Journal of Dairy Research (2003) **70** 257–265. © *Proprietors of Journal of Dairy Research 2003*
DOI: 10.1017/S0022029903006241 *Printed in the United Kingdom*

Effect of cereal grain and fibre supplements on the fatty acid composition of milk fat of grazing dairy cows in early lactation

Chakra Wijesundera[1]*, Zhiping Shen[1], William J Wales[2] and Dawn E Dalley[3]

[1] Food Science Australia, Sneydes Road, Werribee, Vic. 3030, Australia
[2] Department of Natural Resources and Environment, Kyabram Dairy Centre, 120 Cooma Road, Kyabram, Vic. 3620, Australia
[3] Department of Natural Resources and Environment, Agriculture Victoria, Ellinbank, RMB 2460, Warragul, Vic. 3821, Australia

Received 7 September 2001 and accepted for publication 5 September 2002

Two experiments were undertaken to determine the effects of cereal grain and fibre (hay or straw) supplements on the fatty acid composition of milk fat of grazing dairy cows in early lactation. In both experiments, grain supplements significantly increased ($P<0{\cdot}05$) the proportion of the endogenously synthesized 10 : 0–16 : 0 fatty acids. Of the C18 acids, the proportion of 18 : 0 and 18 : 3 was significantly decreased ($P<0{\cdot}05$) by grain supplementation, while that of 18 : 2 was significantly increased ($P<0{\cdot}05$). Irrespective of diet, 18 : 1 *trans*-11 was the most dominant *trans* 18 : 1 isomer in milk fat. In the first experiment, the proportions of the 18 : 1 *trans*-11 isomer and conjugated linoleic acid (CLA, 18 : 2 *cis*-9, *trans*-11) were highest for the pasture-only diets, and significantly ($P<0{\cdot}05$) decreased with grain supplementation. The opposite result was observed in the second experiment, conducted in a different dairy region, suggesting that factors such as the quality of pasture on offer and the physiological state of the cow could affect the content of CLA and *trans* fatty acids in milk fat. In both experiments, there was a significant positive linear relationship between CLA and 18 : 1 *trans*-11. Fibre supplements had little effect on the fatty acid composition of the milk.

Keywords: CLA, *trans* 18 : 1 isomers.

Fat in milk, unlike carbohydrate and protein, varies widely in both amount and composition owing to nutritional factors which are superimposed upon variations due to species, genetics and stage of lactation (Hawke & Taylor, 1995). Nutritional effects specific to fat arise principally because a considerable, but variable, proportion of the fatty acids of milk is derived directly from dietary lipids. Changes in the fatty acid composition of milk can have significant effects on both physical and nutritional functionality of dairy foods.

Over two-thirds of the Australian dairy industry is based in Victoria, where pasture is a low-cost source of feed and seasonal calving is widely practised to maximize pasture use. In the last 15 years, the feeding of concentrates on Victorian dairy farms has risen from less than 100 kg/cow per year to around 1 tonne/cow per year (Doyle et al. 2000). About 80% of farmers now feed grain-based supplements in the dairy at milking time (Grainger et al. 1996). It is estimated that supplements constitute about 25% of the total feed eaten, but this varies from 0 to 74% on irrigated farms in northern Victoria (Armstrong et al. 1998). Supplementary feeds are given to cattle at pasture to raise animal performance above that attainable from pasture alone; to maintain production during periods of pasture shortage; and to overcome limitations in pasture quality at key times of the year (Stockdale, 1999).

Whilst seasonal variations have been reported for the major component acids of Australian milk fat (Parodi, 1970; Thomas & Rowney, 1996), there is little local information on the effects of feed supplements on the composition of milk fat. Recently, two separate feeding experiments were conducted in Victoria to determine the effects of grain supplementation and the provision of fibre (hay or straw) on rumen fermentation and marginal responses of cows grazing perennial ryegrass pastures in early lactation (Wales et al. 2001; D. E. Dalley, unpublished observations). The milk from those same experiments was used to determine the effects of grain and fibre supplements on the component fatty acids of milk fat and the results are reported here.

