambs ($P < 0.001$). The proportions of *c9*-C18:1 and total monounsaturated fatty acids (MUFAs) were higher ($P < 0.001$) in LTL of castrates than rams. The proportions of C18:2n-6 and total n-6 polyunsaturated fatty acids (PUFAs) were lower ($P = 0.001$) in LTL of castrates compared to rams related to differences in IMF content. The proportions of C14:0, C16:0, *c9*-C18:1 and total MUFA were higher ($P < 0.05$), while the proportions of C18:2n-6, C20:4n-6, C20:5n-3, total PUFA, n-6 and n-3 PUFA were lower ($P < 0.05$), in SB than in T×SB lambs, which was related to differences in IMF content. There was a higher ($P < 0.001$) proportion of conjugated linoleic acid (CLA) *c9,t11*-C18:2 in LTL from SB compared to T×SB. The effects of slaughter age on the proportions of fatty acids in LTL did not show a clear trend. The concentration of 4-methylnonanoic acid was higher ($P = 0.002$) in SAT of rams than castrates, particularly in older lambs. Despite the differences in the muscle fatty acid composition due to gender, slaughter age or breed of lambs, the ratio of n-6/n-3 PUFA (≤3.11) was within the dietary recommendation of <4.0 for human health.

Keywords: Branched chain fatty acids, CLA, Lamb meat, Omega-3, PUFA, Ram

1. Introduction

Intramuscular fat (IMF) content and its fatty acid composition influence not only the shelf life, odour and flavour quality of meat but also its nutritional value (Wood et al., 2008; Frank et al., 2016). From a consumer health perspective, a diet high in saturated fatty acids (SFAs) is believed to increase the risk for cardiovascular diseases, while a ratio of polyunsaturated fatty acid (PUFA) to SFA of >0.4 and a ratio of n-6 to n-3 PUFA (n-6/n-3) of <4.0 are believed to be beneficial in the prevention of such diseases (Warren et al., 2008; Wood et al., 2008).

The quantity and quality of fatty acids in meat is influenced by carcass fatness; as carcass fatness increases, neutral lipids (triacyglycerols mainly) in IMF also increase, while polar lipids (phospholipids mainly) remain relatively constant (Nürnberg et al., 1998; Wood et al., 2008). In muscle, triacylglycerols are associated with a high content of monounsaturated fatty acids (MUFAs) and SFAs while phospholipids are associated with having more PUFAs and long chain n-3 and n-6 PUFAs (Nürnberg et al., 1998; De Smet et al., 2004; Wood et al., 2008).

In sheep, compared to castrated males or females (ewes), entire males (rams) have superior production efficiencies and produce leaner carcasses; however, they may yield inferior carcass quality linked to soft, oily and yellow fat with high proportions of unsaturated fatty acids (Jacobs et al., 1972; Seideman et al., 1982). Furthermore, aroma and flavour of meat from rams is less preferred by some consumers than that from castrates or ewes due to ‘sheepy’, ‘muttony’ and ‘sweaty’ characteristics believed to be linked to the presence of higher amounts of branched chain fatty acids (BCFAs), such as the 4-methyloctanoic acid (MOA), 4-ethyloctanoic acid (EOA) and 4-methylnonanoic acid (MNA) (Prescott et al., 2001; Young et al., 2003b; Watkins et al., 2013; Gkarane et al., 2017). Leaner carcasses can also be obtained from younger or lower live weight animals (Santos-Silva et al., 2002; Okeudo and Moss, 2007)

and by breed selection in production (Demirel et al., 2006; Navajas et al., 2008), e.g. purebred Texel has a low IMF content compared to Scottish Blackface (Navajas et al., 2008). Consequently, castration, slaughter age and breed can influence the intramuscular fatty acids and ultimately the sensory and nutritional profile of lamb. This study aimed to determine the effects of gender and slaughter age of lambs from two breeds on the intramuscular fatty acid profile and on BCFAs in subcutaneous adipose tissue (SAT).

2. Materials and Methods

2.1. Animal husbandry, slaughter and sampling

Two hundred lambs (100 Texel × Scottish Blackface (T× SB), 100 Scottish Blackface (SB)), born in March/April 2014, were sourced from six Irish farms, four of which had SB sires (total 20 SB sires) and three had Texel sires (total 15 Texel sires) (Gkarane et al., 2017; Claffey et al., 2018). Within each breed type 50 lambs were castrated within 48 h of birth. Lambs were raised at pasture from birth, weaned at 130 days and selected for slaughter in groups of 40 (10 T× SB rams, 10 T ×SB castrates, 10 SB rams, 10 SB castrates) in October 2014, November 2014, January 2015, March 2015 and April 2015, with the heaviest ram and castrate lambs selected for slaughter at each slaughter date. On selection, lambs were housed individually in slatted pens and, following a 12d adaptation period during which the lambs were gradually introduced to a barley/maize-based concentrate ration and grass silage, they received *ad libitum* a finishing diet consisting of the barley/maize-based concentrate ration (95% dietary dry matter (DM) intake) and grass silage (5% DM intake) for 36 d pre-slaughter. Lambs were maintained in close proximity to, but separate from, cyclic females while at pasture and following housing. At the end of the finishing period, lambs were transported to a commercial abattoir (Gillivan's, Moate, Co. Westmeath, Ireland) for slaughter. The mean ages of the lambs at slaughter in October, November, January, March and April were 196, 242, 293, 344 and 385 days,

