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Influence of pasture and concentrates in the diet of grazing dairy cows on the fatty acid composition of milk

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In five short-term experiments conducted in Victoria in 1997 and 1998, grazing dairy cows were given either pasture alone or pasture supplemented with high-energy concentrates, and the fatty acid profiles of milk fat were measured. We established the effects of these feeds on some aspects of milk fat of importance for human nutrition, but we specifically focused on the hypothesis that conjugated linoleic acid (CLA) concentrations in milk fat increase as pasture intake increases, and decrease as more concentrates are fed. In agreement with previous research, feeding fresh pasture alone resulted in high concentrations (1·0–1·8 g/100 g milk fat) of CLA. When the effect of level of pasture consumption on CLA content was examined, a significant positive relationship ($r^2 = 0{\cdot}35$; $P < 0{\cdot}05$) was obtained. When cereal grain concentrates were used to supplement pasture intake, the CLA content of milk fat generally declined ($P < 0{\cdot}05$), except where the amount of concentrates given led to a marked reduction in total milk fat concentration. The use of cereal grain concentrates also generally resulted in significant ($P < 0{\cdot}05$) increases in medium-chain saturated fatty acids, but always reduced the contribution of butyric acid to milk fat, from 4·5 to 3·9 g/100 g milk fat, on average.

Keywords: Conjugated linoleic acid, cereal concentrates, milk fat.

Dairy farmers in southern Australia and New Zealand are paid principally for the solids component of the milk they produce. Concentrations of fat, protein and lactose in milk are affected by energy intake, type of diet, breed of cow, genetic variation, time of year and stage of lactation. Milk fat is the constituent of milk solids that can be most readily influenced by nutritional manipulation, and is under the farmer's control. Sutton (1989) reported that milk fat concentration can be altered over a wide range, of about 3 percentage units, by nutritional means, but protein can be altered only by about 0·6 percentage units, and lactose can barely be altered at all.

The milk fat of dairy cows has some unique functional and nutritional properties, and modifying its chemical composition and structure can enhance or alter these properties. Although milk fat is high in saturated fatty acids, which have been claimed to contribute to heart disease (Berner, 1993; Chisholm et al. 1996), other components of milk fat are considered to be beneficial for human health (Parodi, 1999, 2001). Two milk components that have received attention recently are the fatty acids, conjugated linoleic acid (CLA) and butyric acid ($C_{4:0}$). The term, CLA, is used for a mixture of positional and geometric isomers of linoleic acid ($C_{18:2}$) that contain conjugated unsaturated double bonds. Recent research shows CLA to be anticarcinogenic (Ip et al. 1994; Aro et al. 2000). The most biologically active isomer of CLA appears to be *cis*-9, *trans*-11 $C_{18:2}$, which accounts for >80% of the isomers of CLA in milk fat (Chin et al. 1992). While meat and organs of ruminant animals contain appreciable amounts of this fatty acid, milk fat is the richest natural source of CLA. Butyric acid, which is uniquely present in ruminant milk, has been claimed to have a role in preventing colon cancer (Perrin et al. 1994). Recent research has shown that the fatty acid profile of the milk fat

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produced by dairy cows can be influenced by dietary manipulation (Murphy, 2000).

The objective of this study was to examine the influence of pasture and concentrates in the diet of grazing dairy cows on some aspects of milk fat composition of particular importance for human nutrition. Specifically, we focused on the hypothesis that CLA concentrations in milk fat increase as pasture intake increases, and decrease as more concentrates are fed.

Materials and Methods

During 1997 and 1998, five experiments were conducted in which grazing dairy cows were given either pasture alone or pasture supplemented with high-energy concentrates. Four of the experiments took place at Kyabram, in northern Victoria (36° 20′ S, 145° 04′ E), and one at Ellinbank, in southern Victoria (38° 30′ S, 146° 00′ E). Some information about these experiments has been summarized in Table 1. More detailed descriptions of pasture mass, botanical composition and pasture allowances in Experiments 1–4 are available in the publications listed in Table 1. For Experiment 5, pre-grazing pasture mass was 2·4 t dry matter (DM)/ha, which comprised 830 g/kg DM as perennial ryegrass (*Lolium perenne* L.), 5 g/kg DM as white clover (*Trifolium repens* L.) and 165 g/kg DM as weeds and dead material. The height of the pasture before grazing according to a rising plate meter was 8·0 cm, and the daily pasture allowance for all treatments was 40 kg DM/cow. The management of cows and grazing, and the measurements made were similar for all the experiments.

In Experiments 1–4, groups of between two and five Friesian cows were offered either pasture alone or the same pasture allowance with one or more levels of supplement. In Experiment 1, cows were offered different pasture allowances, with no supplementation. In Experiment 5, the cows grazed as one group. All cows were multiparous and were blocked on the basis of pre-experimental milk yields, with cows within blocks being randomly allocated to treatments and replicates. Details of the cows, and a summary of feeding treatments, are given in Table 1. For at least 2 weeks before each experiment, information was collected for adjustment of experimental data for between-cow differences. Once an experiment started, all cows received their allocated feeds for about 5–7 weeks, with samples being collected for analysis of milk fat components during the last 3–4 weeks.