*For correspondence; e-mail: Chakra.Wijesundera@foodscience.afisc.csiro.au

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press

Material and Methods

Experimental design

The first feeding experiment was conducted at Kyabram Dairy Centre, Kyabram, Victoria, Australia (36° 20′ S, 145° 04′ E) in spring 1999 (hereafter referred to as the Kyabram experiment). Sixty-three spring-calving Friesian cows were allocated to seven treatment groups. Experimental details are given in Table 1. All cows grazed as a group on irrigated perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) mixed pasture and received a supplement mix consisting of 5 kg dry matter (DM)/cow per day of barley grain plus l kg of a fibre pellet 16 d before the start of the experiment. Thereafter, cows in each treatment group were offered one of the following diets each day: low-pasture (LP), a daily pasture allowance of 20 kg DM/cow; high-pasture (HP), a daily pasture allowance of 40 kg DM/cow; low pasture+fibre pellet, i.e., LP+2·5 kg DM/cow of hay-based fibre pellet; low pasture+fibre cube, i.e., LP+2·5 kg DM/cow of hay-based fibre cube; low pasture+grain, i.e., LP+5·0 kg DM/cow of barley-based pellet; low pasture+grain+fibre pellet, i.e., LP+5·0 kg DM/cow of barley-based pellet+2·5 kg DM/cow of hay-based fibre pellet; and low pasture+grain+fibre cube, i.e., LP+5·0 kg DM/cow of barley-based pellet+2·5 kg DM/cow of hay-based fibre cube. The pasture was perennial ryegrass/white clover (Table 1) and the grain pellet contained, on a DM basis, 75% barley and 25% wheat. Hay was used as the fibre supplement.

Perennial pasture hay was from a single source and was either finely ground and pressed through a 10-mm diameter pelleting die (fibre pellet) or coarsely chopped then pressed through a 30 cm × 30 cm cubing die (fibre cube). Barley grain was from a single source and was rolled and pelleted through the same pelleting die (grain pellet). The same grain and hay were incorporated into pellets (grain+fibre pellet) or cubes (grain+fibre cube). Molasses was used as a binding agent in all supplements. Those cows receiving supplements were given them individually in two equal amounts after the morning and afternoon milkings.

The seven treatments were replicated three times with three cows per replicate. Cows were allocated to replicates by stratified randomization based on initial pre-treatment live weight, milk yield, milk fat and milk protein concentrations and condition score. One replicate of all treatments grazed within the same paddock; groups were separated by electric fences. The feeding experiment lasted for 40 d. Milk was sampled (0·5% v/v) for fatty acid analysis 16, 23 and 30 d after the start of the experimental feeding. A single milk sample per replicate (21 in total) was analysed after pooling of milk from each cow within a replicate over 3 d.

The second feeding experiment was conducted at Agriculture Victoria Ellinbank, Warragul, Victoria, Australia (38° 25′ S, 145° 56′ E) in spring 2000 (hereafter referred to as the Ellinbank experiment). Sixty spring-calving Friesian cows were allocated to five treatment groups. Experimental details are given in Table 1. All cows grazed as a group on perennial ryegrass pasture and were offered daily 5 kg DM/cow of barley and 1·0 kg DM/cow of barley straw for 10 d before the start of the experiment. Each treatment was replicated three times, and there were four cows per replicate. Six days were allowed for the cows to adjust to the experimental regime and diet before measurements began. Cows were offered the following diets daily: low pasture (LPE), a daily pasture allowance of 30 kg DM/cow; low pasture+grain, i.e., LPE+5·0 kg DM/cow of cereal grain; low pasture+straw, i.e., LPE+1·8 kg DM/cow of straw; low pasture+grain+straw, i.e., LPE+5 kg DM/cow of cereal grain+1·8 kg DM/cow of straw; and low pasture+grain+straw+lipid, i.e., LPE+5kg DM/cow of cereal grain+1·8 kg DM/cow of straw+250 g digestible lipid/cow. The pasture was predominantly perennial ryegrass (Table 1), and the grain pellets contained, on a DM basis, 75% barley and 25% wheat. The fatty acid supplement was designed to be rumen-protected and contained predominantly the long-chain saturated fatty acids, 16:0 (32%), 18:0 (46%) and 18:1 (13%). Those cows receiving supplements were given them individually twice a day, the grain in the shed during milking and the straw immediately after milking.

