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Age and nutrition influence the concentrations of three branched chain fatty acids in sheep fat from Australian abattoirs

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Abstract

The characteristic mutton odour, associated with the cooked meat of older sheep, can be problematic for some consumers who find the odour disagreeable. Branch chain fatty acids (BCFAs) are considered to be the main determinants of mutton odour. In this study, the aim was to identify the factors influencing the BCFA content of animals at abattoirs in Australia. Samples of subcutaneous fat from over the chump (*gluteus medius*) were collected from 533 sheep carcasses at abattoirs in New South Wales, Victoria and Western Australia. The carcasses were from sheep differing in age, gender, breed and nutrition. The concentrations of three branched chain fatty acids (BCFAs); namely, 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA) and 4-methylnonanoic acids (MNA), were determined. Statistical modelling showed that, with pre-slaughter nutrition in the model as a random term, BCFA concentrations could be used for discriminating the age of sheep. Fat samples from lamb carcasses had lower MOA and EOA concentrations and a higher concentration of MNA in comparison to hogget and mutton ($P < 0.05$). When nutrition was excluded as a random effect from the statistical model, the MOA and MNA concentrations did not differentiate between lamb, hogget and mutton whereas, for EOA, lamb had a lower concentration than mutton ($P < 0.05$) with hogget intermediate. An interaction existed between age and gender ($P < 0.05$) where female lambs had lower EOA concentrations relative to the mutton but not for castrates.

Keywords: BCFA, sheep category, determination, sheep fat, age, nutrition

1.0 Introduction

The characteristic mutton odour, associated with the cooked meat of older sheep, can result in low consumer acceptance (Young, Lane, Priolo & Fraser, 2003). Branched chain fatty acids (BCFAs), particularly 4-methyloctanoic (MOA) and 4-methylnonanoic acids (MNA), have been implicated as the main compounds responsible for this aroma in cooked ovine meat (Brennand and Lindsay, 1992; Rousset-Akrim, Young & Berdagué, 1997; Young, Berdagué, Viallon, Rousset-Akrim & Theriez, 1997). The concentrations of these two compounds increase in sheep fat as an animal ages (Young, Lane, Podmore, Fraser, Agnew, Cummings & Cox NR, 2006). Recent work (Salvatore, Allen, Butler, Elkins, Pethick & Dunshea, 2007) reported that differences in MOA and MNA concentrations in 8 and 22 month old animals did exist but lower amounts of these BCFAs were found in the older animals compared to the younger ones, contrasting the work reported by Young et al. (2006). This was unexpected as BCFA concentrations are expected to increase with age, and Salvatore et al. (2007) believed this result was due to confounding related to slaughter date and feeding.

Salvatore et al. (2007) demonstrated, for the first time, the influence of breed, gender and age on MOA and MNA concentrations in the fat tissue of Australian sheep. The animals used by Salvatore et al. (2007) were typical of those employed for meat production in Australia, and were part of an experimental flock developed by the Australian Sheep Industry Co-operative Research Centre (Pethick, Warner & Banks, 2007). Given the experimental nature of the flock used by Salvatore et al. (2007), the study was extended to include animals available at commercial abattoirs. For this work, it was intended to collect as much data as possible (e.g. breed, age, gender and finishing diet) for the animals so that the impact of each factor on the BCFA concentrations of the sheep could be identified.

Additionally, a recent Australian Senate inquiry into meat marketing (2008) reported there is some concern that hogget and mutton substitution for lamb may be occurring in the industry. Sheep classification, based on dentition of an animal, occurs prior to slaughter and, once processed, there is no objective method that can be used for the identification of sheep category. If such a test was available then ‘truth in labelling’ could be performed on sheepmeat while it is in the supply chain. Where anomalies were detected (e.g. when meat from older animals is substituted for lamb), ‘trace-

back' activities could then be performed to identify the source of any irregularity. The sample preparation step used in this study, and that of Salvatore et al. (2007), was chosen because it closely replicates the cooking process that ovine fat is subjected to, prior to consumption.

If the BCFA concentrations increase with animal age (Young, Lane, Podmore, Fraser, Agnew, Cummings & Cox, 2006), then it's possible that the measured concentrations could be used to determine age and thus category (i.e. lamb, hogget or mutton) of the animal. Thus chemical analysis would be an effective tool which could be applied to fat from meat taken from anywhere in the supply chain. Thus, rather than having to rely on dentition as a proxy for age (which occurs prior to processing), an alternative method would be available that could be used for determining sheep category. So, in addition to identifying what factors influence the BCFA content in the fat of Australian sheep, an additional aim was to determine whether chemical analysis could be used for classifying sheep age and thus category.

