PE4.107 Chemical basis for discriminating lamb from mutton 395.00

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***Abstract*— The characteristic mutton odour, associated with the cooked meat of older animals, results in low consumer acceptance of sheep meat. Branch chain fatty acids (BCFA's) are considered to be the main determinants of mutton odour. Samples of subcutaneous fat were collected from 533 sheep carcasses at abattoirs in Australia. The samples were taken from sheep differing in age (lamb, hogget, and mutton), gender (wether and female), breed (Merino, Dorpa Lee with various crossbreeds) and nutrition (grain, lucerne, lucerne mixed, native pasture, pasture, pasture plus supplement and saltbush). The levels 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 included as a random term in the model, the levels of MOA and EOA in the fat could be used to discriminate sheep age ($P < 0.05$). Lamb fat had lower levels of MOA and EOA than hogget and mutton. When nutrition was excluded as a random effect, MOA and MNA did not differentiate between lamb, hogget and mutton while EOA was lower in lamb compared to mutton ($P < 0.05$) with hogget as an intermediate. An interaction existed between age and gender ($P < 0.05$) with lower EOA levels found in female lambs relative to hoggets and mutton but not in wethers. In conclusion, as pre-slaughter nutritional history is rarely available at the abattoir, levels of EOA, MNA and MOA levels in fat would not be recommended as an objective test for predicting sheep age and thus category.**

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***Index Terms*— sheep classification, mutton odour, branched chain fatty acids**

I. INTRODUCTION

Mutton substitution for lamb can be an occasional but significant problem in the Australian sheep industry. The characteristic mutton flavour/odour, associated with the cooked meat of older animals, results in low consumer acceptance of sheep meat. The compounds associated with this odour have been identified as branched chain fatty acids (BCFAs) with 4-methyloctanoic (MOA) and 4-methylnonanoic acid (MNA) considered as the two main contributors [1]. Work performed by Salvatore *et al* [2] indicated that the BCFA levels in lamb and mutton were different. This suggested that a BCFA test might be an effective tool for discriminating between lamb and mutton. The study by Salvatore *et al* was performed on animals that came from an experimental flock. Subsequently, a larger study was conducted with the focus was on animals of differing age, breed, sex and pre-slaughter nutrition, sourced from Australian domestic commercial abattoirs in New South Wales, Victoria and Western Australia. 4-Ethyloctanoic acid (EOA), another BCFA, was also included in this study as it has also been implicated as a contributor to mutton odour [3]. The objective of this study was to evaluate whether chemical analysis of BCFAs could be used to determine sheep age and thus sheep category (i.e. lamb, hogget or mutton). This was done with a long term view to have an objective test for the identification of

sheep category in order to validate "truth in labelling" of sheep meat. Such a test could mean that meat substitution may be detected in the supply chain and so help to reduce this practice in the industry.

II. MATERIALS AND METHODS

A. Sample collection and preparation

In total, 533 samples were collected from commercial abattoirs in three states of Australia; New South Wales (180), Victoria (170) and Western Australia (183). Details were also collected on breed (Merino, Dorpa Lee with various crossbreeds), sex (wether and female) and pre-slaughter nutrition (grain, lucerne, lucerne mixed, native pasture, pasture, pasture plus supplement and saltbush). By age group, there were 254 lamb (< 1 year old), 131 hogget (about 1-2 years old) and 148 mutton (> 2 years old) samples.

Subcutaneous fat samples (*ca* 20-30 g) were collected from the chump area (over the gluteus medius) at 24 hours post slaughter and frozen at -20 °C. Prior to analysis, the surface layer of the fat was removed and the remainder cut into smaller portions (*ca* 0.5 cm^3 squares). Molten fat was prepared by heating the cut portions in a microwave oven for approximately 5 min.

Molten fat (1g) was injected into a Unitrex co-distillation unit (SGE, Ringwood) and heated at 200 °C for 1 hr under a flow (200 mL min^{-1}) of nitrogen. The released compounds were purged through the Unitrex unit and collected on a Tenax® trap which 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 (IS, undecanoic acid – C_{11} FA, 1.00 mg kg^{-1}), the sample was derivatised using bisilyltrifluoroacetamide (BSTFA) at 60 °C for 30 min as trimethylsilyl (TMS) esters.

B. Determination of BCFAs by gas chromatography-mass spectrometry

The FA-TMS esters were separated by injection (1 □l) onto a DB5-MS fused silica capillary column (J&W, 30m x 0.25 mm i.d. x 250 □m 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 °C and increased to 325 °C at a rate of 180 °C min^{-1}. The GC oven was held at 75 °C for 2 min then increased to 300 °C at a rate of 10 °C 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 280 °C. Mass spectra were acquired using an ion source temperature of 220 °C 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 °C 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 C_{11} FA-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:

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

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 ($A_{\text{BCFAstandard}}/A_{\text{ISstandard}}$) against BCFA standard concentration where $A_{\text{BCFAstandard}}$ and $A_{\text{ISstandard}}$ are the peak areas of the BCFA standard and internal standard, respectively.