Table 1. Details of the experimental cows and the quality of herbage consumed when grazing pasture and supplemented with grain and/or fibre in the Kyabram (Wales et al. 2001) and Ellinbank experiments

Values are means ± SD for *n* values as stated

	Kyabram (*n*=63)	Ellinbank (*n*=60)
Duration of experiment (d)	40	39
Time of year	Sept–Nov	Aug–Oct
Live weight of cows (kg)	520±41·1	535±45·5
Body condition score	3·6±0·03	4·4±0·04
Stage of lactation (d)	49±14·1	31±12·2
Pasture on offer	(*n*=21)	(*n*=15)
Mass (t DM/ha)	4·1	2·4±0·40
Plate meter height (cm)	11·9	8·8±1·70
Ryegrass (g/kg DM)	507	760
White clover (g/kg DM)	188	10
Weeds (g/kg DM)	194	170
Dead (g/kg DM)	111	60

Milk for fatty acid analyses was sampled 24 and 29 d after the start of experimental feeding. Subsamples of milk (0·5% v/v) from each of the 12 cows receiving the same feed were pooled.

Average milk yield and composition, DM intake, pasture substitution rate and the estimated nutritive value of the feeds consumed by the cows in both experiments are shown in Table 2.

Extraction of milk fat

Milk samples were frozen immediately after milking and stored at −18 °C. When required, the milks were thawed

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press

Table 2. Milk yield and composition, dry matter intake and the nutritive characteristics of the feeds consumed by cows grazing pasture (LP, low pasture; HP, high pasture) and supplemented with grain and/or fibre in the Kyabram (Wales et al. 2001) and Ellinbank experiments

Values are means ± SED for $n=9$ (Kyabram) and $n=12$ (Ellinbank)