2.0 Materials and methods

2.1 Sample collection

In total, 533 samples were collected from abattoirs in New South Wales (180), Victoria (170) and Western Australia (183). Across the samples from the three states, there was some variation in the data collected on each flock of animals sampled. The animals were typical for the particular state and time of collection. The samples from WA were collected from June to November 2007 with some data available on breeds (Merino, South African Merino x Merino, Dorpa Lee with others unspecified) but none on the carcass weight. For Victoria, the samples were collected from March to June 2007 and no data was collected on either breed or carcass weight. The samples from NSW were collected in October 2006 with all carcasses having data on carcass weight and most having data on breed. Pre-slaughter nutrition was categorised into seven groups; grain, lucerne, lucerne mixed, native, pasture, pasture supplement and saltbush (Table 1).

2.2 Sample preparation

Subcutaneous fat samples (*ca* 20-30 g) were collected from the chump area (over the *gluteus medius*, to minimise carcase damage) at 24 hours post slaughter and

frozen at -20 ℃ until needed. Prior to analysis, the surface layer of the fat was removed and the remainder cut into small portions (*ca* 0.5 cm^3 squares). Molten fat was prepared by heating the cut portions in a microwave oven for approximately 5 mins.

Molten fat (1 g) was injected into a Unitrex co-distillation unit (SGE, Ringwood) and heated at 200 ℃ for 1 hr under a flow (200 mL min^{-1}) of nitrogen. Each batch of ten samples included one spiked recovery sample containing the internal standard (undecanoic acid). The released compounds were purged through the Unitrex unit and collected onto a trap. The trap, consisting of Tenax®, a glass wool plug and anhydrous sodium sulphate, was then eluted with 5 mL diethyl ether:hexane (20:80). The organic phase was concentrated to 1 mL and, after the addition of the internal standard (1.00 mg kg^{-1}), the sample was treated with bisilyltrifluoroacetamide at 60 ℃ for 30 min and the BCFAs were derivatised as their trimethyl esters.

2.2.1 Reagents

Solvents used were of pesticide grade quality. 4-Methyloctanoic (MOA), 4-methylnonanoic (MNA), 4-ethyloctanoic (EOA) and undecanoic acids were purchased from Sigma-Aldrich (Castle Hill) and were of >98% purity. Nitrogen and helium were ultra-high purity grade (Linde, Altona). All other reagents were of analytical reagent grade.

2.3 Gas chromatography-mass spectrometry analysis

The fatty acid-TMS esters were separated by injection (1 µL) onto a DB5-MS fused silica capillary column (J&W, 30m x 0.25 mm i.d. x 250 µm film thickness) in a Varian 3400 gas chromatograph (GC) and detected by a Saturn 2000 ion trap mass spectrometer operating in full scan mode. The septumless programmable injector (SPI) was programmed starting at 45 ^oC and increased to 325 ^oC at 180 ^oC min^{-1}. The GC oven was held at 75 ℃ for 2 min then increased to 300 ℃ at 10 ℃ min^{-1} and held at this temperature for 8 min. Helium was used as the carrier gas at a constant pressure of 105 kPa. The mass spectrometer transfer line was at 280 ℃. Mass spectra were acquired using an ion source temperature of 220 ℃ and an electron multiplier voltage of 2400 V. The mass spectrometer was calibrated using FC43 (Varian, Inc., Springvale).

Quantitation of the BCFAs was performed using the Varian Saturn Workstation 2000 software. For calibration, the standards were in the range of 0.02 to 1.00 mg kg^{-1} and the standard solutions were derivatised using bisilyltrifluoroacetamide at 60 ℃ for 30 min. The following ions were used for quantitation; MOA-TMS ester, *m*/*z* = 215.0, EOA-TMS ester, *m*/*z* = 229.0, MNA-TMS ester, *m*/*z* = 229.0 and the internal standard, undecanoic acid-TMS ester, *m*/*z* = 243.0, respectively. The concentrations were determined using external quantitation. The calculation of concentration for a given BCFA was made using:

$$[\text{BCFA}]\ (\text{mg kg}^{-1}) = \frac{A_{\text{BCFAsample}}}{A_{\text{IS sample}}} \times k$$

where $A_{\text{BCFAsample}}$ is the peak area of the BCFA in the sample, A_{ISsample} is the peak area of the internal standard in the sample and *k* is the slope of a linear calibration curve with intercept set to zero. The calibration curve was formed by plotting the ratio of BCFA standard peak area to peak area of the internal standard ($A_{\text{BCFA standard}}/A_{\text{IS standard}}$) against BCFA standard concentration where $A_{\text{BCFA standard}}$ and $A_{\text{IS standard}}$ are the peak areas of the BCFA and internal standards, respectively.

