FATTY ACID COMPOSITION OF PASTURES

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SUMMARY
The long chain fatty acid (LCFA) concentration and composition of herbage from 3 mixed-species pastures grown at Kyabram, Victoria, were determined for a small number of samples harvested at a nominal grazing height. The 3 pasture types were perennial ryegrass (*Lolium perenne* L., 51-61%), Persian clover (*Trifolium resupinatum* L., 37-76%) and subterranean clover (*Trifolium subterraneum* L., 39-84%). Based on a small data set, we estimate that cows grazing perennial ryegrass, Persian clover or subterranean clover-type pastures could consume herbage containing 18-22, 14-27 and 20-34 g LCFA/kg DM, respectively, with half to two thirds of this as 18:3 fatty acid. Time of year appeared to be an important factor in determining the total concentration and composition of LCFA in pastures. There was a positive linear relationship between the total concentration of LCFA and concentration of crude protein in harvested herbage, with this relationship apparently independent of botanical composition of pastures.

Keywords: pastures, long chain fatty acid concentration, long chain fatty acid composition

INTRODUCTION
Variation in total intake and composition of fatty acids consumed from forages and other sources can alter the composition and concentration of fat in milk, and can affect fertility, body condition score and incidence of disease in cows (Thatcher and Staples 2000; Bauman and Griinari 2003; Stockdale 2001; Azain 2003). Variation in the composition of milk fat also has implications for human health and its suitability for use in different markets as an ingredient (Berner 1993; Papalois *et al.* 1996). The ability of farm managers to manipulate the total intake and composition of fatty acids by cows has the potential to improve production efficiency and the value of milk (Dewhurst *et al.* 2003).

Lipids from forages are the primary source of lipids in the diet of cows in most dairy production systems in south-eastern Australia. Forages tend to be high in linolenic (18:3), linoleic (18:2) and palmitic (16:0) acids, although the relative concentrations vary with species, variety and conservation technique (Mayland *et al*. 1976; Dewhurst *et al.* 2003). In general, the total concentration of LCFA in DM, and the concentration of 18:3 in total LCFA, will decrease with increasing intensity of light, temperature and physiological maturity (Hawke 1973) while the total concentration of LCFA in DM will increase in response to management techniques that promote rapid vegetative growth (Barta 1975). In fresh herbage, this variation is positively correlated with changes in the concentration of crude protein (Mayland *et al*. 1976).

While there is good information on the concentration of metabolisable energy, crude protein and neutral detergent fibre in pastures grazed by dairy cows in Victoria (Cohen and Doyle 2000; www.Target10.com.au; Heard *et al.* 2002), information is limited on the concentration and composition of fatty acids. The objective of this paper is to describe the concentrations of fatty acids in fresh herbage representative of that consumed by cows grazing pastures typical of those grown at Kyabram, in the irrigation area of northern Victoria.

METHODS AND MATERIALS
Samples of herbage harvested from 3 mixed-species pastures grown at Kyabram (36°20´S, 145°04´E) were taken between August 2001 and December 2002. The 3 pasture types, classified by the predominant species, were perennial ryegrass (*Lolium perenne* L.), Persian clover (*Trifolium resupinatum* L.) and subterranean clover (*Trifolium subterraneum* L.) (Table 1).

For samples of annual clover pastures, two 0.245 m^2 quadrats of pasture were selected that were similar in botanical composition (assessed visually) and height (± 1.5 cm) as measured with a rising plate meter (Earle and McGowan 1979). One quadrat was harvested to ground level and the other to 4 cm using hand shears and a cutting height guide set at 4 cm. For perennial ryegrass pastures, samples of herbage from an area 10 to 20 m^2 were harvested to 4-6 cm (nominal grazing height) using an auto-

scythe. At the same time, at least five 0.245 m^2 quadrats of pasture representative of this material were harvested to ground level and combined to form a single representative sample. All samples were placed in a freezer (-20°C) within about 2 h of sampling, and were subsequently thawed in a refrigerator (3°C) just prior to sub-sampling and analysis for botanical and chemical composition.

A sub-sample from each sample cut to ground level was taken to determine botanical composition by sorting into ryegrass, clover, other live (other), and dead material, followed by drying at 100°C to determine the contribution of each category to total DM. A sub-sample was taken from material cut to nominal grazing height, freeze dried and milled through a 0.5 mm screen prior to determination of total nitrogen (N), using a Leco FP-428 (Leco Australia Pty Ltd), and the composition and total concentration of fatty acids eluting between C10 to C20, according to the method of Sukhija and Palmquist (1988), with the exception that methylene chloride was used in place of benzene as the extracting solvent. The DM content at 100°C of each freeze-dried sample was also determined and used to adjust the total concentrations of fatty acids and N in freeze-dried matter to that in dry matter determined at 100°C.

