Volatile Branched Chain Fatty Acids in Fat From Two Sheep Breeds

O. A. Young[1], M. P. Agnew[1] & T. J. Fraser[2]
[1]MIRINZ, P.O. Box 617, Hamilton, N. Z. & [2]AgResearch, Lincoln, N. Z.

Introduction

The Merino breed is anecdotally stronger in flavour than others, although there is no experimental evidence to confirm this. Moreover, the term 'flavour' as used here is vague. It could refer to sheepmeat flavour (Wong *et al.*, 1975), pastoral flavour (Berry *et al.*, 1980), or some other ill-defined flavour characteristic of the breed. Of these possibilities, sheepmeat flavour is amenable to a direct chemical measure of the volatile branched chain fatty acids (BCFAs). These fatty acids, often 4-methyl-branched with 9 or 10 carbon atoms, are strongly characterising of sheep and goat meats (Brennand *et al.*, 1989). We measured the concentration of these fatty acids in fat of Merino and Coopworth sheep of the same age, sex and dietary history.

Materials and Methods

Lambs were born in spring on the same median date for both breeds (n = 2 x 10), castrated, weaned onto ryegrass/clover, and finally grazed together for six weeks before slaughter at 35 weeks. The day after slaughter, subcutaneous backfat was sampled at the 13th rib, taking care to recover the entire fat layer. Perirenal fat was also recovered.

The fat was frozen to –20°C until ready for analysis. After tempering to –5°C, adhering lean tissue was removed with a scalpel and the clean fat was finely chopped with razor blade. Accurately weighed 1 g samples was blended in a Polytron for 30 seconds with 16 ml of methylene chloride and 0.5 g of anhydrous sodium sulphate. The homogenate was filtered through paper (Whatman 4) and the residue washed with 2 ml aliquots of methylene chloride to 20 ml final. A 2 ml subsample was evaporated at 30°C under a gentle stream of nitrogen. 2-Methyloctanoic acid (5 μg), a gift of C.B. Johnson, Palmerston North, was added as an internal standard prior to methylation. For calibration, known quantities of 4-methyloctanoic and 4-methylnonanoic acids (Lancaster Synthesis, U.K.) were added to screwcap tubes. The esterified and/or free fatty acids in each tube were methylated with 2 M methanolic sodium hydroxide (0.1 ml, 100°C, 10 min.) followed by 2 % sulphuric acid in methanol (2 ml, 100°C, 60 min.) The mixture was shaken with 2 ml of saturated NaCl and 1 ml of pentane. The pentane phase was recovered, and the mixture rewashed with pentane. After drying with sodium sulphate, the pentane was evaporated with nitrogen at room temperature.

Aliquots of the methyl esters in fresh volumes of pentane were injected, then resolved on BPX gas chromatographic columns (SGE, Australia), using temperature profiles to achieve clear resolution of interesting esters, which were detected by flame ionization (HP 5890, BPX70) for longer chain fatty acids, or by a mass spectrometer (MD800, Fisons, BPX5) in selective ion mode, m/z = 74, 87 and 88 to detect the shorter chain BCFAs and the internal standard. Saturated BCFAs with 17 carbon atoms were selected with m/z = 284. Longer chain fatty acids were expressed as area percent of the flame ionization profile, while the shorter chain BCFAs were expressed as μg BCFA (as the free acid) per g of chopped fat after corrections for the internal standard and calibration. Means were compared by *t*-tests.

Results

The mean carcass weight for Coopworth was higher than for Merino, 17.6 *vs* 14.1 kg ($P < 0.001$). Although fat thickness was not measured, it was obvious that the Coopworth lambs had more subcutaneous fat.

In preliminary work to identify the BCFAs in a mixture with other shorter chain fatty acids, the total ion mode was used for mass spectral detection. However, as was anticipated, when lambfat esters were analysed, selective ion monitoring was essential to detect measurable quantities of the BCFAs. This was because these acids are present in very low concentrations. The Kovats' indices on a BPX5 column for several methyl BCFAs and straight chain fatty acids are shown in Table 1.

Table 1. Kovats' indices and ion abundances of several fatty acid methyl esters resolved on a BPX5 column.

Fatty acid methyl ester	Kovats' index	Relative proportions[¶] of three diagnostic ions (%)		
		74	87	88
Octanoic	1139	69	28	3
2-Methyloctanoic	1169	2	8	90
4-Methyloctanoic	1190	42	46	12
Nonanoic	1228	67	30	3
4-Methylnonanoic	1274	68	31	1
Decanoic	1318	64	33	3

[¶] Data are means from the subcutaneous fat of two Coopworth and two Merino lambs.

The 88 ion was useful in identifying 2-methyloctanoic acid, the internal standard for quantitation of two species-characteristic BCFAs in subcutaneous and perirenal fat (Table 2).

Table 2. Concentration of BCFAs in sheepfats (μg/g) from two breeds.

Breed	Subcutaneous fat		Perirenal fat	
	4-Methyloctanoic[¶]	4-Methylnonanoic	4-Methyloctanoic	4-Methylnonanoic
Coopworth	172 ± 25	14.1 ± 2.3	26.4 ± 3.0	2.2 ± 0.8
Merino	71 ± 7	7.4 ± 1.4	19.0 ± 2.6	2.1 ± 0.6
Effect of breed	***[†]	*	NS	NS

[¶] Data are means of 10 lambs per breed ± standard error. [†] ***, $P < 0.001$; *, $P < 0.05$; NS, not significant.