2.4 Statistical analysis

The data was tested in a similar way to Salvatore et al. (2007) with the log variates of BCFA concentration ($\log_{10}$ (EOA + 0.075), $\log_{10}$ (MNA + 0.0003) and $\log_{10}$ (MOA + 0.05)) related to effects and interactions of gender and breed (as fixed effects) while adjusting for abattoir, sampling date and nutrition (as random effects) using restricted maximum likelihood (REML) models. The most parsimonius model, for each fixed variate, was chosen using Wald tests accompanied by approximate *F* statistics (Kenward & Roger, 1997). The random terms selected for all modelling (abattoir, sampling date and nutrition) were the most appropriate given the structure of the data where other random terms, main or interaction, were confounded with these terms, i.e. animal source confounded with sampling date. Log_{10} (y + c) transformations were needed to ensure that the amount of residual variation did not change with the increase in the mean and *c* was selected to give the best fit for the transformed data. After selection of the appropriate model, specific pairs of means were compared using the SEDLSI procedure (Genstat 2003). The SEDLSI procedure computed a least significant interval (LSI, or error bar) that overlaps when there is no significant difference between back transformed data, or that is disjoint (i.e. does not overlap) where there are significant differences (Hannah & Quigley, 1996). The computation

was performed using a table of treatment means and the corresponding standard error of distance (SED) to generate a value, δ, such that $[\delta_i + \delta_j] \cong$ SED. All analyses were performed using Genstat. Boxplots were produced using R, version 2.4.1 (R Development Core Team, 2006). Estimates of the mean BCFA concentrations for pre-slaughter nutrition category were derived as best linear unbiased predictors (BLUPs) since pre-slaughter nutrition was fitted as a random effect. There were 533 values (254 lamb, 131 hogget and 148 mutton) used in the data set when the nutrition category was not considered as a factor while, when the nutrition class was adjusted for, 333 values (206 lamb, 48 hogget and 79 mutton values) were used for the analysis.

3.0 Results and discussion

3.1 BCFA concentrations

This study, and the earlier one by Salvatore et al. (2007), represents a novel application of the Unitrex apparatus and the related sweep co-distillation technique. These units were originally developed in the late 1980's to isolate pesticides from complex matrices in the analysis of organophosphate and organochloride pesticides in meat and dairy products (Tekel' & Hatrík, 1996). For the present work, these units were used to simulate the cooking conditions typically involved with roasting sheepmeat. As the unit is a closed system, the purge of nitrogen and entrapment using Tenax®, allowed for the convenient capture of the thermally labile compounds resulting from heating (and hydrolysis) of ovine fat.

Figure 1 shows a total ion chromatogram of the compounds purged from a molten lamb fat sample using a Unitrex sweep co-distillation unit with the fatty acids measured as trimethylsilyl (TMS) esters. The main components in the total ion chromatogram were the TMS esters of hexadecanoic (palmitic, $C_{16:0}$), octadecenoic (oleic, $C_{18:1}$) and octadecanoic (stearic, $C_{18:0}$) fatty acids which result from hydrolysis of the triacylglycerol component of the fat, and are the major fatty acids in sheep fat. Typical ranges for these fatty acids are ~ 20 to 30 g per 100g of the total fatty acid content (Wood, Enser, Fisher, Nute, Sheard, Richardson, Hughes & Whittington, 2008). In contrast, BCFAs were present in the ovine fat samples at much lower concentrations. This can be clearly seen in the inset of Figure 1 which shows an expanded region of the chromatogram, indicating the retention times of the BCFAs as TMS esters.

The BCFA concentrations increased with age with lower concentrations being found in lamb (< 1 yr), higher concentrations present in mutton (> 2 yr) and the concentrations in hogget (> 1 yr and < 2 yr) as intermediate between lamb and mutton (Figure 2). This is not surprising since BCFA concentrations in sheep fat are expected to change with an animal's age, increasing as an animal grows older (Young & Braggins 1999). This study substantiates this view and confirms the work of others (Ha & Lindsay 1990; Sutherland & Ames 1996; Young et al. 2006). MOA was the most abundant of the BCFAs with the median MOA concentration being almost two-fold higher than EOA, and ten-fold higher than MNA. Young et al. (2006) found that MOA was higher than MOA. EOA was not reported in that study. EOA and MNA were not correlated (r^2 = 0.18) but there is evidence that both EOA and MNA were correlated with MOA (r^2 = 0.64 and 0.73, respectively, P < 0.05). The positive relationship between MOA and MNA has been previously found in 8 and 22 month old animals (Salvatore et al., 2007) as well as in rams of different ages (Young et al., 2006).