In Experiments 1–4, all treatment and replicate groups grazed separately and, in all experiments, cows were given fresh strips of pasture twice each day. Supplements were given individually to cows twice each day, immediately after milking, at about 07.00 and 16.00, and before returning to the pasture. While at pasture, back-grazing beyond the current day's allocation was prevented. In Experiments 1–4, cows had access to water at milking times only (King & Stockdale, 1981).

Cows were milked twice daily, and individual yields were recorded. Cows were weighed immediately after a morning milking, either daily or twice a week, and their body condition scores were assessed at the beginning and end of an experiment according to the 8-point scale of Earle (1976). DM intakes of supplements were measured daily for each cow, and samples of that offered and refused were dried at 100 °C for 24 h to determine DM content. The amount of pasture eaten by each group of cows was assessed every day using a sward-sampling technique similar to that described by Stockdale & King (1983). A rising plate meter (Earle & McGowan, 1979; Stockdale, 1984) was used to estimate pre- and post-grazing herbage masses. Each day, 20–50 rising plate meter height measurements were taken for all treatments. Calibration quadrats (0·245 m^2) were cut to ground level with hand shears in Experiments 1–4, and with a shearing hand-piece in Experiment 5, to allow conversion of meter reading to herbage mass. Separate regression equations were developed for pre- and post-grazing samples in each experiment.

In all experiments, samples of the herbage (about 0·04 m^2) offered to cows in each treatment were cut to ground level each day, dried at 60 °C for 72 h and, after bulking on a weekly basis and grinding through a 1 mm screen, were analysed for pepsin/cellulase *in vitro* digestibility (IVDMD) (Clarke et al. 1982). In addition, samples from Experiments 1–4 were analysed for total nitrogen (N) (Leco Australia, Castle Hill, NSW), while those from Experiment 5 were analysed using a micro-Kjeldahl method. Neutral-detergent fibre was determined by a modification of the technique described by Van Soest et al. (1991): the amylase step was excluded because the samples filtered freely. Samples of the concentrates offered were analysed as for herbage, except that digestibility *in vitro* was determined using a rumen fluid technique (Tilley & Terry, 1963). Crude protein concentration was calculated as N % × 6·25.

In each experiment, a bulked sample of at least a litre of milk was collected from each cow over a period of 2–6 d (the period was consistent within an experiment), which was frozen as it was collected. The milk was thawed, and the fat separated before analysis. The cream layer was skimmed and transferred into a glass flask. The cold cream was churned into butter by vigorous shaking of the flask, then the butter was removed and transferred to a glass centrifuge tube. The butter was melted at 70 °C before being centrifuged at 1000 ***g*** for 10 min to separate the fat layer. The centrifuge was heated to approximately 65 °C to stop the fat from solidifying during spinning. In Experiments 1 and 2, analyses were conducted on pooled milk from treatment groups whereas from Experiments 3, 4 and 5, the milks from individual cows were analysed.

The fatty acid composition of the milk fat was determined in duplicate by capillary gas–liquid chromatography (GLC), based on the method of Bannon et al. (1985). Milk fat was melted at 60 °C for 30 min and mixed.

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Table 1. Some details of the experiments in which the fatty acid composition of milk fat was monitored

	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
Reference	Wales et al. (1999)	Walker et al. (2001)	Stockdale (2000)	Stockdale (2000)	Dalley et al. (2001)
Duration (d)	38	50	35	35	25
Time of year	Oct–Nov	Feb–Apr	Sep–Oct	Nov–Dec	Sep–Oct
Number of cows	24	54	32	48	24
Feeding treatments	1. 20†	1. 0‡	1. 25+0§	1. 25+0‖	1. 0¶
(see footnotes for descriptors)	2. 70	2. 3	2. 25+5	2. 25+5	2. 300 g $NaHCO_3$
		3. 5	3. 50+0	3. 50+0	3. 6
		4. 7	4. 50+5	4. 50+5	4. 6+300 g $NaHCO_3$
		5. 9			
		6. 11			
Cows per treatment	6	9	4	6	6
Daily milk yield, kg/cow	28·5	22·3	25·4	30·1	26·9
Milk fat concentration, g/kg	35·5	38·9	38·5	39·0	38·3
Milk protein concentration, g/kg	29·3	32·9	30·0	29·5	34·6
Live weight, kg	525	549	476 & 551	486 & 618	521
Body condition, units	3·9	4·3	3·4 & 5·2	4·1	Not measured
Stage of lactation, d	36	167	21	46	29
Pasture on offer					
In vitro digestibility, g/kg DM	724 & 759	624	776	729	765
Crude protein, g/kg DM	155 & 148	121	166	117	263
Neutral detergent fibre, g/kg DM	532 & 488	648	499	577	507
Concentrates					
In vitro digestibility, g/kg DM	—	888	835	859	871
Crude protein, g/kg DM	—	132	109	113	107
Neutral detergent fibre, g/kg DM	—	179	213	197	157
Starch, g/kg DM	—	446	471	474	Not measured

† Two herbage daily allowances (20 and 70 kg DM/cow) at each of two contrasting herbage masses. There were no supplementary feeding treatments in this experiment

‡ Herbage daily allowance was 25 kg DM/cow in all treatments, with a range (0–11 kg DM/cow) of pelleted concentrates (750 g/kg barley, 250 g/kg wheat plus 16 g/kg sodium bicarbonate and 40 g/kg minerals and vitamins) offered