	Kyabram								Ellinbank					
	LP	HP	LP+fibre pellet	LP+fibre cube	LP+grain	LP+grain+ fibre pellet	LP+grain+ fibre cube	SED	LP	LP+grain	LP+straw	LP+grain+ straw	LP+grain+ straw+lipid	SED
Daily milk yield (kg/cow)	$20{\cdot}7^{c}$	$24{\cdot}2^{a,b}$	$22{\cdot}1^{b,c}$	$20{\cdot}1^{c}$	$26{\cdot}2^{a}$	$25{\cdot}6^{a}$	$25{\cdot}7^{a}$	1·46	$29{\cdot}4^{b}$	$32{\cdot}4^{a}$	$27{\cdot}8^{b}$	$32{\cdot}1^{a}$	$32{\cdot}0^{a}$	1·08
Milk fat (g/kg)	38·0	36·8	37·5	37·5	35·7	37·3	34·3	2·39	$42{\cdot}0^{a}$	$38{\cdot}0^{b}$	$42{\cdot}4^{a}$	$39{\cdot}1^{b}$	$39{\cdot}5^{b}$	0·81
Milk protein (g/kg)	28·0	30·0	28·1	28·4	30·3	30·8	28·8	1·01	$31{\cdot}4^{c}$	$32{\cdot}6^{a,b}$	$31{\cdot}9^{b,c}$	$32{\cdot}7^{a}$	$32{\cdot}2^{a,b}$	0·32
Daily pasture intake (kg DM/cow)	$11{\cdot}2^{b}$	$15{\cdot}6^{a}$	$10{\cdot}9^{b,c}$	$10{\cdot}6^{b,c,d}$	$10{\cdot}3^{c,d}$	$10{\cdot}1^{c,d}$	$10{\cdot}0^{d}$	0·40	$13{\cdot}3^{a}$	$12{\cdot}2^{c}$	$12{\cdot}9^{b}$	$11{\cdot}8^{d}$	$11{\cdot}8^{d}$	0·14
Daily grain intake (kg DM/cow)	—	—	—	—	4·5	4·8	4·9	—	0	5·0	0	4·9	4·8	0·11
Daily intakes of Hay (Expt 1) or Straw (Expt 2) (kg DM/cow)	—	—	2·4	2·1	—	2·4	2·5	—	0	0	0·4	0·6	0·8	0·09
Forage : concentrate ratio	1·0	1·0	1·0	1·0	0·70	0·72	0·72	—	1·0	0·71	1·0	0·71	0·72	0·008
Digestibility of diet (g/kg DM)	837^{a}	837^{a}	$787^{b,c}$	780^{c}	840^{a}	815^{b}	$793^{b,c}$	10·9	854^{a}	$845^{a,b}$	$840^{a,b}$	834^{b}	831^{b}	7·4
Neutral detergent fibre in the diet (g/kg DM)	460^{b}	$437^{b,c}$	493^{a}	507^{a}	370^{e}	413^{d}	$417^{c,d}$	8·9	422^{b}	353^{d}	446^{a}	379^{c}	385^{c}	9·8
Crude protein in the diet (g/kg DM)	212^{b}	227^{a}	193^{c}	188^{c}	188^{c}	171^{d}	172^{d}	4·4	250^{a}	219^{b}	259^{a}	226^{b}	220^{b}	6·8

Within rows, treatment means without a common superscript letter are significantly different ($P<0{\cdot}05$)

at 4 °C and homogenized (Foss Electric Milko Tester) at the rate of 1 l milk/12 min. The freshly homogenized milk (90 ml) was mixed with chloroform (50 ml) and methanol (100 ml), more chloroform (50 ml) and water (10 ml) were added, and the mixing continued for a further 10 min and 20 min respectively after each addition. The mixture was then centrifuged at 1400 ***g*** at 4 °C for 30 min. The chloroform layer was separated, dried over anhydrous sodium sulphate, and the solvent was distilled off in a vacuum rotary evaporator to recover the milk fat.

Fatty acid analysis

Milk fats were *trans*-esterified with 2 M-potassium hydroxide in methanol using the procedure of Bannon et al. (1985). The resulting fatty acid methyl esters (FAME) were analysed on a SP-2560 fused silica capillary column (100 m × 0·25 mm i.d., 0·2 μm film; Supelco, Bellafonte, PA, USA) using a Perkin Elmer Model Autosystem XL Gas Chromatograph (GC) equipped with a flame-ionization detector. A constant helium flow (1·0 ml/min) was maintained through the column by an electronic flow controller. The oven temperature was programmed from 100 °C 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 and maintained at 200 °C for a further 20 min. The injector and detector temperatures were held at 220 °C and 240 °C respectively. Each milk fat sample for GC analysis was esterified and analysed in duplicate. A hexane solution of FAME (0·5 μl) was injected using an autosampler at a split ratio of 100 : 1. Peak areas were integrated using Turbochrom Work Station software (version 6.1.1.0.0, Perkin Elmer Corporation). Peak areas for individual FAME were normalized by comparison with those for standard milk fat (CRM164, Bureau of European Communities).