Crude protein (CP) was calculated from total nitrogen (CP = N × 6.25). Total LCFA is the sum of 16:0, 18:0, 18:1, 18:2 and 18:3. With 1 exception, all other peaks eluting between C10 and C20 on each chromatogram comprised less than 0.5% of the total area. They were excluded from further analysis due to the high degree of error associated with estimating their contribution to total LCFA. The exception was a compound that eluted with a retention time similar to that of 10:1 and an area of the elution peak similar to that of 16:0. This compound was later determined to be a 5-carbon keto acid when analysed using a gas chromatograph/mass spectrometer, and was also excluded from the analysis on this basis. The relationship between the concentration of CP and total LCFA in samples was determined by simple linear regression (Genstat 6).

Table 1. Botanical composition (g/kg DM) of herbage from 3 types of pastures cut to ground level.

Harvest date	Botanical Composition			
	Ryegrass	Clover[A]	Other	Dead
	Perennial Ryegrass			
28-Sep-01	587	260	67	87
18-Oct-01	512	372	81	35
18-Oct-02	607	333	24	36
	Persian Clover			
03-Jun-02	172	448	349	32
24-Jun-02	309	483	123	85
13-Aug-02	264	519	96	120
13-Aug-02	43	758	120	79
29-Aug-01	287	368	285	60
18-Sep-02	436	462	21	81
	Subterranean clover			
24-Jun-02	30	766	11	193
15-Aug-02	0	838	81	81
15-Aug-02	406	516	3	75
28-Aug-02	47	700	220	33
10-Sep-02	0	849	55	96
26-Sep-02	350	391	0	259
15-Oct-02	0	803	40	157

[A] The category clover in perennial ryegrass pastures was usually white clover (*Trifolium repens* L.), while the category ryegrass in the 2 clover type pastures was usually short rotation ryegrass (*Lolium perenne* × *multiflorum*).

RESULTS

The composition and total concentration of LCFA for each sample are given in Table 2. Herbage from perennial ryegrass pastures harvested above 4-6 cm height contained an average of 20.1 g LCFA/kg DM. Herbage from Persian and subterranean clover pastures harvested above 4 cm height contained an average of 22.9 g and 27.5 g LCFA/kg DM, respectively, however, values were higher in June and early August and appeared to decline in late August and September. Qualitative examination of the data indicates that annual clover pastures contained similar concentrations of total LCFA to the perennial ryegrass type pastures in late September and October.

The principal LCFA in all pastures was 18:3 with concentrations in the annual clover pastures 2.1 to 4.8 times that of 16:0, and 2.3 to 5.1 times that of 18:2, these being the next most quantitatively

significant fatty acids in all pastures. The lowest ratios of 18:3 to 16:0 or 18:2 for annual clover pastures occurred in late August and September. The ratios of 18:3 to 16:0 and 18:2 for the perennial ryegrass pastures were 2.5-3.3 and 3.2-3.5, respectively. The concentrations of 18:0 and 18:1 were low compared with the other fatty acids, and appeared to increase in the annual clover pastures in late August and September.

There was a significant positive linear relationship between the concentration of CP and total LCFA in DM using all samples harvested above nominal grazing height:

$$\text{Total LCFA (g/kg DM)} = 0.11\ (\pm\ 0.015) \times \text{CP (g/kg DM)} + 0.06$$

($P<0.001$; $100r^2 = 72.0$; r.s.d. = 2.87; n = 16).

Table 2. Composition (g/kg total LCFA) and total concentration (g/kg DM) of LCFA from 3 types of pastures cut to a rising plate meter height of 4-6 cm.