§ Herbage daily allowances were about 25 and 50 kg DM/cow, with either 0 or 5 kg DM of pelleted concentrates (750 g/kg barley, 250 g/kg wheat). The four treatments were applied to either thin or fat cows

‖ Herbage daily allowances were about 25 and 50 kg DM/cow, with either 0 or 5 kg DM of pelleted concentrates (750 g/kg barley, 250 g/kg wheat)

¶ Herbage daily allowance was 40 kg DM/cow in all treatments, with either 0 or 6 kg DM of pelleted concentrates (750 g/kg barley, 250 g/kg wheat) ± sodium bicarbonate (direct into the rumen)

An aliquot of fat in hexane (5% v/v) was methylated with a potassium hydroxide/methanol mixture, and 0·5 µl was injected into a Varian 3400 GLC (Varian Instrument Group, Palo Alto, CA 94304, USA) using a BPX70 fused silica column (25 m × 0·25 mm, SGE, Ringwood, VIC 3134, Australia) fitted with a flame ionization detector. A constant flow of He (1·0 ml/min) through the column was maintained by an electronic flow controller. The oven temperature was programmed to increase from 100 to 130 °C at 3 deg C/min, then to 170 °C at 5 deg C/min, where it was held for 20 min. Finally, the oven temperature was increased to 200 °C at 10 deg C/min where it was maintained for a further 20 min. The injector and detector temperatures were held at 220 and 240 °C, respectively. The peaks were integrated using Varian Star Integration software (Varian Associates Inc, Palo Alto, California CA 94304, USA), following identification after comparison with a reference mixture (Bureau of European Communities, CRM 164), and correction for response factors.

Statistical analyses

In all experiments except Experiment 5, results of each analyte were analysed by analysis of variance using Genstat 5. Since pre-experimental data were collected in Experiment 5, treatment results were corrected for between-cow

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Table 2. Effects of pasture allowance and pasture mass on the intake of grazing dairy cows and on the fatty acid composition (g/100 g) of milk fat in Experiment 1 (Wales et al. 1999)

Values are means with SED for $n=8$

	Treatment main effects				Statistics		
	Pasture allowance (PA)		Pasture mass (PM)			Significance	
	Low	High	Low	High	SED	PA	PM
Pasture daily intake, kg DM/cow	8·5[a]	17·9[b]	11·7[a]	14·7[b]	1·14	**	*
Daily milk yield, kg/cow	23·3[a]	28·4[b]	24·5	27·3	1·84	*	NS
Milk fat concentration, g/kg	35·3	36·6	36·3	35·6	1·88	NS	NS
$C_{4:0}$	4·6	4·5	4·5	4·6	0·21	NS	NS
$C_{6:0}$–$C_{10:1}$	6·2[a]	6·8[b]	6·3	6·8	0·17	*	NS
$C_{12:0}$	2·6[a]	3·0[b]	2·7[a]	2·9[b]	0·05	**	*
$C_{14:0}$	10·2[a]	11·1[b]	10·4[a]	10·8[b]	0·13	**	*
$C_{16:0}$	26·8	28·4	28·1	27·2	1·74	NS	NS
$C_{12:0}$–$C_{16:0}$	39·6	42·5	41·2	40·9	1·90	NS	NS
$C_{18:0}$	11·2[a]	12·2[b]	11·5[a]	11·9[b]	0·07	**	*
$C_{18:1}$ (*cis* and *trans*)	26·5	23·8	25·7	24·7	1·14	NS	NS
$C_{18:2}$	1·4	1·4	1·4	1·4	0·05	NS	NS
$C_{18:3}$	0·6	0·6	0·6	0·6	0·05	NS	NS
Conjugated linoleic acid (*cis* 9, *trans* 11)	1·4	1·4	1·4	1·4	0·07	NS	NS
Total saturated fatty acids	65·2	69·6	67·2	67·7	1·66	NS	NS

For main effects, values within a row without common superscripts are significantly different ($P<0·05$)
*$P<0·05$; **$P<0·01$; NS, not significant, $P>0·05$

differences by using analysis of covariance. Except for Experiment 2, all experiments were factorial designs, where main effects and all interactions were tested for significance. Experiment 1 was a 2*2 factorial with two replicates, Experiment 3 was a 2*2*2 factorial with two replicates, and Experiments 4 and 5 were both 2*2 factorials with four replicates. Since no interaction was statistically significant at $P<0·05$, only main effects are presented. Experiment 2 had six treatments, with three replicates, in a dose response design that allowed it to be analysed by analysis of variance or regression analysis. The number of df associated with the error term in each experiment was 3, 10, 7, 9 and 9 in Experiments 1–5, respectively. Results from all experiments for treatments where no concentrates were fed were included in a regression analysis to determine the relationship between CLA concentration and pasture intake.

Results

In Experiment 1, pasture intake increased as both pasture allowance and pre-grazing pasture mass increased (Table 2). While there were no effects of treatment on gross milk fat composition, increasing the intake of pasture increased some of the saturated fatty acids, with pasture allowance having a greater effect than pasture mass (Table 2). However, there was no effect of treatment on the concentration of total saturated fatty acids.