A recent Australian study (Salvatore et al., 2007) found lower concentrations of MOA were present in older animals (22 months) compared to 8 month old lambs which contrasts the result found in this study and the commonly accepted view that BCFA concentrations increase with age. Salvatore et al. (2007) reported that the median MOA concentrations at 8 and 22 months were 0.084 and 0.041 mg kg^{-1} respectively; whereas the median MOA concentrations for lamb and mutton were 0.10 and 0.15 mg kg^{-1} in the present study. The difference reported by Salvatore et al. (2007) was believed to be related to confounding of slaughter date and diet. Assuming that the animals in the present work are similar in an age to those sampled by Salvatore et al. (2007) this explanation seems feasible and is not related to the measurement technique which is the same in both studies. The earlier study used animals derived from an experimentally designed flock where animal management had been controlled (Hopkins, Stanley, Martin & Gilmour, 2007). There was some variation in the diet of these animals where the older animals received a higher ratio of legume silage to total concentrate which might have affected BCFA production in the rumen (Salvatore et al., 2007).

This is the first time that a large scale survey (> 500 samples) of BCFA content in the fat of sheep of different breeds and finishing diets available at abattoirs in Australia and, possibly, elsewhere. Previous workers have restricted their studies to single animals (Wong, Johnson & Nixon, 1975; Ha & Lindsay, 1990; Brennand & Lindsay,

1992) or experimental cohorts with lower sample numbers of fixed breed and diet (Johnson, Wong, Birch & Purchas, 1977; Salvatore et al*.,* 2007; Sutherland & Ames, 1995; Sutherland & Ames, 1996; Young et al*.*, 1997; Young et al*.*, 2003; Young et al*.*, 2006). This work is an extension of the study by Salvatore et al. (2007) who reported on the factors that influenced the BCFA concentrations measured in an experimental cohort of animals of known breed and pre-slaughter nutrition (Hopkins et al*.*, 2007). It was anticipated that, examining a wider range of breeds and finishing diet, would provide more definitive information on the BCFA concentrations in a representative cross-section of the Australian meat sheep flock.

3.2 Factors affecting BCFA concentrations

Statistical analysis, with restricted maximum likelihood (REML) models, was used to determine how the factors gender, breed and pre-slaughter nutrition influenced the measured BCFA concentration. The first model related the variate, $\log_{10}$(MOA+0.05) to breed, gender and slaughter age of sheep but, after adjustment for abattoir and day of slaughter as random effects, no statistical significance was found for this model (P = 0.885, Table 2). This was also the case for MNA where the variate $\log_{10}$ (MNA + 0.0003) was related to the same factors. Yet, for EOA, a relationship between the variate, $\log_{10}$ (EOA + 0.075), and the factors was found (P = 0.024) with the EOA concentration increasing with age. This is seen in Figure 3 which shows a plot of the transformed means as a function of slaughter age. A notable difference existed between the mean for lamb and mutton with hogget overlapping both groups. For EOA, an interaction between gender and age was observed with higher concentrations present in older females compared to the other age groups (Figure 4). This trend was not apparent in males across the age groups (Figure 4). This contrasts other work where higher BCFA concentrations are generally associated with male compared to female animals (Young & Braggins 1999).

A wide range of finishing diets was observed for the data set (Table 1) and, where appropriate, pre-slaughter nutrition was grouped into simpler categories. The introduction of pre-slaughter nutrition as a random term to the model had a significant effect for each BCFA; the P values for MOA, EOA and MNA were respectively 0.009, 0.008 and 0.056 (Table 2). For MOA and EOA, hogget had the highest value with mutton as an intermediate and lamb having the lowest concentration. For MNA, hogget also had the highest mean concentration but no statistical difference was present between lamb and mutton. It is unclear why, after adjustment for on-farm

nutrition, hogget had the highest concentrations of MOA and EOA, as this contrasts the work of Young et al. (2006).