The GC oven temperature program was selected such that the short-chain fatty acids could be detected while providing satisfactory resolution for *trans* 18 : 1 isomers. The major fatty acids were identified by comparison of their GC retention times with those of authentic standards. The *trans* fatty acid isomers were identified by comparison of the GC elution pattern with that reported by Ratnayake (1998) for the same SP-2560 column. A 100-m, SP-2560 column, which was used in the present study, is an efficient GC column for the separation of *cis-trans* 18 : 1 isomers (Ratnayake, 1998).

Gas Chromatography/Mass Spectrometry (GC/MS)

Identification of the unsaturated acids, including the *trans* fatty acid isomers, was further substantiated by re-analysis of the methyl esters by gas chromatography/mass spectrometry (GC/MS) on the same SP-2560 column as that used for GC, using a Hewlett Packard Model 6890/ 5973 MSD GC/MS system. The injector temperature, split ratio and the oven temperature program were the same as for GC above. The helium flow through the column was maintained at 1·0 ml/min by an electronic flow controller, and the MS was operated in the electron ionization mode at an energy level of 70 eV and an ion source temperature of 180 °C. The MS was scanned from mass 29 to 400 with a cycle time of 2 scans/s. The instrument was operated; data acquired and processed using MS-Chemstation software.

Data processing and statistical analysis

Results were subjected to analysis of variance using Genstat 5 statistical software. SED between treatment means was calculated using a probability of $P \leqslant 0{\cdot}05$ for a significant difference. In the case of the Ellinbank experiment, the results for replicate groups of cows were pooled before analysis. In order to estimate the error variance between such pooled samples, it was assumed that the real treatment-by-day interaction was negligible and that the observed treatment-by-day interaction was actually an estimate of the error variance.

In the statistical analysis of data in Table 2, a replicate or grazing group of three cows (Kyabram experiment) or four cows (Ellinbank experiment) was the experimental unit with the seven (Kyabram experiment) or five (Ellinbank experiment) treatments replicated three times. Treatments were compared by analysis of variance using Genstat 5, with differences between means established at $P<0{\cdot}05$.

Results

The fatty acid compositions of the milk fats from the Kyabram and Ellinbank experiments are shown in Tables 3 and 4 respectively. In both experiments there were small differences between treatments in the proportion of 4 : 0–8 : 0 acids. However, the supplementation of pasture with either grain or fibre had no effect ($P>0{\cdot}05$) on the total proportions of these acids. Grain, but not fibre, supplements significantly increased ($P<0{\cdot}05$) proportions of 10 : 0–16 : 0 acids compared with pasture-only treatments. Lipid supplementation in the Ellinbank experiment significantly increased ($P<0{\cdot}05$) the proportion of 16 : 0. The proportion of 18 : 0 significantly decreased ($P<0{\cdot}05$) with grain supplementation. The increase in the saturated acids 10 : 0–16 : 0 by grain supplementation was accompanied by a significant reduction ($P<0{\cdot}05$) in 18 : 1 9*c*. Fibre had no significant effect ($P>0{\cdot}05$) on 18 : 1 9*c*.

The main polyunsaturated fatty acids of the milk fats were 18 : 2 (9*c*, 12*c*), 18 : 3 (9*c*, 12*c*, 15*c*) and conjugated linoleic acid (CLA, 18 : 2 9*c*, 11*t*). In both experiments, 18 : 2 (9*c*, 12*c*) significantly increased ($P<0{\cdot}05$) while 18 : 3 (9*c*, 12*c*, 15*c*) significantly decreased ($P<0{\cdot}05$) with grain supplementation.