The fatty acid profiles of the milk fat in Experiment 2 are shown in Table 3. As the amount of concentrates consumed increased, the proportions of $C_{6–10}$, $C_{12:0}$, $C_{14:0}$ and $C_{18:2}$ all increased, while that of $C_{18:0}$ decreased. In contrast, the proportions of $C_{4:0}$, $C_{12:0–16:0}$, $C_{18:1}$ and CLA changed curvilinearly with concentrate intake (CI). The significant curvilinear regression for CLA is given in Fig. 1, and is described by the following equation:

$$\text{CLA(g/100 g milk fat)} = 1·56 - 0·18(\pm 0·035)\text{CI} + 0·015(\pm 0·003)\text{CI}^2$$
$$r^2 = 0·60 (P<0·01).$$

In all cases, the curvilinearity seemed to be associated mainly with the treatment in which the greatest amount of concentrates was eaten, and that treatment also induced a sharp decline in the total concentration of milk fat (Table 3).

In Experiment 3, fat cows produced milk with a higher fat concentration than did thin cows; moreover, the milk fat from the fatter cows had higher proportions of $C_{18:1}$ fatty acid and lower proportions of $C_{14:0}$ and $C_{12:0–16:0}$ (Table 4). Increasing the level of pasture feeding increased the proportion of $C_{12:0}$, $C_{14:0}$, $C_{18:3}$ and CLA (Table 4). Supplementing the diet with concentrates led to a decline in the proportions of $C_{4:0}$, $C_{18:0}$, $C_{18:1}$ and CLA whilst most of the other saturated short-, medium- and long-chain fatty acids increased, as did $C_{18:2}$ (Table 4). The milk from individual cows was analysed in this experiment and the

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Table 3. Effects of concentrate supplementation on the fatty acid composition (g/100 g) of milk fat produced by grazing dairy cows in Experiment 2 (Walker et al. 2001)

Values are means with SED for $n=9$

	Concentrates offered daily kg DM/cow						Statistics	
	0	3	5	7	9	11	SED	Significance
Pasture daily intake, kg DM/cow	$12{\cdot}1^{d}$	$12{\cdot}0^{d}$	$11{\cdot}2^{c}$	$10{\cdot}6^{bc}$	$10{\cdot}4^{b}$	$9{\cdot}2^{a}$	0·33	**
Concentrate intake, kg DM/d	0	3·0	5·0	7·0	9·0	10·4	—	—
Daily milk yield, kg/cow	$12{\cdot}4^{a}$	$15{\cdot}6^{b}$	$18{\cdot}3^{c}$	$19{\cdot}9^{cd}$	$20{\cdot}7^{d}$	$21{\cdot}9^{d}$	1·06	**
Milk fat concentration, g/kg	$44{\cdot}8^{b}$	$42{\cdot}1^{b}$	$40{\cdot}9^{b}$	$40{\cdot}5^{b}$	$41{\cdot}8^{b}$	$32{\cdot}5^{a}$	1·78	**
$C_{4:0}$	$4{\cdot}4^{b}$	$4{\cdot}5^{b}$	$5{\cdot}0^{b}$	$4{\cdot}8^{b}$	$4{\cdot}5^{b}$	$3{\cdot}7^{a}$	0·28	*
$C_{6:0}$–$C_{10:1}$	$5{\cdot}7^{a}$	$6{\cdot}4^{a}$	$7{\cdot}9^{b}$	$8{\cdot}4^{b}$	$8{\cdot}3^{b}$	$7{\cdot}2^{ab}$	0·55	**
$C_{12:0}$	$2{\cdot}4^{a}$	$2{\cdot}9^{a}$	$3{\cdot}6^{b}$	$4{\cdot}0^{b}$	$4{\cdot}0^{b}$	$4{\cdot}1^{b}$	0·26	**
$C_{14:0}$	$10{\cdot}1^{a}$	$10{\cdot}9^{ab}$	$11{\cdot}6^{bc}$	$12{\cdot}3^{c}$	$11{\cdot}7^{bc}$	$12{\cdot}0^{c}$	0·35	**
$C_{16:0}$	30·1	31·4	32·0	34·1	32·7	29·9	1·45	NS
$C_{12:0}$–$C_{16:0}$	$42{\cdot}6^{a}$	$45{\cdot}2^{ab}$	$47{\cdot}3^{bc}$	$50{\cdot}3^{c}$	$48{\cdot}4^{bc}$	$46{\cdot}0^{b}$	1·61	*
$C_{18:0}$	$11{\cdot}3^{d}$	$10{\cdot}5^{cd}$	$8{\cdot}9^{c}$	$7{\cdot}7^{bc}$	$6{\cdot}5^{ab}$	$5{\cdot}9^{a}$	0·77	**
$C_{18:1}$ (*cis* and *trans*)	$24{\cdot}2^{c}$	$22{\cdot}5^{bc}$	$20{\cdot}3^{ab}$	$18{\cdot}2^{a}$	$18{\cdot}7^{a}$	$21{\cdot}7^{b}$	1·28	**
$C_{18:2}$	$1{\cdot}3^{a}$	$1{\cdot}3^{a}$	$1{\cdot}5^{b}$	$1{\cdot}7^{c}$	$2{\cdot}0^{d}$	$2{\cdot}7^{e}$	0·06	**
$C_{18:3}$	0·5	0·5	0·4	0·4	0·5	0·4	0·03	NS
Conjugated linoleic acid (*cis* 9, *trans* 11)	$1{\cdot}5^{c}$	$1{\cdot}3^{bc}$	$1{\cdot}1^{ab}$	$1{\cdot}0^{a}$	$1{\cdot}1^{ab}$	$1{\cdot}4^{c}$	0·11	**
Total saturated fatty acids	$67{\cdot}7^{ab}$	$69{\cdot}7^{bc}$	$72{\cdot}0^{cd}$	$73{\cdot}9^{d}$	$70{\cdot}6^{bc}$	$66{\cdot}3^{a}$	1·38	**