respectively. A total of 198 animals were presented for slaughter (two SB rams died over the course of the experiment). After slaughter, carcasses were chilled overnight and transported to Teagasc, Food Research Centre, Ashtown, Dublin 15, Ireland for dissection. Mean carcass weights (±standard deviation) for the SB and T×SB animals of 20.8 (±1.89) and 25.7 (±2.43) kg, respectively, and for the rams and castrates of 23.2 (±3.28) and 23.3 (±3.31) kg, respectively, were recorded. Details of the production and carcass traits are published in Claffey et al. (2018). The LTL was excised from each carcass, cut into 2.5 cm thick steaks, vacuum packed, aged for 8 d at 4 °C and frozen at−20 °C until required for analysis. From the same region, SAT samples were collected and stored in the same way as LTL. The study was carried out under licence from the Irish Government Department of Health and all procedures used complied with national regulations concerning experimentation on farm animals All animal procedures used in this study were conducted under experimental license from the Irish Health Products Regulatory Authority (HPRA) in accordance with the European Union protection of animals used for scientific purposes regulations 2012 (S.I. No. 543 of 2012).

2.2. Intramuscular fat content measurement

Samples of LTL were thawed overnight at 4°C and homogenized using a Kenwood CH180 Compact Mini Chopper (Kenwood, Hampshire, UK). The IMF contents were determined using the SMART Trac Rapid Fat Analyzer (CEM Corporation, NC, USA) as described in Gkarane et al. (2017).

2.3. Muscle fatty acid analysis

The microwave-assisted extraction of total fatty acids and subsequent conversion to fatty acid methyl esters (FAMEs) was carried out based on the method of Brunton et al. (2015). A 1 g portion of LTL was placed into a perfluoroalkoxy alkane (PFA) vessel. Using a MARS

6 Express 40 position Microwave Reaction System (microwave; CEM Corporation, Matthews, NC, USA), saponification was performed by adding 10 ml of 2.5% KOH (VWR Chemicals, Leuven, Belgium) in methanol (Fisher Scientific, Loughborough, UK) into the PFA vessel, heating to 130°C over 4 min and holding at that temperature for a further 4 min while stirring. Esterification was carried out by adding 15 ml of 5% acetyl chloride (Sigma-Aldrich, Darmstadt, Germany) in methanol to the cooled reaction PFA vessel and heating to 120°C over 4 min with a 2 min holding time. Following addition of 20 ml saturated NaCl (Fisher Scientific) to aid phase separation, FAMEs were partitioned into 10 ml pentane and an aliquot (1.5 ml) of the pentane layer was transferred to a GC vial containing anhydrous Na_2SO_4 (Fisher Scientific). The separation of FAMEs was performed on a Varian 3800 GC fitted with a FID detector and equipped with a Varian 8400 autosampler, using a CP-Sil 88 capillary column (100 m length, 0.25 mm internal diameter, 0.2 µm film thickness (Chromapack, The Netherlands)). The carrier gas used was hydrogen at a flow rate of 1 ml/min. The injector was set to splitless mode. The injector and flame ionization detector (FID) were kept at constant temperatures of 250°C and 260°C, respectively. The column oven temperature was held at 70°C for 4 min, increased at 8°C/min to 110°C, increased at 5°C/min to 170°C and held for 10 min, and finally increased at 4°C/min to 240°C and held for 10.50 min. The total run time was 59 min. The peaks were identified using a 37 FAME standard mix (Supelco Inc., Bellefonte, PA), while individual standards were used to identify FAMEs not present in the mix (Matreva, Inc., Pleasant Gap, PA). Results for each FAME were expressed as least square means of the proportion of the total fatty acids (g fatty acid/100g of total fatty acids).