C. Statistical analysis

The data was tested in a similar way to [2] 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 sampling date, sex, abattoir, breed, and nutrition using restricted maximum likelihood (REML) models. The most parsimonius model, for each fixed variate, was chosen using Wald tests accompanied by approximate F statistics [4]. 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. After the selection of the appropriate model, specific pairs of means were compared using the SEDLSI procedure in GenStat [5]. Given a variate or table of parameter estimates (typically treatment means) and a corresponding standard error of distance (SED) or variance-covariance matrix, the SEDLSI procedure computes a value, d_i, such that [d_i + djj] □ SED and constructs least significant intervals (LSIs) for graphical presentation of the means with the data back transformed and presented on the original scale. LSIs are intervals (or error bars) that are designed to overlap where there is no significant difference between estimates, and to be disjoint where there are significant differences [6]. All analyses were performed using GenStat.

III. RESULTS AND DISCUSSION

Figure 1 shows a total ion chromatogram (TIC) of the compounds purged from a molten lamb fat sample using a Unitrex sweep co-distillation unit with the fatty acids (FAs) measured as trimethylsilyl (TMS) esters. The main components in the TIC were hexadecanoic (palmitic, $C_{16:0}$), octadecenoic (oleic, $C_{18:1}$) and octadecanoic (stearic, $C_{18:0}$) FA-TMS esters. Typical ranges for these FAs are ~ 20 to 30 g per 100g of the total fatty acid content [6]. In contrast, BCFAs were present in the ovine fat samples at much lower levels. This can be clearly seen in the inset of Figure 1 which shows an expanded region of the TIC, indicating the retention times of the BCFAs as TMS esters.

For MOA and MNA, when nutrition was excluded from the analysis, no difference was found in the levels of these BCFAs in lamb, hogget and mutton (P = 0.885 and 0.195, respectively; Table 1). Yet, for EOA, a relationship between the variate, $\log_{10}$ (EOA + 0.075), and the factors was found to exist (P = 0.024) with the mean EOA concentration increasing with age (Figure 2). The result for EOA is in accord with the generally accepted view that BCFA concentrations in ovine fat increases with an animal's age [8-11]. MOA and MNA would also be expected to increase with age but this was not the case. MOA was the most abundant BCFA while MNA was the least abundant with EOA as an intermediate. MOA has been reported elsewhere to be more abundant in ovine fat compared to MNA [11]. For EOA, an interaction between gender and age was observed with higher levels present in older females compared to the other age groups (Figure 3). This trend was not apparent in males across the age groups (Figure 3). This is also a contrast to other work where higher BCFA levels are generally associated with male animals compared to female [8].

Introducing pre-slaughter nutrition as a random term to the model had an effect for each BCFA (Table 1). For the log variates of MOA, EOA and MNA, diet had a significant influence on the BCFA concentrations for each age group (P = 0.009, 0.008 and 0.056, respectively). The mean value for MOA and EOA was highest for hogget with mutton as an intermediate and lamb having the lowest mean concentration. For MNA, hogget also had the highest mean concentration but no statistical difference was present between lamb and mutton. Given that hogget lies between lamb and mutton, the reason for this higher, unexpected result is unclear.

BCFA levels measured in ovine fat were not sufficient for classifying sheep category. Pre-slaughter nutrition is a factor in the development of a statistical model that relates BCFA concentrations to sheep age and thus whether an animal is lamb, hogget or mutton. It would be difficult though to use this technique to classify sheepmeat samples into an age class, since it would be difficult to get details on an animal's finishing diet at this end of the supply chain. Other strategies, e.g. fingerprint profiling [12], exist which, combined with modern statistical techniques [13], can be applied to the measured total ion chromatograms (TICs). This has been investigated and is reported in an accompanying paper [14].

IV. CONCLUSION

The chemical analysis of the branched chain fatty acids, EOA, MOA and MNA, in ovine fat was not sufficient to discriminate lamb from hogget or mutton. Provided that pre-slaughter nutrition was known, the levels of MOA and EOA (but not MNA) could be used to differentiate sheep category (lamb from hogget and mutton). Practically, though, it is unlikely that this technique could be used for sheep classification as accessibility to pre-slaughter information reduces as meat product travels through the supply chain.