Values within a row without common superscripts are significantly different ($P<0{\cdot}05$)
*$P<0{\cdot}05$; **$P<0{\cdot}01$; NS, not significant, $P>0{\cdot}05$

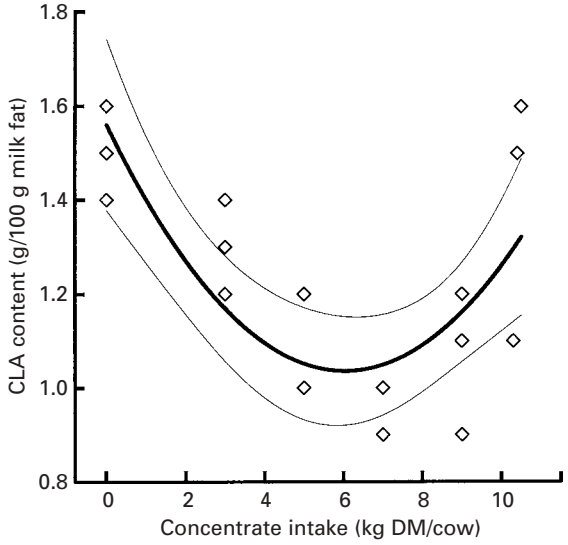

Fig. 1. The effect of daily concentrate intake on the concentration of conjugated linoleic acid (CLA) in milk fat. The heavy line is the curve of best fit and the lighter curves indicate the 95% confidence interval about the regression.

SD about the mean CLA concentrations averaged ±0·33 g/100 g in the milk fat of unsupplemented cows and ±0·18 g/100 g when concentrates were fed. These values equated to 21 and 15% of the means, respectively. The only difference between Experiments 3 and 4 in the effects of supplementation on fatty acid proportions was that there was no effect in Experiment 4 on $C_{18:0}$ whilst $C_{18:3}$ declined when concentrates were eaten, and pasture allowance had no affect on any fatty acid in Experiment 4 (Table 5). The milk fat from individual cows in Experiment 4 showed that the SD about mean CLA concentrations averaged ±0·17 g/100 g in the milk fat of cows eating the unsupplemented diet and ±0·07 g/100 g for cows consuming concentrates, these values equating to 15 and 8% of the means, respectively.

Despite a substantial reduction in milk fat concentration when concentrates were given in Experiment 5, there were few significant effects on the proportions of individual fatty acids (Table 6). Proportions of $C_{12:0}$ and $C_{18:2}$ increased, while those of $C_{4:0}$ and $C_{18:0}$ decreased. However, while there were few significant responses attributed to feeding concentrates in this experiment, there were also no contradictions in response between experiments. The milk from individual cows was also analysed in this experiment and the SD about mean CLA concentrations averaged ±0·77 g/100 g in the milk fat of cows eating the unsupplemented diet and ±0·79 g/100 g in cows consuming concentrates. These values equated to 47 and 34% of the means, respectively. The addition of a buffer to the diet had little effect on fatty acid composition (Table 6).

When the CLA results for all the unsupplemented diets from the five experiments were combined, there was a significant, positive, linear relationship between the CLA concentration in milk fat and daily pasture intake

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Table 4. Effects of pasture allowance, concentrate supplementation and body condition score of grazing dairy cows on the fatty acid composition (g/100 g) of milk fat produced in Experiment 3 (Stockdale, 2000)

Values are means with SED for $n=16$

	Treatment main effects						Statistics			
	Body condition (BC)		Pasture allowance (PA)		Supplements (Suppl.)			Significance		
	Thin	Fat	Low	High	No	Yes	SED	BC	PA	Suppl.
Pasture daily intake, kg DM/cow	16·7	16·7	14·1^{a}	19·3^{b}	17·9^{b}	15·5^{a}	0·61	NS	**	**
Concentrate intake, kg DM/d	2·4	2·3	2·4	2·3	0	4·7	—	—	—	—
Daily milk yield, kg/cow	29·4	30·5	28·7^{a}	31·2^{b}	29·0^{a}	30·9^{b}	0·72	NS	*	*
Milk fat concentration, g/kg	35·7^{a}	39·0^{b}	38·1	36·7	37·9	36·9	1·37	*	NS	NS
$C_{4:0}$	4·2	4·3	4·2	4·2	4·5^{b}	3·9^{a}	0·13	NS	NS	**
$C_{6:0}$–$C_{10:1}$	8·1	7·9	7·8	8·2	7·7^{a}	8·3^{b}	0·20	NS	NS	*
$C_{12:0}$	4·2	4·0	4·0^{a}	4·3^{b}	3·6^{a}	4·6^{b}	0·12	NS	*	**
$C_{14:0}$	12·2^{b}	11·4^{a}	11·6^{a}	12·1^{b}	11·3^{a}	12·4^{b}	0·13	**	**	**
$C_{16:0}$	29·9	28·2	29·4	28·6	27·5^{a}	30·6^{b}	0·72	NS	NS	**
$C_{12:0}$–$C_{16:0}$	46·4^{b}	43·6^{a}	45·0	45·0	42·4^{a}	47·5^{b}	0·80	*	NS	**
$C_{18:0}$	9·0	10·0	9·4	9·5	10·5^{b}	8·4^{a}	0·44	NS	NS	**
$C_{18:1}$ (*cis* and *trans*)	20·3^{a}	22·6^{b}	21·7	21·1	23·3^{b}	19·5^{a}	0·80	*	NS	**
$C_{18:2}$	1·4	1·5	1·5	1·4	1·3^{a}	1·6^{b}	0·10	NS	NS	*
$C_{18:3}$	0·6	0·6	0·5^{a}	0·6^{b}	0·6	0·6	0·02	NS	*	NS
Conjugated linoleic acid (*cis* 9, *trans* 11)	1·4	1·3	1·2^{a}	1·5^{b}	1·5^{b}	1·2^{a}	0·09	NS	*	**
Total saturated fatty acids	70·8	68·9	69·7	70·0	68·3^{a}	71·5^{b}	0·85	NS	NS	**