Harvest date	Fatty Acid					
	16:0	18:0	18:1	18:2	18:3	Total
			Perennial Ryegrass			
28-Sep-01	218	23	40	171	547	20.4
18-Oct-01	213	24	48	172	543	18.4
18-Oct-02	179	36	24	170	591	21.5
			Persian Clover			
03-Jun-02	163	19	16	154	649	27.3
24-Jun-02	166	13	9	133	679	26.7
13-Aug-02	193	15	13	157	622	24.0
13-Aug-02	177	16	13	141	658	25.3
28-Aug-02	195	30	41	146	588	21.2
29-Aug-01	191	21	28	157	604	22.3
18-Sep-02	224	47	39	210	480	13.7
			Subterranean clover			
24-Jun-02	145	22	10	155	667	33.6
15-Aug-02	144	23	10	139	683	31.9
15-Aug-02	144	25	18	128	685	26.8
10-Sep-02	150	23	12	133	682	32.0
26-Sep-02	179	23	20	170	610	19.7
15-Oct-02	198	32	36	156	578	21.2

DISCUSSION

The range in the total concentration of LCFA in samples harvested above nominal grazing height from perennial ryegrass pastures was low (18 to 22 g/kg DM) compared with values reported in other studies. For example, Elgersma *et al.* (2003) report a range in total concentration of LCFA of 22 to 29 g/kg DM in perennial ryegrass pastures over a full growing season at Wageningen University, The Netherlands. Dewhurst *et al.* (2002) report a range in total concentration of LCFA of 21 to 35 g/kg DM in perennial ryegrass pastures over a full growing season at Aberystwyth, UK. Molloy *et al.* (1978) report a range of 29 to 48 g/kg DM in perennial ryegrass/white clover pastures over a full growing season at Palmerston North, NZ. Differences between locations may be due to differences in variety, maturity and/or the environment under which pastures were grown (Elgersma *et al.* 2003), however, it would be useful to conduct some comparative testing of samples between laboratories used in more recent studies to ensure consistency in reporting of results between sites.

The average proportion of total LCFA as 18:3 in the perennial ryegrass pastures sampled in this study was also lower than values reported by Dewhurst *et al.* (2002) and Elgersma *et al.* (2003) (0.56 *v.* 0.66 and 0.73, respectively). Hawke (1973) reported considerable variation in the proportion of total LCFA as 18:3 in samples of ryegrass and lucerne. This variation was inversely associated with the intensity of light and/or temperature during growth, and was reported to be more influential on composition of LCFA than physiological maturity. While there are no data to compare levels of light intensity and temperatures experienced during growth of pastures in each of the respective studies cited here, it is probable that both were higher during growth of pastures at Kyabram given well-established differences in climate between sites.

Lower proportions of 18:3 in total LCFA in the annual clover pastures, particularly in late August and September, did not appear to be related to changes in botanical composition. As suggested above, changes in light intensity and/or temperature may also be responsible for changes in the composition of LCFA in these pasture types. However, there appear to be limited data available to compare the

effects of factors such as environment or physiological maturity on the concentration or composition of LCFA for these pasture types, and it is not possible to assess the relative importance of each in this study.

The linear relationship between the concentration of total LCFA in DM and that of CP is in good agreement with the findings of Barta (1975) (slope of 0.10 ± 0.009), for combined data for 6 grass species, and Molloy *et al.* (1978) (slope of 0.09 ± 0.039) for perennial ryegrass/white clover pastures. This is not surprising as most of the crude protein in plant leaf material is usually true protein associated with membrane lipids, particularly the chloroplasts (Bauchart *et al.* 1984). However, there was a large difference in the height of the intercept between studies (0.06 in this study *v.* 16.9 and 17.2 in the 2 studies mentioned above). An intercept close to zero, as was found in this study, suggests most of the LCFA in leaf material is associated with protein. A positive intercept suggests that some LCFA occurs in leaf material that is not associated with protein. One possible explanation for this discrepancy is the existence of consistent errors in the estimation of the total concentration of LCFA in samples between laboratories. It is possible that both Barta (1975) and Molloy *et al.* (1978) overestimated the total LCFA in their forage samples. The technique they used assumed the total concentration of carboxyl groups (meq/mL) following an extraction of lipid from a sample of herbage was equivalent to the concentration of LCFA in the original sample. This assumption may not always be valid, as demonstrated by the presence of significant quantities of a 5-carbon keto acid appearing in lipid extracts of samples analysed in this study.

Time of year appears to be more important than botanical composition in determining the concentration and composition of LCFA herbage harvested above a nominal grazing height. There is a strong positive linear relationship between the concentration of crude protein and total LCFA in herbage with this relationship, apparently also independent of the botanical composition of pastures sampled in this study. Comparative testing of samples between laboratories used by different research groups would help to ensure consistency in reporting of results for the composition and total concentration of LCFA's in pastures grown at different sites.

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