In the Kyabram experiment, grain supplementation significantly reduced ($P<0{\cdot}05$) both CLA and 18 : 1 *trans*-11, whereas the opposite effect was observed in the Ellinbank

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press

Table 3. Fatty acid composition (weight percentage) of the milk fat of grazing (LP, low pasture; HP, high pasture) dairy cows supplemented with barley and hay (Kyabram experiment). Fatty acids are denoted in short-hand; for example, 18 : 1 16*t* represents C18 fatty acid with a trans double bond at the Δ16 position

Values are means ± SED for $n=9$

Fatty acids	LP	HP	LP+fibre pellet	LP+fibre cube	LP+grain	LP+grain+ fibre pellet	LP+grain+ fibre cube	SED
Saturated acids								
4 : 0	4·03^{a,b}	4·03^{a,b}	3·93^{a,b}	4·26^{a}	3·68^{b,c}	3·41^{c}	3·92^{a,b}	0·194
6 : 0	2·42	2·43	2·35	2·38	2·49	2·47	2·43	0·092
8 : 0	1·29^{b}	1·30^{a,b}	1·22^{b}	1·24^{b}	1·46^{a}	1·46^{a}	1·34^{a,b}	0·075
Total 4 : 0–8 : 0	7·74	7·76	7·79	7·88	7·62	7·33	7·70	0·270
10 : 0	2·52^{b,c}	2·53^{b,c}	2·35^{c}	2·40^{c}	3·25^{a}	3·31^{a}	2·91^{a,b}	0·226
12 : 0	2·65^{c}	2·72^{b,c}	2·47^{c}	2·48^{c}	3·54^{a}	3·68^{a}	3·26^{a,b}	0·256
14 : 0	9·83^{b}	10·01^{b}	9·89^{b}	9·66^{b}	11·78^{a}	12·07^{a}	11·26^{a}	0·564
Total 10 : 0–14 : 0	15·00^{c}	15·27^{b,c}	14·72^{c}	14·54^{c}	18·57^{a}	19·06^{a}	17·43^{a,b}	1·029
16 : 0	27·51^{b}	26·78^{b}	28·50^{b}	27·01^{b}	31·14^{a}	31·47^{a}	32·56^{a}	0·966
18 : 0	12·24^{a}	12·43^{a}	12·99^{a}	12·32^{a}	9·99^{b}	9·38^{b}	9·47^{b}	0·621
cis-Unsaturated acids								
10 : 1 9*c*	0·26^{a,b}	0·27^{a}	0·21^{c}	0·23^{b,c}	0·29^{a}	0·27^{a}	0·29^{a}	0·016
14 : 1 9*c*	0·87^{b,c}	0·93^{a,b}	0·71^{d}	0·76^{c,d}	1·02^{a,b}	1·06^{a}	1·06^{a}	0·067
16 : 1 9*c*	1·36	1·28	1·33	1·40	1·32	1·41	1·49	0·057
18 : 1 9*c*[1]	19·91^{a}	19·97^{a}	19·81^{a,b}	20·91^{a}	16·40^{c}	15·75^{c}	16·65^{b,c}	1·446
18 : 2 9*c*,12*c*	0·75^{c}	0·65^{d}	0·79^{c}	0·78^{c}	0·96^{a,b}	0·98^{a}	0·89^{b}	0·033
18 : 3 9*c*,12*c*,15*c*	0·65^{a}	0·60^{a}	0·60^{a}	0·61^{a}	0·48^{b}	0·47^{b}	0·43^{b}	0·037
trans-Unsaturated acids								
16 : 1 9*t*	0·04	0·04	0·03	0·04	0·04	0·04	0·04	0·004
18 : 1 6*t*–8*t*	0·22	0·22	0·20	0·20	0·20	0·21	0·19	0·011
18 : 1 9*t*	0·15^{a}	0·14^{a,b}	0·13^{b,c}	0·14^{a,b}	0·12^{c}	0·13^{b,c}	0·12^{c}	0·007
18 : 1 11*t*[2]	3·54^{a}	3·74^{a}	3·00^{b,c}	3·44^{a,b}	2·55^{c,d}	2·71^{c,d}	2·46^{d}	0·211
18 : 1 12*t*–14*t*	0·22^{a,b,c}	0·24^{a,b}	0·22^{a,b,c}	0·20^{c}	0·24^{a,b}	0·25^{a}	0·21^{b,c}	0·012
18 : 1 16*t*	0·28	0·35	0·28	0·25	0·27	0·26	0·22	0·037
Total *trans* 18 : 1	4·41^{a}	4·71^{a}	3·83^{b,c}	4·23^{a,b}	3·38^{c,d}	3·56^{c,d}	3·19^{d}	0·220
18 : 2 9*c*, 11*t* (CLA)	1·44^{a,b}	1·64^{a}	1·14^{d}	1·41^{b,c}	1·10^{d}	1·22^{c,d}	1·17^{d}	0·097