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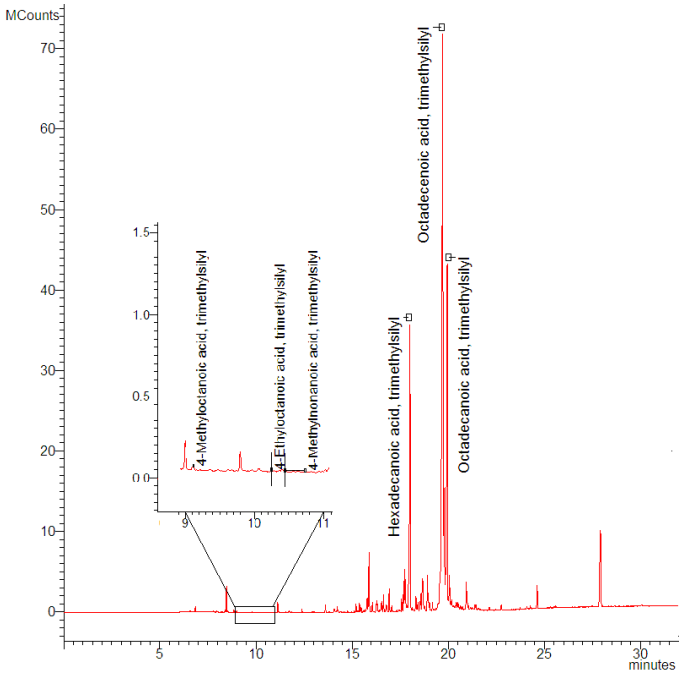

Figure 1. Representative total ion chromatogram (TIC) of compounds purged from a lamb fat sample as trimethylsilyl esters showing retention times of the three main fatty acids. The inset shows an expanded region of the TIC indicating the retention times of the branched-chain fatty acids as TMS esters.

Table 1. Predicted mean concentrations (mg kg^{-1}, back-transformed from log variate) for branched chain fatty acid (BCFA) of different sheep age with the inclusion and exclusion of nutrition as a random term in the statistical analysis.

Effect	BCFA[A]	Lamb	Hogget	Mutton	*P*-value
No nutrition	MOA	0.131	0.139	0.133	0.884
	EOA	0.060^{a}	0.071^{ab}	0.076^{b}	0.024
	MNA	0.011	0.011	0.008	0.195
Nutrition	MOA	0.107^{a}	0.197^{b}	0.147^{b}	0.009
	EOA	0.054^{a}	0.090^{b}	0.076^{b}	0.008
	MNA	0.008^{d}	0.020^{e}	0.003^{d}	0.056

[abc]Different letters within a row denote a significant difference ($P < 0.05$) [de]Different letters within a row denote a significant difference ($P < 0.1$) [A]MOA = 4-Methyloctanoic acid, EOA = 4-Ethylnonanoic acid, MNA = 4-Methylnonanoic acid

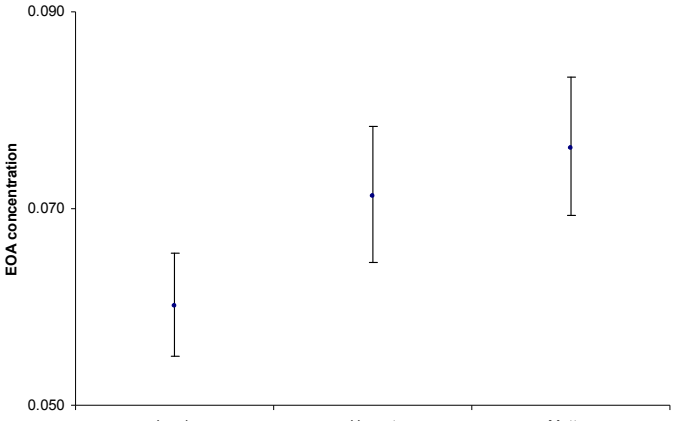

Figure 2. Plot of predicted mean concentration (mg kg^{-1}, back-transformed from log variate) for 4-ethyloctanoic acid (EOA) against sheep age without nutrition in the model as a random effect. ± least significant intervals (LSIs).

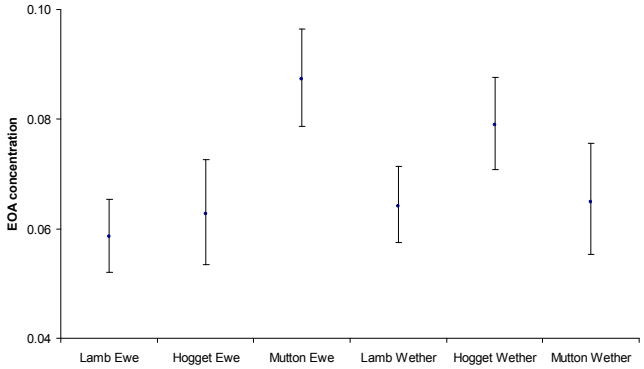

Figure 3. Plot of predicted mean concentrations (mg kg^{-1}, back-transformed from log variate) for 4-ethyloctanoic acid (EOA) against sheep age (lamb, hogget and mutton) and gender (female, wether) without nutrition in the model as a random effect. ± least significant intervals (LSIs). Significance of fixed terms in final model – Sex, $P = 0.848$ Age $P = 0.011$ Sex.Age $= 0.004$ with model as Sex*Age

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