For main effects, values within a row without common superscripts are significantly different ($P<0·05$)
*$P<0·05$; **$P<0·01$; NS, not significant, $P>0·05$

Table 5. Effects of pasture allowance and concentrate supplementation of grazing dairy cows on the fatty acid composition (g/100 g) of milk fat produced in Experiment 4 (Stockdale, 2000)

Values are means with SED for $n=24$

	Treatment main effects				Statistics		
	Pasture allowance (PA)		Supplements (Suppl.)			Significance	
	Low	High	No	Yes	SED	PA	Suppl.
Pasture daily intake, kg DM/cow	11·3^{a}	13·4^{b}	13·6^{b}	11·1^{a}	0·29	**	**
Concentrate intake, kg DM/d	2·0	2·1	0	4·1	—	—	—
Daily milk yield, kg/cow	24·3^{a}	26·7^{b}	25·0^{a}	26·1^{b}	0·47	**	*
Milk fat concentration, g/kg	36·1	36·8	36·2	36·6	0·77	NS	NS
$C_{4:0}$	4·5	4·4	4·6^{b}	4·3^{a}	0·07	NS	**
$C_{6:0}$–$C_{10:1}$	7·0	7·2	6·6^{a}	7·6^{b}	0·17	NS	**
$C_{12:0}$	3·2	3·3	2·8^{a}	3·7^{b}	0·14	NS	**
$C_{14:0}$	11·1	11·5	10·7^{a}	11·9^{b}	0·33	NS	**
$C_{16:0}$	29·6	31·5	28·8^{a}	32·2^{b}	1·36	NS	*
$C_{12:0}$–$C_{16:0}$	43·9	46·3	42·4^{a}	47·8^{b}	1·69	NS	*
$C_{18:0}$	10·3	10·0	11·0	9·4	0·70	NS	NS
$C_{18:1}$ (*cis* and *trans*)	23·3	21·0	24·0^{b}	20·3^{a}	1·07	NS	*
$C_{18:2}$	1·3	1·3	1·2^{a}	1·4^{b}	0·04	NS	**
$C_{18:3}$	0·6	0·6	0·6^{b}	0·5^{a}	0·02	NS	**
Conjugated linoleic acid (*cis* 9, *trans* 11)	1·0	1·1	1·2^{b}	0·9^{a}	0·06	NS	**
Total saturated fatty acids	69·3	71·3	68·2^{a}	72·4^{b}	1·19	NS	**

For main effects, values within a row without common superscripts letters are significantly different ($P<0·05$)
*$P<0·05$; **$P<0·01$; NS, not significant, $P>0·05$

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Table 6. Effects of concentrate supplementation, and the inclusion of a buffer in the diet, of grazing dairy cows on the fatty acid composition (g/100 g) of milk fat produced in Experiment 5 (Dalley et al. 2001)

Values are means with SED for $n=12$

	Treatment main effects				Statistics		
	Grain		Buffer			Significance	
	No	Yes	No	Yes	SED	Grain	Buffer
Pasture daily intake, kg DM/cow	16·6[b]	12·7[a]	14·7	14·6	0·91	**	NS
Concentrate intake, kg DM/d	0[a]	5·4[b]	3·1	2·3	0·46	**	NS
Daily milk yield, kg/cow	25·6	28·1	26·6	27·2	1·31	NS	NS
Milk fat concentration, g/kg	41·8[b]	34·3[a]	38·4	37·7	2·48	*	NS
$C_{4:0}$	4·5[b]	3·5[a]	3·8	4·1	0·23	**	NS
$C_{6:0}$–$C_{10:1}$	6·5	7·0	7·0	6·5	0·48	NS	NS
$C_{12:0}$	2·8[a]	3·7[b]	3·5[b]	3·0[a]	0·22	**	*
$C_{14:0}$	9·2	10·0	9·9	9·2	0·49	NS	NS
$C_{16:0}$	24·5	23·6	24·4	23·6	1·34	NS	NS
$C_{12:0}$–$C_{16:0}$	36·5	37·3	37·9	35·9	1·82	NS	NS
$C_{18:0}$	12·1[b]	7·8[a]	9·2	10·7	1·00	**	NS
$C_{18:1}$ (*cis* and *trans*)	28·3	26·6	26·2	28·7	1·37	NS	NS
$C_{18:2}$	1·3[a]	3·0[b]	2·6	1·8	0·58	*	NS
$C_{18:3}$	0·9	0·9	0·9	0·9	0·08	NS	NS
Conjugated linoleic acid (*cis* 9, *trans* 11)	1·6	2·3	1·9	2·0	0·40	NS	NS
Total saturated fatty acids	62·6	58·5	61·2	59·9	2·88	NS	NS