2.4. Subcutaneous adipose tissue branched chain fatty acid analysis

The microwave-assisted extraction as in Section 2.3 was also used for the FAME preparation of the BCFAs. Stock solutions of the BCFA standards (MOA, EOA and MNA) in

methanol, and internal standard (IS; C23:0) in chloroform (10 mg/ml) (Sigma-Aldrich, Darmstadt, Germany), were prepared. A 0.1 g portion of SAT was placed in a PFA vessel with 100 µl IS and subjected to the same procedure as in Section 2.3. For peak identification (by matching retention times) and quantification purposes, an aliquot of each of the BCFA standards, with the IS, was also derivatised. The separation of FAMEs was carried out on a PerkinElmer Clarus 580 GC fitted with a FID detector (PerkinElmer, Massachusetts, USA) using a ZB-5 column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). The carrier gas was hydrogen at a flow rate of 1 ml/min. The injection volume was 1 µl at a 5:1 split ratio. The temperature of the injector and detector were 200°C and 260°C, respectively. The oven temperature was set at 50°C for 5 min and was increased at 5°C/min to 240°C and held for 20 min. Quantification was described in Gravador et al. (2018) and results were expressed as the least square mean of the absolute concentration of total BCFAs in SAT (µg BCFA/g fat).

2.5. Statistical Analysis

All data were analysed using SAS v9.4. Data diagnostics for normality was performed on the IMF content, all intramuscular fatty acid data (g/100g total fatty acids) or the BCFA data (µg/g SAT) and, for data where the residuals were not normally distributed, a Box-Cox transformation in PROC TRANSREG was used to determine the appropriate lambda value for the transformation (Fahey et al., 2007). Following this, ANOVA, using lamb as the experimental unit, was performed using a mixed model (PROC MIXED) with gender (rams and castrates), slaughter age (October, November, January, March and April), breed (SB and T×SB) and their interactions as fixed effects and individual lamb as a random effect. The intramuscular fatty acid data were also analysed using IMF as a covariate. Means were considered significant at $P < 0.05$.

3. Results

The IMF content was higher (*P< 0.001*) in castrates than in rams and was higher (*P< 0.001*) in SB compared to T×SB, but there was no difference (*P > 0.05*) due to slaughter age (Table 1). There were significant gender by age interactions (*P < 0.05*; Table 2) whereby rams had higher muscle proportions than castrates of: *t11*-C18:1 (*P < 0.001*) in October; *c11*-C18:1 (*P = 0.044*) in January, March and April; conjugated linoleic acid (CLA) *c9,t11*-C18:2 (*P = 0.002*) in October; total n-6 PUFA *(P = 0.044)* in January and March; and n-6/n-3 ratio (*P = 0.04*) in November and March. On the other hand, castrates had higher muscle proportions than rams of: CLA *c9,t11*-C18:2 (*P = 0.002*) in April and CLA *t10,c12*-C18:2 *(P = 0.017)* in January. There was a significant breed by age interaction for muscle proportions of C14:0 (*P = 0.033*) and C15:0 (*P = 0.050*) (Table 2), whereby C14:0 was higher in muscle of SB than T×SB lambs in October, November and January, while C15:0 was higher in SB than T×SB in April. Significant interactions between gender, age and breed (*P < 0.05*) were observed for the muscle proportions of C15:0, C16:0, C17:0, CLA *c9,t11*-C18:2, C20:4n-6, total SFA, total n-6 PUFA and n-6/n-3 ratio.

There were lower (*P < 0*.05) muscle proportions of *c9*-C18:1 and total MUFA but higher (*P < 0.05*) proportions of C15:0, C16:1, *t10*-C18:1, C18:2n-6, total PUFA and PUFA/SFA (P/S) ratio in rams than in castrates (Table 1). There was a decrease (*P < 0.05*) in muscle proportions of C18:3n-3, C20:3n-6, C20:5n-3 and total SFA with age of lambs. On the other hand, there was an increase (*P < 0.05*) in muscle proportions *t10*-C18:1, *c9*-C18:1 and total MUFA with age of lambs (Table 1). Muscle proportions of C20:4n-6 and total n-3 PUFA were affected (*P < 0.05*), but not linearly, by age of lambs (Table 1). Muscle from SB lambs contained higher (*P < 0.05*) proportions of *c9*-C18:1, total MUFA but lower (*P < 0.05*) C18:0, *t10*-C18:1, C18:2n-6, C20:4n-6, C20:5n-3, total PUFA, P/S ratio and n-3 PUFA than muscle from T×SB lambs (Table 1).

4-Methyloctanoic acid, EOA and MNA were detected in 99%, 47% and 59% of SAT samples, respectively. A significant gender by age interaction (*P = 0.002;* Figure 1) was observed for MNA, whereby the concentration in SAT of rams was higher than in castrates in January and April samples but not at other slaughter ages. The effect of gender on MOA concentration approached significance (*P = 0.062*), whereby a numerically higher concentration in SAT of rams than castrates was observed (Table 3). The effect of age on MOA concentration approached significance (*P = 0.096*), but no clear trend was observed. On the other hand, no effects (*P > 0.05*) of gender or age on EOA concentration were found. Overall, there were no differences (*P > 0.05*) in the concentration of any BCFA in SAT between SB and T×SB lambs (Table 3).