The mean concentration of both BCFAs was much higher in the subcutaneous fat ($P < 0.001$), and the mean concentration in both fat depots was numerically higher in Coopworth, significantly so in subcutaneous fat. When BCFA data for subcutaneous fat from the two breeds were independently correlated, the Pearson coefficients were around 0.63, but the significance was low ($P \leq 0.06$). By comparison, the equivalent correlations in perirenal fat were essentially random ($P \leq 0.72$).

In addition to the six compounds identified in the chromatographic profile between 1100 and 1350 Kovats' units (Table 1), there were about another 18 peaks revealed by two or more of the three selected ions. As judged by ion proportions and relative retention times, these were very possibly isomers of methyl- (or ethyl-) branched acids, like 4-ethyloctanoic acid and others (Brennand & Lindsay, 1992). Based on total ion counts (three ions only) their concentrations were significantly higher in Coopworth subcutaneous fat than in Merino.

The fatty acid composition of longer chain fatty acids in subcutaneous fat is expressed as area percent in Table 3. The greatest numerical and significant difference occurred with stearic acid, much lower in Merino, causing Merino fat to be more oily to the touch.

Table 3. Area percent[¶] of longer chain fatty acid methyl esters from subcutaneous fat of two sheep breeds.

Breed	Fatty acid													
	14:0	14:1	15:0	15:1	16:0	16:1	17:0 (14-Me)	17:0 (15-Me)	17:0	18:0	18:1	18:2	18:3	Saturated/ unsaturated
Coopworth	2.9	0.26	0.67	0.22	22	1.4	0.46	0.76	1.80	29	31	1.2	1.4	1.63
Merino	5.1	0.28	0.80	0.24	22	1.5	0.43	0.75	1.53	23	33	1.3	1.2	1.45
Effect of breed[†]	***	NS	**	NS	NS	NS	NS	NS	**	***	*	NS	NS	*

[¶]Values are means, 10 lambs per breed. Percentages do not add to 100 because smaller fatty acids are not shown and some unidentified peaks were probably not fatty acids. Standard errors are not shown for clarity. [†] See Table 2; **, $P < 0.01$.

Two methyl-branched long chain fatty acids are included in Table 3. Other C17 methyl-branched fatty acids were detected with ion m/z = 284. These acids, probably the 4-, 6-, and 8-methyl isomers (Johnson & Purchas, 1997), were present in lesser quantities. If the identities are valid, all the C17 branched chain fatty acids eluted between C16:0 and C17:0 on a BPX5 column.

Discussion

The use of selective ion monitoring has obviated the need to concentrate BCFAs by the laborious procedure of simultaneous distillation and extraction (SDE), first applied to sheepfat BCFAs by Wong *et al.* (1975). The selective ion method has the advantage that methyl esterification in one tube is sufficient preparation for fatty acids present in the tens of parts per million range, like the volatile BCFAs, and for fatty acids present in quantities 3000-fold higher, like stearic acid.

4-Methyloctanoic and 4-methylnonanoic acids were present in the two fat depots of Coopworth and Merino lambs in quantities higher than previously encountered in several studies (see e.g., Purchas *et al.*, 1986; Brennard & Lindsay, 1992). This could have been due to differences in methodology. However, the lower concentration of the BCFAs in perirenal fat is consistent with other studies, and is due to the different metabolic origin of the perirenal fat, more directly from diet rather than from *in situ* synthesis which generates the BCFAs (Scaife & Garton, 1975; Ha & Lindsay, 1990; Brennard & Lindsay, 1992). This difference might explain why the concentrations of the two BCFAs were uncorrelated in perirenal fat.

Johnson & Purchas (1997) found good correlations between 4-methyloctanoic and methylhexadecanoic acids (not *iso-* or *anteiso-*) in subcutaneous fat of lambs, and this formed a basis for measuring the characterising BCFAs without SDE procedures. These longer chain branched acids were probably detected in the present study but were not measured.

It is widely recognised that compared with other breeds, Merinos have an oily subcutaneous fat. This is reflected in the breed's fatty acid composition (Cramer *et al.*, 1970) and was confirmed in the present study.

Returning to core question as to why Merinos are considered 'stronger' flavoured than other breeds, it seems unlikely that BCFAs are responsible because their concentrations were higher in Coopworth, a common meat-producing breed. Nonetheless, the Merino is a slow-maturing breed, and at maturity, BCFA concentrations might equal or exceed those in other breeds. However, there are three other plausible explanations: Merino is primarily a wool breed. So long as a Merino is adequately producing wool it is likely to be held on-farrn rather than slaughtered. As a result, Merinos at slaughter are likely to be older and therefore more strongly flavoured. Also, Merino lambs are prone to the high pH condition at slaughter (Young *et al.*, 1993; Hopkins *et al.*, 1996), which can adversely affect flavour. Finally, the higher concentration of unsaturated fats might lead to stronger odours and flavours.

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