[1] Contains small amounts of 18 : 1 6*c*–10*c* and 18 : 1 15*t* isomers
[2] Contains small amounts of 18 : 1 10*t*
Within rows, treatment means without a common superscript letter are significantly different ($P<0·05$)

experiment. In the Ellinbank experiment, 18 : 1 *trans*-11 was also significantly increased ($P<0·05$) by supplementation of pasture with straw. In the Kyabram experiment, this acid was unaffected ($P>0·05$) by the addition of cubed hay but was significantly decreased ($P<0·05$) by the addition of pelleted hay. Grain supplementation had no effect ($P>0·05$) on the other *trans*-18 : 1 or *trans*-16 : 1 isomers.

For all dietary treatments, there was a significant positive linear relationship between the proportions of CLA and the 18 : 1 *trans*-11 isomer (Fig. 1). There was no difference in this relationship between the Kyabram and Ellinbank experiments.

Discussion

The 18 : 1 *trans*-15 was the only isomer that remained unresolved from the *cis* 18 : 1 isomers on the SP-2560 column. This was not a problem as the 18 : 1 *trans*-15 isomer is a minor component of ruminant fats or partially hydrogenated vegetable oils; its level normally does not exceed 1·3–1·5% of the total *trans* 18 : 1 isomers (Ratnayake, 1998). Some of the *trans* 18 : 1 isomers, however, did not separate from each other. Under the temperature program used in this study, the 18 : 1 *trans*-10 was poorly resolved from 18 : 1 *trans*-11; thus the 18 : 1 *trans*-11 amounts shown in Tables 3 and 4 might contain small amounts of 18 : 1 *trans*-10. The 18 : 1 *trans*-11 isomer is the predominant *trans* 18 : 1 isomer of ruminant fats (Parodi, 1976; Wolf et al. 1998). The 18 : 1 *trans*-12, 18 : 1 *trans*-13 and 18 : 1 *trans*-14 isomers also co-eluted under the conditions used in this study and are reported as a group in Table 3 and Table 4.

The fatty acids of milk fat arise from two sources. The 4 : 0–14 : 0 acids and approximately one-half of 16 : 0 are synthesized *de novo* in the mammary gland from short-chain fatty acids arising from microbial digestion of

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press

Table 4. Fatty acid composition (weight percentage) of the milk fat of grazing (LP, low pasture) dairy cows supplemented with barley, straw and lipid (Ellinbank experiment). Fatty acids are denoted in short-hand; for example, 18 : 1 16*t* represents C18 fatty acid with a *trans* double bond at the Δ16 position