4. Discussion

4.1. Gender effects

The lower IMF content in rams compared to castrates could be due to higher feed conversion efficiency and more rapid gain of rams than castrates (Seideman et al., 1982). The higher muscle proportion of *c9*-C18:1, accounting for over 37% of the total fatty acids, in castrates than in rams, is in agreement with the higher proportions of C18:1 found by Solomon et al. (1990) in *longissimus* muscle of castrates in comparison to rams and cryptorchids. Animal gender affects carcass fatness, which in turn influences the muscle fatty acid composition (Nürnberg et al., 1998). When the lipid content in muscle increases, the proportion of neutral lipids, which are high in SFA and MUFA content also increases (De Smet et al., 2004; Wood et al., 2008). Thus, the higher muscle proportion of total MUFA in castrates than rams in the current study may be a consequence of the higher IMF content in castrates. Furthermore, the higher IMF in castrates can explain the numerically higher muscle proportions of C18:0 and total SFA in castrates than rams.

An increase in IMF leads to a decrease in the ratio of P/S due to the dilution of phospholipids with triacylglycerols or to the difference in the ratio of polar and neutral lipids. The preferential deposition of C18:2n-6 in membrane phospholipids, and equal deposition of C18:3n-3 between triacylglycerols and phospholipids (De Smet et al., 2004), could explain the higher proportion of C18:2n-6 in rams with lower IMF than castrates, and the absence of gender effects on the proportion of C18:3n-3 observed in the current study. In agreement with this, Tichenor et al. (1970) reported a higher proportion of C18:2n-6 in perirenal and subcutaneous fat of rams than castrates, and it was suggested that since lambs were fed the same diet, results indicated the involvement of a hormone-related mechanism for preferential deposition and/or mobilization of fatty acids. The higher proportions of total PUFA and n-6 PUFA in muscle of rams compared to castrates reflect differences in C18:2n-6, the main contributor to total PUFA and n-6 PUFA in muscle. The higher proportion of PUFA and n-6 PUFA in muscle of rams than castrates and the lack of gender differences in proportions of total SFA and n-3 PUFA led to the higher ratios of P/S and n-6/n-3 in ram muscle compared to castrate muscle. Despite the presence of gender effects on the ratios of P/S and n-6/n-3 in muscle, the values obtained in the current study for all samples were outside the recommended value of >0.4 for P/S but within the recommended value of <4.0 for n-6/n-3 (Wood et al., 2008).

When the proportions of *c9*-C18:1 and total MUFA were adjusted to similar IMF contents (Supplementary Table 1), both fatty acids remained higher ($P = 0.003$ and $P = 0.012$, respectively) in castrates than in rams. On the other hand, when the proportions of C18:2n-6, total n-6 PUFA and total PUFA were adjusted to similar IMF contents (Supplementary Table 1), the effect of gender on C18:2n-6 and total n-6 PUFA approached significance ($P = 0.079$ and $P = 0.055$, respectively), while the effect of gender on total PUFA was not significant ($P > 0.05$). In a study of Monteiro et al. (2006), when the fatty acid percentages in muscle of bulls and steers were adjusted to similar IMF content, fatty acid profiles remained different between

genders, which was attributed to a castration effect probably linked to hormonal differences between bulls and castrates. According to Cinci et al. (2000), the effect of gender on the fatty acid composition in rats could be due to differences in gene expression and/or in activity of enzymes involved in fatty acid synthesis, desaturation or elongation.

4.2. Age effects

Generally, an increase in age or weight at slaughter is associated with an increase in IMF content (Nürnberg et al., 1998; Okeudo and Moss, 2007), in contrast to the current observations. In this study, the birth dates of the lambs were similar but the heaviest or the early maturing lambs were slaughtered first, while the lightest or later maturing lambs were allowed to reach the desired slaughter weight, i.e. at an older age. The absence of slaughter age effects on IMF could be due to the selection of the later maturing/fattening lambs for slaughter at the later dates, thus attaining comparable IMF content at slaughter (Thornton and Tume, 1987).

In a study of Okeudo and Moss (2007), IMF content increased linearly with slaughter weights, but the trend was not linear for all muscle fatty acids; it was suggested that each class of fatty acid varies in a different way. In our study it was observed that as lambs age, the muscle proportions of C14:0 and C16:0 decreased. Tejeda et al. (2008) reported a decrease in proportion of C12:0 and C14:0 with age, but an increase in proportion of C16:0 and a constant proportion of C18:0 and total SFA in *longissimus lumborum*. In agreement, in our study the muscle proportion of C18:0 was not affected by age of lambs.

The increase in muscle proportions of *c9*-C18:1 and total MUFA with age of lambs observed here could be attributed to the effect of maturity of animals on the activity of enzymes involved in fatty acid synthesis, rather than IMF content, which was not significantly affected by age of lambs. Stearoyl-CoA desaturase enzyme (SCD) catalyses the synthesis of MUFA from SFA and of CLA *c9,t11*-C18:2 from *t11*-C18:1 (Smith et al., 2006; Barton et al., 2011).