For main effects, values within a row without common superscripts are significantly different ($P<0·05$)
*$P<0·05$; **$P<0·01$; NS, not significant, $P>0·05$

(PI) (Fig. 2), as described by the following regression equation:

$$\text{CLA(g/100 g milk fat)} = 0·88 + 0·17(\pm 0·060)\text{PI};$$
$$r^2 = 0·35 (P<0·05);\ n=17.$$

Discussion

Our results support the hypothesis that increasing the consumption of pasture increases the concentration of CLA in milk fat. When the results for the pasture alone treatments from all the experiments reported here were combined, there was a positive relationship between level of pasture intake and CLA concentration. The CLA concentration in the milk fat of pasture-fed cows can be two (1·09 *v.* 0·46 g/100 g milk fat; Kelly et al. 1998b) to five (2·21 *v.* 0·39 g/100 g milk fat; Dhiman et al. 1999a) times higher than that in cows given total mixed rations. Thus, not only is pasture in itself important for obtaining high CLA concentrations, but increasing the consumption of pasture in pasture-only diets can lift CLA levels further. The *cis*-9-*trans*-11-CLA isomer is the most common form in milk and is an intermediate of biohydrogenation of linoleic acid by the rumen bacterium, *Butyrivibrio fibrisolvens* (Kepler & Tove, 1967). Changes in substrate supply and extent of biohydrogenation affect the supply of intermediates and end-products of biohydrogenation, thus affecting the CLA concentration in ruminant products (Kelly et al. 1998a; Dhiman et al. 1999b). Since bacteria change the *cis*-11 of linoleic acid ($C_{18:2}$) to the *trans*-11 of CLA in the rumen, concentrations depend not only on levels of linoleic acid in the feed, but probably also on the retention time of feed in the rumen. As intake increases, the mean retention time

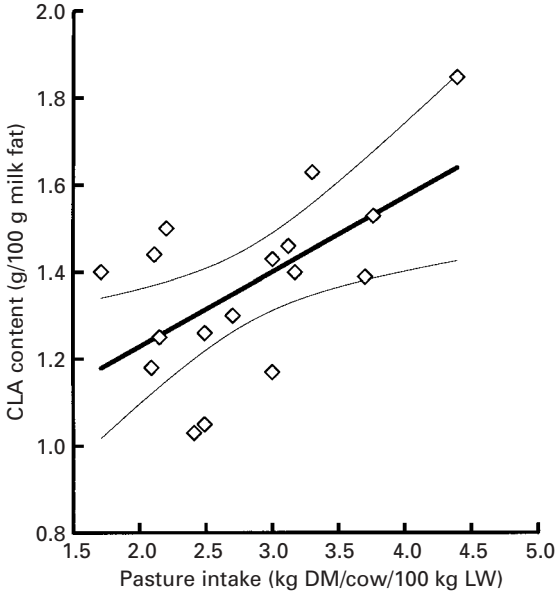

Fig. 2. The effect of daily pasture intake, where no concentrates were fed, on the concentration of conjugated linoleic acid (CLA) in milk fat. Results from all experiments have been pooled. The heavy line is the curve of best fit and the lighter curves indicate the 95% confidence interval about the regression.

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of feed in the rumen decreases (AFRC, 1993). Feed moves out of the rumen more rapidly as pasture intake increases and may reduce the extent of biohydrogenation, thereby resulting in higher concentrations of CLA.

Although the relationship between pasture intake and CLA concentration was statistically significant, it was not particularly strong. However, it is perhaps not surprising that a clearer relationship was not obtained because there are reports of large variations in CLA concentration between individual cows on the same diet (e.g. Kelly et al. 1998b). Certainly in Experiments 3, 4 and 5, there was generally more variation in CLA concentration in the milk fat of cows consuming pasture alone than there was when concentrates were included in the diet. Although some, if not most, of the CLA appearing in milk is the result of direct transfer of CLA absorbed from the gut, there is now evidence that the cow herself can synthesize CLA from *trans*-11 linoleic acid through delta-9 desaturase (Corl et al. 1998). It is possible that some of the variation in CLA content of milk fat seen between individual animals eating a similar diet may be due to differences in the cows ability to produce CLA from *trans*-11 linoleic acid.

It is also possible that some variation in the relationship is associated with variation in the pastures used in these studies, which varied in metabolizable energy from 8·6 to 11·2 MJ/kg DM. While most of the experiments reported here were conducted in spring with ryegrass/white cover pastures, the cows in Experiment 2 grazed pastures in early autumn in which paspalum (*Paspalum dilatatum*) predominated (Walker et al. 2001). Dhiman et al. (1999a) suggested that type of forage could affect the resulting CLA concentration. When they compared lucerne hay with grass hay, however, they found that there were no significant differences between treatments in CLA content (mean of 0·81 g CLA/100 g milk fat). The effect of composition of fresh pasture on CLA concentrations needs further investigation.