These results indicate that an animal's finishing diet prior to slaughter significantly impacts on the BCFA concentrations found in sheep fat. This has been observed by other workers (Enser, Nute, Wood, Berge, Zygoyiannis, Thorkelsson, Piasentier & Fisher, 2000; Johnson et al., 1977; Wong et al., 1975; Young et al., 1997; Young et al., 2003) who reported that elevated BCFA concentrations were associated with animals fed either grain or concentrate compared to pasture-fed animals prior to slaughter. The effect of diet on the BCFA concentrations has been suggested to be due to increased propionate formation in the rumen resulting from the high soluble carbohydrate content in the grain and concentrate feedstock (Young et al. 1997; Young et al. 2003). This also implies that diet may impact on the eating quality of the cooked meat from animals finished on diets based on either grain or concentrate. This, of course, is speculative and needs further work to confirm. However if it is true then this could have some useful implications for abattoirs which process animals from the local region as, where information is available on the animals' finishing diet and BCFA content, it would be possible to predict the eating quality of meat from these animals. With some markets, there are consumers who have a low acceptance of the cooked meat which comes from older sheep due to the mutton odour (Young & Braggins 1999), since BCFAs are responsible for this aroma, knowledge of pre-slaughter nutrition could be used to predict the quality of cooked meat available in that market place.

Given that pre-slaughter nutrition plays a significant role in the BCFA content of sheep fat, an exploratory assessment was made to determine the effects of each nutrition category on the estimated mean BCFA concentration associated with each group (Table 3). Since nutrition was included as a random term in the statistical analysis, it was only possible to provide these values as estimates. BCFA concentrations were found to be higher in the subcutaneous fat of sheep grazing 'native pasture', 'saltbush' and 'lucerne mixed' compared to the other categories. Lucerne has been reported to increase the concentration of MOA in fat from animals finished on lucerne compared to ryegrass (Young & Braggins 1999) which would explain the elevated BCFA concentrations found with the 'lucerne mixed' category. It would also be reasonable to expect that higher concentrations would be found with 'lucerne' but this was not the case. As noted above, the use of grain would also be expected to increase BCFA concentrations but this was not observed in this

provisional analysis. Young and Braggins (1999) note that grain cereals differ in their propensity to generate BCFAs, which might help to explain the results of this study. It is also interesting to note the higher concentrations found with the 'native pasture' and 'saltbush' categories. This would imply that, given the relationship between BCFAs and mutton odour, the aroma and flavour of the cooked meat from these animals would be more mutton-like, assuming the quality of the native pasture is similar to saltbush. While an aroma has been detected in cooked meat from lambs grazed on saltbush, its presence did not impact on the flavour of the cooked meat (Hopkins and Nicholson 1999); a result which has been confirmed by others (Pearce, Masters, DeBoer, Rintoul & Pethick, 2003; Pearce, Norman, Wilmot, Rintoul, Pethick & Masters, 2008; Pearce, Pethick & Masters, 2008). It should also be noted that there have been no previous reports of the BCFA concentrations in sheep fat taken from animals fed on either native pasture or saltbush.

It can be seen that the results of this study contrast, at times, to those reported by other workers. Young and Braggins (1999) noted that relatively little work had been done establishing the relationship between diet and sheepmeat odour/flavour. This suggests that further work is required to elucidate what seems to be, a complex relationship between diet and sheepmeat flavour. One means of understanding this relationship would be to perform a similar study using sheep with more regulated finishing diets. The present work was aimed to survey animals available at abattoirs which procure sheep from a variety of different farms and diverse feeding regimes, making regulation of the animal's final feeding diet difficult.

On their own, BCFA concentrations measured in ovine fat are not sufficient for classifying sheep category. Pre-slaughter nutrition is a significant factor in the development of a statistical model that relates BCFA concentrations to animal age, lamb, hogget or mutton. It would be difficult to use this technique to classify sheepmeat samples, say, at retail as it would be difficult to obtain the details on an animal's finishing diet at this stage of the chain. Other strategies, e.g. fingerprint profiling (Ryan & Robards, 2006), exist which, combined with modern statistical techniques (Hastie, Tibshirani & Friedman, 2009), can be applied to the measured total ion chromatograms resulting from the separation and measurement of all compounds by gas chromatography/mass spectrometry. This is currently being investigated and shows great promise (Watkins, Clifford, Allen, Rose, Warner, Dunshea, & Pethick, 2009).

4.0 Conclusion

The chemical analysis of the branched chain fatty acids, EOA, MOA and MNA, in an ovine fat sample taken from a carcase was not sufficient to discriminate lamb from hogget or mutton. Provided that pre-slaughter nutrition was known, the concentrations of MOA and EOA (but not MNA) could be used to differentiate sheep category (lamb from hogget and mutton). Practically, though, it would be unlikely that this technique could be used for sheep classification as the ability to access pre-slaughter information becomes reduced as meat travels through the supply chain.