An effect of age of the bovine on the gene expression of SCD in SAT was demonstrated (Martin et al., 1999), thus the increase in C18:1 and MUFA with age of lambs in the current study could be a result of an increase in activity of SCD or their accumulation in muscle with age. In agreement with this, the proportion of unsaturated fatty acids in fat was reported to increase with live weight of lambs (Jacobs et al., 1972) specifically the *c9*-C18:1, despite the absence of differences in IMF content between animals (Tejeda et al., 2008). Similarly, an increase in percentages of C18:1 and MUFA were observed as cattle grow older (Huerta-Leidenz et al., 1996; Barton et al., 2011). The higher proportion of *t11*-C18:1 and CLA *c9,t11*-C18:2 in muscle of rams compared to castrates in October (younger lambs), is in agreement with the higher content of *t11*-C18:1 in bulls than heifers, of similar age, observed by Barton et al. (2011). The authors suggested that the different rates of *de novo* fatty acid biosynthesis are due to different activities of lipogenic enzymes and/or their regulation, and partly due to the SCD mRNA levels (Barton et al., 2011).

Prior to finishing period on concentrates, all lambs were fed pasture, and the lower proportion of C18:3n-3 in April samples could be partly due to a seasonal decrease in pasture quality, since grass is a rich source of this fatty acid. The C18:3n-3 in muscle is utilized in the synthesis of long chain n-3 PUFA in phospholipid (Warren et al., 2008), which could explain the similar trend observed for the muscle proportion of C20:5n-3 and total n-3 PUFA, which decreased with slaughter age. In agreement with the current results, Okeudo and Moss (2007) and Huerta-Leidenz et al. (1996) found that the proportion of C18:3n-3 in muscle and fat of animals decreased with slaughter age/weight. The absence of an effect of slaughter age on the muscle proportion of C18:2n-6 is consistent with the findings of Santos-Silva et al. (2002). The higher total n-6 PUFA in muscle of rams slaughtered in January and March than castrates could partly be explained by the lower IMF content (%) in rams than castrates (2.43 *vs* 3.32 in January

and 2.64 *vs* 3.24 in March). Despite the differences, the n-6/n-3 ratios were low (≤3.11; Table 2), which is within the dietary recommendation (Woods and Fearon, 2009).

4.3. Breed effects

The higher IMF content in SB in comparison to T×SB lambs is in agreement with a report by Navajas et al. (2008), where higher IMF was found in Scottish Blackface than Texel muscle. Due to the increase in IMF triacylglycerols resulting in an increase in animal fatness, SFA and MUFA contents also increase (De Smet et al., 2004; Demirel et al., 2006), which could explain the observed higher muscle proportion of C14:0 in SB lambs than T×SB, specifically in October, November and January samples, and of C15:0 in April samples. Similarly, the higher proportions of C16:0, *c9*-C18:1 and total MUFA observed in the SB muscle is also consistent with higher IMF in these lambs than in T×SB lambs. In fact, when these fatty acids were adjusted to similar IMF content (Supplementary Table 1), the effects of breed on the muscle proportions of C14:0, C15:0, C16:0, *c9*-C18:1 and total MUFA was not significant ($P > 0.05$).

The major site for CLA synthesis is adipose tissue (Bauman et al., 2000); consequently the proportion of CLA *c9,t11*-C18:2 was also higher in muscle of SB lambs in comparison to T×SB lambs. But when the level of CLA *c9,t11*-C18:2 was corrected for similar IMF content (Supplementary Table 1), the breed effect remained significant ($P = 0.003$), which suggests that it could be that the SCD mRNA expression was more pronounced in T×SB tissues; the distribution of SCD has been shown to be different between breeds of animals (Bauman et al., 2000; Wachira et al., 2002; Taniguchi et al., 2004). In the current study, the relatively higher proportions of C18:2n-6, C20:4n-6, C20:5n-3, total PUFA, n-6 and n-3 PUFA in muscle of T×SB than in SB lambs, could be explained by the lower IMF in T×SB and the associated higher phospholipid to triacylglycerol ratio (De Smet et al., 2004; Demirel et al., 2006; Wood

et al., 2008). In agreement with the current results, Demirel et al. (2006) also reported that for breeds of lambs with low IMF, higher proportions of PUFA (C18:2n-6, C18:3n-3, C20:5n-3, C22:5n-3 and C22:6n-3, P/S and n-6/n-3 except for C20:4n-6) were found in *longissimus thoracis*. The absence of breed effects on the proportions of total SFA and the higher total PUFA in T×SB resulted in a higher P/S ratio T×SB than SB. However, using IMF as a covariate (Supplementary Table 1), the effects of breed on the proportions C18:2n-6, C20:4n-6, C20:5n-3, total PUFA, n-6 and n-3 PUFA in the muscle were not significant ($P > 0.05$) which suggests that breed effects on these fatty acids were mainly due to the differences in IMF contents of SB and T×SB.