When cows consumed either one-third, two-thirds, or their entire diet as perennial pasture, with lucerne (*Medicago sativa*) hay and concentrates making up the balance in the first two treatments, the CLA concentration increased in a linear fashion from 0·89 to 1·43 and 2·21 g/100 g of milk fatty acids, respectively (Dhiman et al. 1999a). In much of the research reported here, cereal grain concentrates reduced the CLA content of milk fat. In Experiment 2, however, in which the daily consumption of concentrates ranged from 3·0–10·4 kg DM/cow, the response in CLA was curvilinear, as described in Fig. 1. We suggest that the reduction in the CLA concentration in milk fat in response to increased intake of concentrates was primarily due to reductions in the contribution of herbage to the diets. This may have increased the synthesis *de novo* of long chain fatty acids in the mammary gland relative to the uptake of CLA from plasma. However, at higher concentrate intakes, where the gross milk fat concentration declined sharply, from about 4·2 to 3·3 g/100 g milk, CLA concentrations recovered to almost the same level as that recorded for cows consuming pasture alone. This result is consistent with the lack of response seen in Experiment 5, where there was also a considerable decline in milk fat concentration when concentrates were given.

The increase in CLA concentration from feeding concentrates may have resulted from greater outflow rates from the rumen, thereby interrupting biohydrogenation, as mentioned above. In addition, diets that result in a low rumen fluid pH, particularly in response to high intakes of starch, can reduce the numbers of bacteria engaged in biohydrogenation (Gerson et al. 1985). However, another explanation concerns the milk fat depression. There is more than one hypothesis that has been put forward to explain the milk fat depression caused by high intakes of dietary starch. The glucose-insulin theory attributes milk fat depression to a reduced supply of lipid precursors to the mammary gland as a result of altered rumen fermentation patterns (reduced acetate+butyrate to propionate ratio) and of insulin-induced repartitioning of lipid precursors from udder to body. This would reduce lipogenesis *de novo* from acetate and butyrate. Alternative theories for milk fat depression assume a direct inhibition of milk fat synthesis within the mammary gland by compound(s) derived directly from the diet or following ruminal metabolism of dietary components. A number of recent studies have implicated *trans* fatty acids arising from incomplete rumen biohydrogenation of poly-unsaturated fatty acids, leading to an increased supply of these fatty acids to the mammary gland, as a major cause of milk fat depression (e.g. Gaynor et al. 1995; Griinari et al. 1998; Bauman & Griinari, 2001; Offer et al. 2001). Whilst *trans* fatty acids in milk were inversely correlated with milk fat concentration ($r=-0{\cdot}63$), Offer et al. (1999) found that concentrations of CLA and total *trans* acids in milk were highly positively correlated ($r=0{\cdot}91$). Therefore, the results of Experiments 2 and 5, in which milk fat was significantly depressed when considerable quantities of concentrates were given, may not be particularly surprising.

The medium-chain saturated fatty acids, $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$, are nutritionally undesirable because in man they increase the cholesterol content of the low-density lipoproteins, which are thought to be associated with an increased risk of coronary heart disease. The amounts of these medium-chain saturated fatty acids were 41·1, 46·6, 45·0, 45·1 and 36·9 g/100 g milk fat in Experiments 1, 2, 3, 4 and 5, respectively. Experiment 5 was conducted earliest in spring while Experiment 2 was carried out in early autumn. In general, feeding concentrates resulted in an increase in the medium-chain saturates, probably because one of the dietary factors that affects the supply of acetic acid from the rumen for fatty acid synthesis *de novo* is the forage to concentrate ratio (Murphy, 2000). In general, C_{18} fatty acids appearing in milk fat are absorbed from the blood stream, and originate from the diet or from triglycerides mobilized from adipose tissue (Moore & Christie, 1981; Murphy, 2000). The C_{16} fatty acids arise almost equally from the diet and from synthesis *de novo* in

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the mammary gland while the C_4–C_{14} fatty acids are derived almost entirely from synthesis *de novo* from acetic acid and β-hydroxybutyrate within the mammary gland (Moore & Christie, 1981; Murphy, 2000).

Of the short chain fatty acids, $C_{6–10}$ do not appear to affect human health, whereas it has been claimed that $C_{4:0}$ (butyric acid) plays a role in preventing colon cancer (Perrin et al. 1994; Parodi, 1999, 2001). In the present experiments, pasture intake had no effect on the butyric acid concentration of milk fat. However, cereal grain concentrate supplements to pasture consistently produced milk fat with lower concentrations of butyric acid although, in summer/autumn, this only occurred at very high intakes of concentrates. Mackle et al. (1997) also reported that milk concentrations of butyric acid decreased when pasture was supplemented with maize grain. While effects of season and stage of lactation are compounded in these studies, together they appeared to have no effect on the concentrations of butyric acid in the milk fat of pasture-fed cows.

The dietary manipulations we used were those that are most common in southern Australian dairy systems. The results suggest that feeding cows plenty of good quality pasture with minimal quantities of cereal grain-based concentrates will result in milk with the healthiest fatty acid profiles. The inclusion of other supplements in the diet, such as full-fat soyabeans or other oilseeds, may have to be considered if the aim is to manipulate fat composition to a greater degree. In addition, since we used only spring-calving cows, the effect of altering the time of calving should be considered in future research.

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