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Table 1. Nutrition categories indicating pre-slaughter diet

Nutrition category	Includes
Grain	Lamb finishing pellets
Lucerne	-
Lucerne mixed	Lucerne with oat paddock *or* clover/pasture *or* mixed grain supplement
Native	Mixed native pasture, native and improved pasture
Pasture	Paddock, hay, rye grass, clover, straw
Pasture supplement	Pasture with barley/hay, finishing pellets, oats/lupins
Saltbush	Old man salt bush/burr

Table 2. Predicted mean concentrations (mg kg^{-1}, back transformed from log variate) for three branched chain fatty acids for lamb (< 1 yr), hogget (> 1 yr and < 2 yr) and mutton (> 2 yr) with the inclusion (nutrition) and exclusion of nutrition (no nutrition) as a random term in the statistical analysis.

Effect	BCFA[d]	Lamb[e]	Hogget	Mutton	*P*-value
	MOA	0.131 ± 0.014	0.139 ± 0.020	0.133 ± 0.015	0.884
No nutrition	EOA	0.060 ± 0.005[a]	0.071 ± 0.007[ab]	0.076 ± 0.007[b]	0.024

	MNA	0.011 ± 0.002	0.011 ± 0.003	0.008 ± 0.002	0.195
Nutrition	MOA	0.107 ± 0.018[a]	0.197 ± 0.053[b]	0.147 ± 0.024[b]	0.009
	EOA	0.054 ± 0.009[a]	0.090 ± 0.016[b]	0.076 ± 0.014[b]	0.008
	MNA	0.008 ± 0.002[a]	0.020 ± 0.013[b]	0.008 ± 0.003[a]	0.056

[abc]Different letters within a row denote a significant difference

[d]MOA = 4-methyloctanoic acid, EOA = 4-ethylnonanoic acid, MNA = 4-methylnonanoic acid

[e]Mean ± least significant interval

Table 3. Estimated branched chain fatty acid concentrations (mg kg^{-1}) for pre-slaughter nutrition category (grain, lucerne, lucerne mixed, native pasture, pasture, pasture plus supplement and saltbush). (No measure of variation can be given as nutrition was treated as a random effect).

Pre-slaughter nutrition	MOA[a]	EOA	MNA
Grain	0.140	0.063	0.011
Lucerne	0.143	0.083	0.007
Lucerne mixed	0.221	-[b]	0.003
Native	0.279	0.123	0.019
Pasture	0.131	0.073	0.009
Pasture supplement	0.151	0.066	0.013
Saltbush	0.206	0.125	0.017

[a]MOA = 4-methyloctanoic acid (P = 0.001), EOA = 4-ethylnonanoic acid (P = 0.005), MNA = 4-methylnonanoic acid (P = 0.252)

[b]not estimated

Figure captions

Figure 1. Representative total ion chromatogram arbitrary units - AU) of compounds purged from a lamb fat sample as trimethylsilyl (TMS) esters showing retention times of the three main fatty acids. AU means arbitrary units. The inset shows an expanded region of the TIC indicating the retention times of the branched-chain fatty acids as TMS esters.

Figure 2. Schematic plots of individual animal readings of 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA) and 4-methylnonanoic (MNA) acids for the different age groups (lamb (< 1 yr), hogget (between 1 and 2 yr), and mutton (> 2 yr)). The box spans the interquartile range of the values, so that the middle 50% of the data lie within the box, with the line indicating the median. The perpendicular lines extend to the most extreme data values within the inner 'fences', which are at a distance of 1.5 times the interquartile range beyond the quartiles, or the maximum value if that is smaller.

Figure 3. Predicted mean concentrations (mg kg^{-1}, back-transformed from log variate, ± least significant interval) for 4-ethyloctanoic acid (EOA) against sheep age with no adjustment for nutrition in the model (P = 0.024).

Figure 4. Plot of predicted mean concentrations (mg kg^{-1}, back-transformed from log variate, ± least significant interval) for 4-ethyloctanoic acid (EOA) against sheep age (lamb, hogget and mutton) and gender (ewe, wether) with no adjustment for nutrition in the model. Significance of fixed terms in final model – Gender, P = 0.848 Age P = 0.011 Gender.Age P = 0.004 with model as Gender.Age

Figure 1.