4.4. Branched chain fatty acids in subcutaneous adipose tissue

The concentrations of total BCFAs quantified in SAT of lambs in the current study were similar to the values reported in SAT of lambs fed pasture, maize or lucerne-based diets (Young et al., 2003a), and of lambs finished in ryegrass, lucerne, or brassica forages (Frank et al., 2016). High levels of BCFAs have been related to typical barnyard/milky/sour/sheepmeat flavours (Prescott et al., 2001) and lamb odour notes (Young et al., 2003b); the concentration of total BCFAs was also positively correlated with lamb flavour liking and overall liking (Frank et al., 2016).

In the current study, the SAT of lambs contained more MOA in comparison to EOA or MNA, consistent with the trend found by others (Sutherland and Ames, 1996; Young et al., 2003b; Frank et al., 2016). The higher concentration of MNA in SAT of rams in comparison to castrates for January slaughter coincided with the results of a quantitative descriptive sensory analysis (QDA) done on LTL of the same lambs used in this study (Gkarane et al., 2017). In the QDA, muscle from rams scored higher than castrates for off-flavours and manure/faecal aroma in January (and November) samples (Gkarane et al., 2017). For lambs slaughtered in

April, the higher concentration of MNA in SAT of rams compared to castrates could be due the sexual maturity of the lambs. In agreement with this, the BCFA concentrations in fat of 12-week old lambs did not differ between genders, but when lambs reached sexual maturity (30-week old) higher MOA and MNA were found in subcutaneous fat of rams than castrates (Sutherland and Ames, 1996). The difference in the concentration of BCFA due to gender in lambs could be to the result of high levels of testosterone in rams, which influences the BCFA formation (Sutherland and Ames, 1996; Salvatore et al., 2007). Furthermore, in agreement with the current findings, Young et al. (2006) found that in more mature animals both MNA and MOA concentrations were higher in rams than in castrates. According to Young et al. (2003b), BCFA concentrations are quite variable, even between animals fed the same diets. Moreover, Salvatore et al. (2007) found a higher concentration of MOA in SAT of 8-month old than 22-month old lambs, which could be attributed to the confounding effects of diets and slaughter age.

5. Conclusion

The effects of gender, slaughter age and breed of lambs on fatty acid proportions in muscle are significant but numerically small. Lambs from rams or T×SB that have low IMF content favouring the deposition of PUFA in muscle, while lambs from castrates or SB that have high IMF content favouring the deposition of MUFA in muscle. In lambs with comparable IMF content, the changes in muscle fatty acid proportion with slaughter age show no clear trends. The total MNA concentration in SAT is higher in rams than castrates, particularly as they mature.

Conflict of Interest

There are no conflicts of interest for the work entitled: 'Effects of castration and slaughter age on the fatty acid composition of ovine muscle and adipose tissue from two breeds'.

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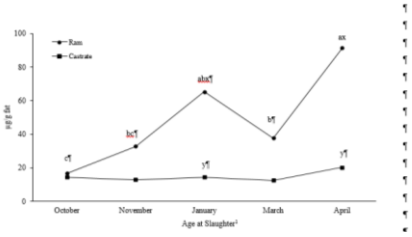

Fig. 1. Gender by age interaction (*P = 0.002*) for the concentration 4-methylnonanoic acid concentration in subcutaneous adipose tissue of lambs.

[1] October (196 days), November (242 days), January (293 days), March (344 days), April (385 days)

[a,b,c] values assigned different letters between ages differ significantly (*P ≤ 0.05*)

[x,y] values assigned different letters between genders differ significantly (*P ≤ 0.05*)

Table 1 Least square mean of intramuscular fat (IMF; g/100g of muscle) and proportions (g/100g total fatty acids) of total fatty acids in *longissimus thoracis et lumborum* muscle of lambs as affected by gender, age and breed.

	Gender			Age at slaughter[1]						Breed[2]	
	Ram	Castrate		October	November	January	March	April		SB	T×SB
IMF	2.61	3.19		2.68	2.82	3.04	3.14	2.83		3.28	2.52
Fatty acids											
C14:0	2.14	2.08		2.72^{a}	2.08^{bc}	2.13^{b}	1.78^{c}	1.85^{bc}		2.25	1.98
C14:1	0.06	0.05		0.05	0.05	0.06	0.05	0.06		0.06	0.05
C15:0	0.32	0.29		0.35^{a}	0.29^{b}	0.33^{ab}	0.28^{b}	0.29^{b}		0.30	0.31
C15:1	0.07	0.06		0.09	0.04	0.04	0.12	0.05		0.06	0.07
C16:0	22.9	22.9		23.8^{a}	23.2^{ab}	23.3^{ab}	22.1^{c}	22.3^{bc}		23.1	22.8
C16:1	1.79	1.65		1.75	1.70	1.63	1.71	1.81		1.76	1.68
C17:0	1.11	1.08		1.09	1.14	1.11	1.07	1.08		1.04	1.15
C18:0	14.3	14.7		14.5	14.6	14.9	14.5	14.3		14.3	14.8
t6-8-C18:1	0.22	0.19		0.19	0.20	0.20	0.25	0.18		0.21	0.20
t9-C18:1	0.29	0.27		0.27	0.31	0.27	0.29	0.25		0.29	0.26
t10-C18:1	1.03	0.70		0.84^{b}	0.73^{b}	0.69^{b}	0.88^{ab}	1.17^{a}		0.66	1.06
t11-C18:1	1.46	1.42		1.52^{ab}	1.52^{ab}	1.75^{a}	1.32^{bc}	1.10^{c}		1.52	1.37
t12-C18:1+*t13*-C18:1	0.22	0.19		0.17	0.17	0.21	0.30	0.16		0.23	0.17
c9-C18:1	37.6	39.1		36.3^{c}	38.4^{b}	38.3^{b}	38.8^{ab}	40.1^{a}		38.9	37.9
c11-C18:1	1.26	1.15		1.19^{ab}	1.04^{b}	1.09^{b}	1.34^{a}	1.37^{a}		1.16	1.25
c13-C18:1	0.10	0.10		0.09	0.10	0.10	0.10	0.11		0.10	0.10
t16-C18:1	0.13	0.14		0.14^{ab}	0.13^{ab}	0.15^{a}	0.12^{b}	0.11^{b}		0.13	0.13

C18:2n-6	3.85	3.29		3.57	3.37	3.22	3.88	3.80		3.26	3.88
C18:3n-3	0.76	0.77		0.73^{b}	0.82^{ab}	0.89^{a}	0.81^{ab}	0.56^{c}		0.76	0.76
CLA *c9,t11*-C18:2	0.53	0.52		0.64^{a}	0.51^{ab}	0.60^{a}	0.49^{ab}	0.39^{b}		0.59	0.46
CLA *t10,c12*-C18:2	0.02	0.02		0.02	0.02	0.03	0.02	0.02		0.02	0.02
C20:2	0.02	0.02		0.01	0.03	0.02	0.03	0.03		0.02	0.03
C22:0	0.03	0.01		0.02	0.01	0.01	0.06	0.00		0.03	0.01
C20:3n-6	0.23	0.22		0.36^{a}	0.21^{b}	0.16^{b}	0.20^{b}	0.19^{b}		0.16	0.29
C20:3n-3	0.00	0.02		0.00^{c}	0.03^{a}	0.01^{ab}	0.01^{bc}	0.00^{c}		0.01	0.01
C20:4n-6	1.64	1.49		1.94^{a}	1.51^{ab}	1.25^{b}	1.65^{ab}	1.45^{ab}		1.30	1.83
C20:5n-3	0.56	0.51		0.62^{a}	0.54^{ab}	0.52^{ab}	0.56^{ab}	0.44^{b}		0.49	0.58
C22:5n-3	0.61	0.57		0.54	0.64	0.58	0.63	0.55		0.56	0.62
C22:6n-3	0.22	0.23		0.24	0.24	0.21	0.23	0.18		0.21	0.24
SFA	39.4	39.8		41.0^{a}	39.9^{ab}	40.3^{a}	38.3^{b}	38.4^{b}		39.7	39.5
MUFA	39.4	40.8		38.1^{c}	40.1^{b}	39.9^{b}	40.5^{ab}	41.9^{a}		40.6	39.6
PUFA	7.45	6.65		7.47	6.90	6.50	7.54	6.84		6.48	7.62
P/S	0.19	0.17		0.18	0.17	0.16	0.20	0.18		0.16	0.19
n-6 PUFA	5.79	5.07		5.95^{a}	5.19^{ab}	4.71^{b}	5.82^{ab}	5.52^{ab}		4.77	6.08
n-3 PUFA	2.20	2.14		2.17^{a}	2.33^{a}	2.25^{a}	2.29^{a}	1.77^{b}		2.05	2.26
n-6/n-3	2.73	2.45		2.84^{a}	2.36^{bc}	2.07^{c}	2.68^{ab}	2.99^{a}		2.28	2.89
Unknown/Others	4.70	4.20		4.50	4.51	4.77	4.81	4.58		4.76	4.52

[1] October (196 days), November (242 days), January (293 days), March (344 days), April (385 days),

[2] Scottish Blackface (SB), Texel x Scottish Blackface (T×SB)

[a,b,c] Means assigned different superscripts differ significantly between ages ($P \leq 0.05$)

CLA - conjugated linoleic acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; P/S - PUFA to SFA ratio; n-6/n-3 - n-6 PUFA to n-3 PUFA ratio; n-6 PUFA

- sum of C18:2n-6, C20:2, C20:3n-6, C20:4n-6; n-3 PUFA - sum of C18:3n-3, C20:3n-3, C20:5n-3, C22:5n-3, C22:6n-3.