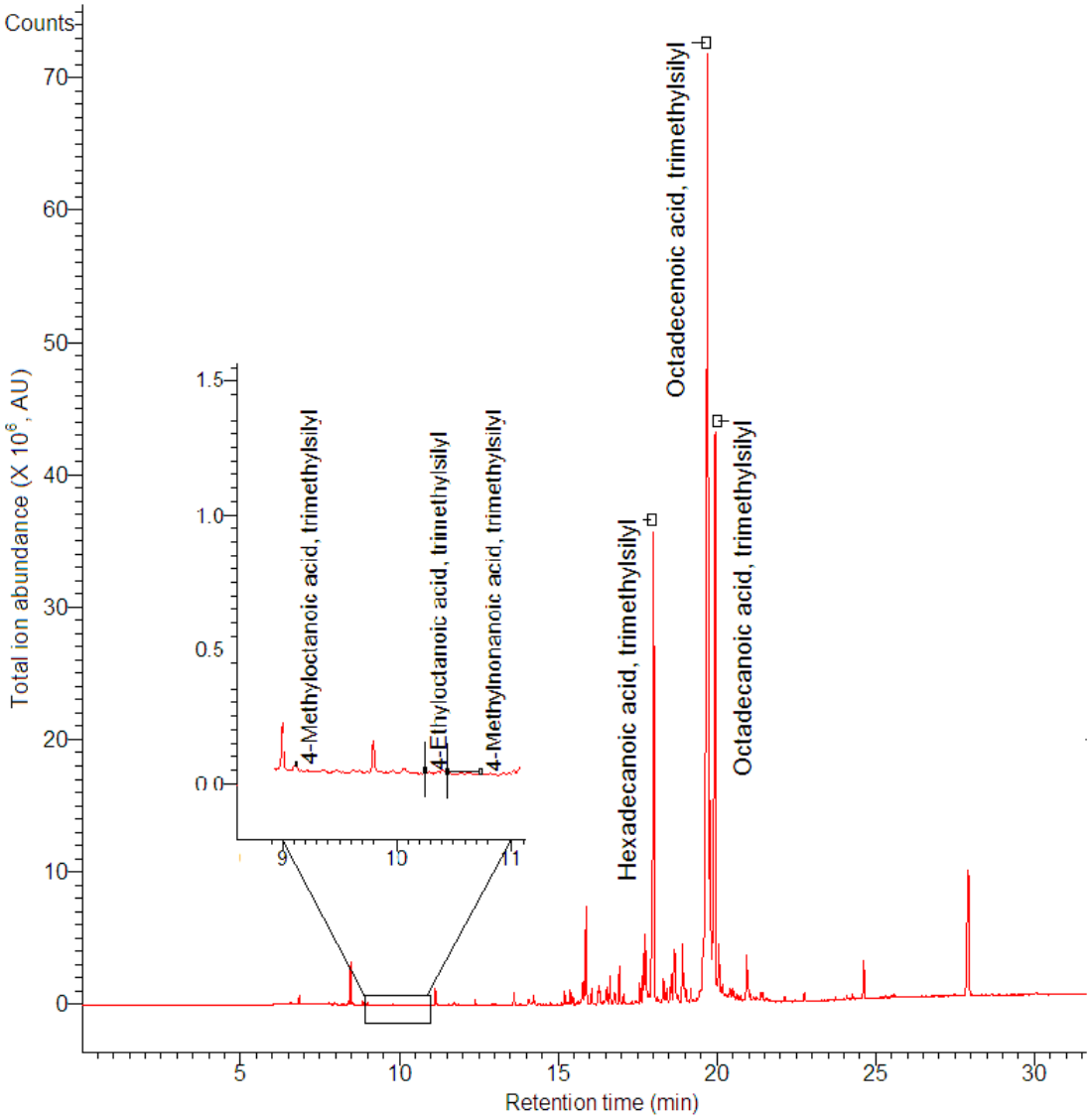

Figure 2

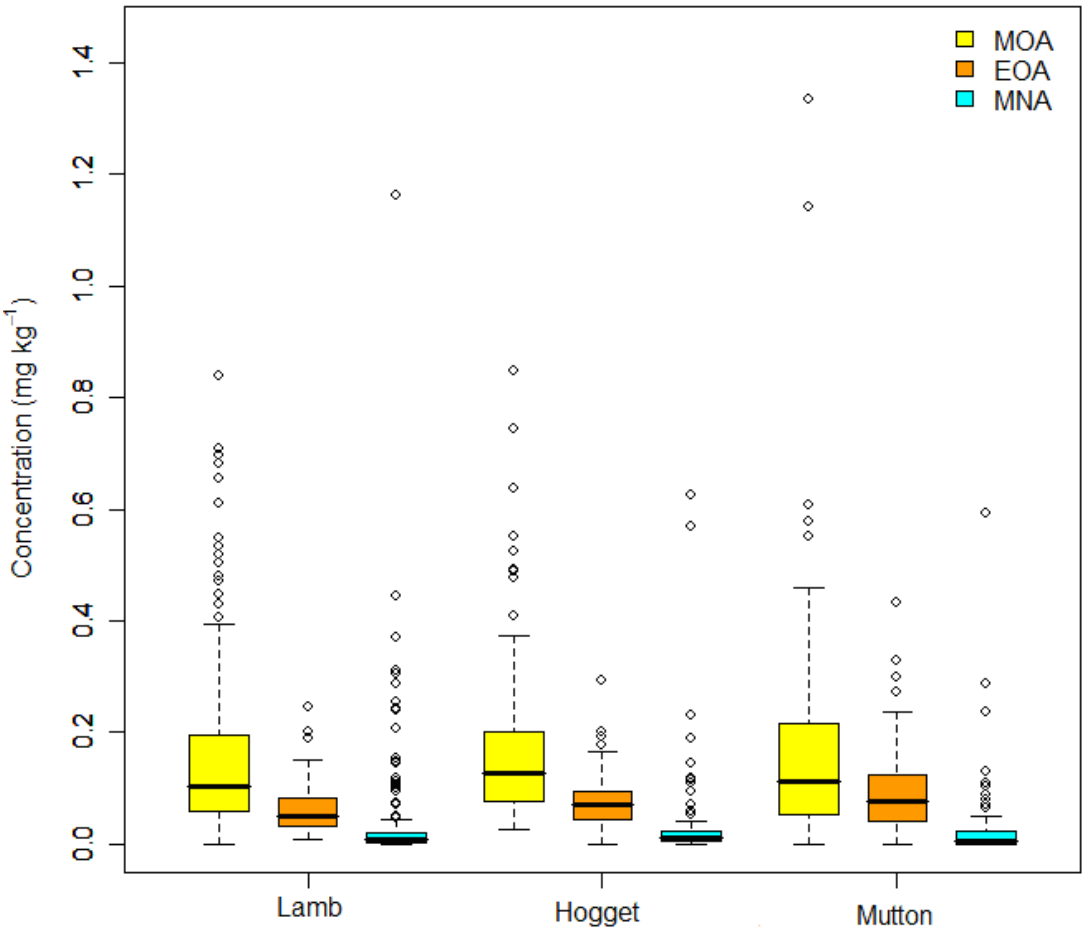