Table 2 Significant interactions for the proportions of total fatty acids (g/100g total fatty acids) in *longissimus thoracis et lumborum* muscle of lambs between gender, breed and age.

	Gender (G) R/C[1]		Breed (B)[2]		Age at slaughter (A)[3]				
					October	November	January	March	April
C14:0			SB		3.03^{ax}	2.25^{bx}	2.32^{bx}	1.81^{c}	1.82^{c}
			T×SB		2.41^{ay}	1.92^{by}	1.94^{by}	1.74^{b}	1.88^{b}
C15:0			SB		0.38^{a}	0.30^{bc}	0.33^{ab}	0.29^{c}	0.31^{bcx}
			T×SB		0.32^{a}	0.28^{b}	0.32^{a}	0.27^{b}	0.28^{by}
t11-C18:1	R				1.79^{ax}	1.63^{a}	1.75^{ac}	1.24^{ac}	0.82^{c}
	C				1.19^{by}	1.44^{ab}	1.75^{a}	1.38^{ab}	1.38^{ab}
t12-C18:1+ *t13*-C18:1	R				0.08^{by}	0.17^{a}	0.20^{a}	0.47^{a}	0.17^{a}
	C				0.25^{x}	0.17	0.22	0.14	0.16
c11-C18:1	R				1.17^{c}	0.99^{c}	1.78^{ax}	1.45^{bx}	1.48^{bx}
	C				1.21^{a}	1.08^{b}	1.00^{by}	1.23^{ay}	1.17^{ay}
CLA *c9,t11*-C18:2	R				0.76^{ax}	0.53^{b}	0.57^{b}	0.48^{b}	0.32^{cy}
	C				0.51^{aby}	0.49^{b}	0.62^{a}	0.50^{ab}	0.46^{bx}
CLA *t10,c12*-C18:2	R				0.02^{ab}	0.03^{a}	0.02^{aby}	0.02^{ab}	0.01^{b}
	C				0.01^{b}	0.01^{b}	0.04^{ax}	0.03^{ab}	0.03^{ab}
n-6 PUFA	R				5.38^{ab}	4.97^{b}	5.00^{abx}	6.02^{ax}	5.59^{ab}
	C				5.75^{a}	4.32^{b}	3.58^{by}	4.65^{ay}	4.78^{a}
n-6/n-3	R				2.70^{b}	2.59^{bx}	2.17^{b}	3.07^{ax}	3.11^{a}
	C				2.39^{a}	2.13^{by}	1.97^{b}	2.28^{by}	2.86^{a}

[1] Rams (R), Castrates (C)

[2] Scottish Blackface (SB), Texel × Scottish Blackface (T×SB)

[3] October (196 days), November (242 days), January (293 days), March (344 days), April (385 days)

[a,b,c] Means assigned different superscripts within rows differ significantly between ages ($P \leq 0.05$)

[x,y] Means assigned different superscripts within columns differ significantly between genders/breeds ($P \leq 0.05$)

[4] Significant G × B × A ($P < 0.05$) interactions were observed for the muscle proportions of C15:0, C16:0, C17:0, CLA *c9,t11*-C18:2, C20:4n-6, total SFA, total n-6 PUFA and n-6/n-3 ratio.

CLA - conjugated linoleic acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; P/S - PUFA to SFA ratio; n-6/n-3 - n-6 PUFA to n-3 PUFA ratio; n-6 PUFA - sum of C18:2n-6, C20:2, C20:3n-6, C20:4n-6; n-3 PUFA - sum of C18:3n-3, C20:3n-3, C20:5n-3, C22:5n-3, C22:6n-3.

Table 3 Least square mean concentrations (μg/g) of branched chain fatty acids (BCFAs) in subcutaneous adipose tissue of lambs as affected by gender, age and breed.

BCFA	Gender (G)			Age at slaughter (A)[1]						Breed (B)[2]	
	Ram	Castrate		October	November	January	March	April		SB	T×SB
4-methyloctanoic acid	147	119		111	132	134	119	169		142	124
4-ethyloctanoic acid	3.49	3.32		3.30	2.84	3.78	3.87	3.24		3.03	3.78
4-methylnonanoic acid	48.8	14.8		15.6^{b}	22.8^{b}	39.7^{a}	25.0^{b}	55.8^{a}		31.6	32.0

[1] October (196 days), November (242 days), January (293 days), March (344 days), April (385 days)

[2] Scottish Blackface (SB), Texel × Scottish Blackface (T×SB)

[3] There were no significant effects of breed and no significant G×B, G×A (except for MNA, *P = 0.002*), A× B or B × G ×A interactions.

[a,b] Means assigned different superscripts differ significantly between ages (*P ≤ 0.05*)