Figure 3

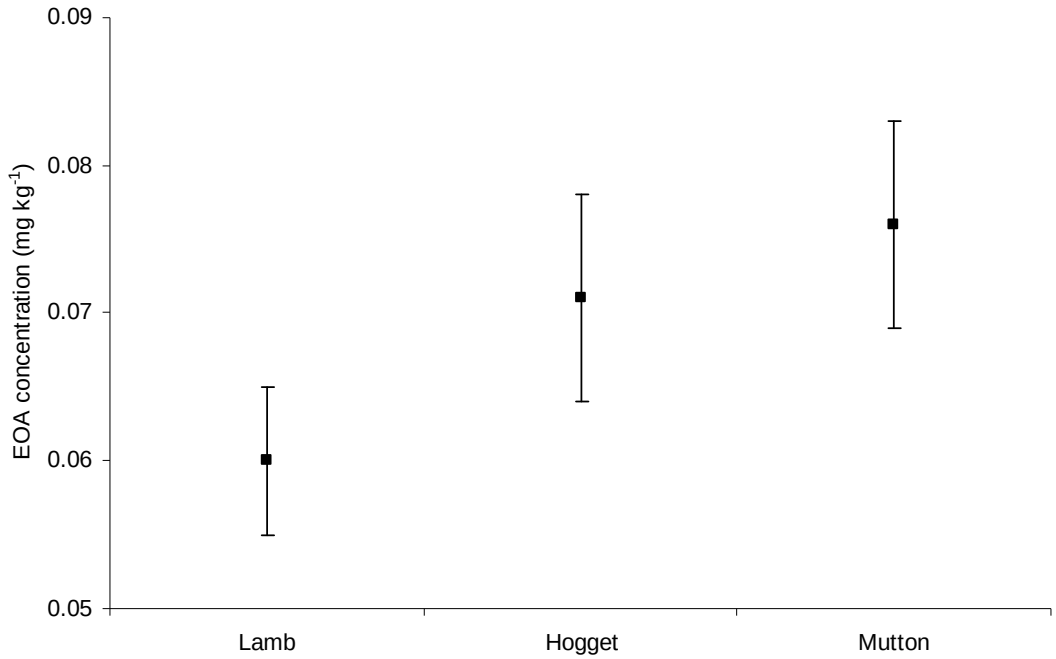

Figure 4