Values are means ± SED for $n=2$

Fatty acids	LP	LP+grain	LP+straw	LP+grain+ straw	LP+grain+ straw+lipid	SED
Saturated acids						
4 : 0	4·68^{a,b}	4·40^{b,c}	4·94^{a}	4·26^{c}	4·44^{b,c}	0·113
6 : 0	2·72	2·84	2·75	2·76	2·75	0·090
8 : 0	1·53	1·74	1·51	1·71	1·64	0·065
Total 4 : 0–8 : 0	8·93	8·98	9·20	8·74	8·83	0·256
10 : 0	3·14^{b}	4·06^{a}	3·00^{b}	4·03^{a}	3·63^{a}	0·166
12 : 0	3·15	4·29	3·01	4·28	3·79	0·183
14 : 0	9·82^{c}	11·53^{a}	9·51^{c}	11·72^{a}	10·56^{b}	0·179
Total 10 : 0–14 : 0	16·10^{c}	19·88^{a}	15·53^{c}	20·03^{a}	17·98^{b}	0·506
16 : 0	24·04^{c}	26·38^{b}	24·23^{c}	25·75^{b}	27·63^{a}	0·393
18 : 0	13·61^{a}	10·63^{b}	13·68^{a}	10·90^{b}	10·37^{b}	0·505
cis Unsaturated						
10 : 1 9*c*	0·28^{c}	0·31^{b}	0·30^{b}	0·31^{b}	0·34^{a}	0·009
14 : 1 9*c*	0·85^{d}	0·91^{b,c}	0·89^{c,d}	0·96^{b}	1·08^{a}	0·018
16 : 1 9*c*	0·99^{b}	0·83^{d}	0·90^{c,d}	0·96^{b,c}	1·10^{a}	0·030
18 : 1 9*c*[1]	21·34^{a}	16·11^{c}	20·75^{a}	17·70^{b,c}	18·44^{b}	0·584
18 : 2 9*c*,12*c*	0·58^{b}	0·88^{a}	0·52^{b}	0·90^{a}	0·84^{a}	0·047
18 : 3 9*c*,12*c*,15*c*	0·90^{a}	0·70^{b,c}	0·77^{b}	0·74^{b}	0·61^{c}	0·039
trans Unsaturated						
16 : 1 9*t*	0·15^{b,c}	0·16^{a,b}	0·18^{a}	0·13^{c,d}	0·12^{d}	0·008
18 : 1 6*t*–8*t*	0·20^{b}	0·26^{a}	0·20^{b}	0·23^{a,b}	0·25^{a}	0·012
18 : 1 9*t*	0·12^{c}	0·16^{a}	0·13^{b,c}	0·15^{a,b}	0·15^{a,b}	0·008
18 : 1 11*t*[2]	2·89^{b}	3·93^{a}	3·70^{a}	3·00^{b}	2·67^{b}	0·181
18 : 1 12*t*–14*t*	0·20^{c}	0·24^{a,b}	0·23^{a,b}	0·25^{a}	0·22^{b,c}	0·007
18 : 1 16*t*	0·36	0·36	0·31	0·34	0·34	0·029
Total *trans* 18 : 1	3·80^{b}	4·94^{a}	4·57^{a}	3·96^{b}	3·62^{b}	0·164
18 : 2 9*c*, 11*t* (CLA)	1·21^{b}	1·57^{a}	1·51^{a}	1·29^{b}	1·22^{b}	0·077

[1] Contains small amounts of 18 : 1 6*c*–10*c* and 18 : 1 15*t* isomers
[2] Contains small amounts of 18 : 1 10*t*
Within rows, treatment means with unlike superscripts are significantly different ($P<0·05$)

carbohydrates in the rumen. The remaining 16 : 0 and virtually all of the C18 acids are derived from circulating blood lipids. Blood lipids may derive from the diet or from fatty acids mobilized from triacylglycerol stores within adipose tissue (Grummer, 1991; Hawke & Taylor, 1995; Kennelly, 1996).

In the present experiment, cereal grain supplements for cows grazing pastures based on perennial ryegrass significantly increased the proportion of the 10 : 0–14 : 0 acids in the milk fat. This could be attributed to increased availability of ruminal volatile fatty acids for endogenous synthesis of these acids, and most probably reflects the changes in energy balance of the cows as observed by Auldist et al. (1998). Cows on the low-pasture treatments in the current experiment were consuming insufficient energy to sustain the measured level of milk production without mobilizing body tissue. When grain was included in the diet, DM and energy intake both increased significantly, so reducing the negative energy balance. Mackle et al. (1997) also reported an increase in the proportion of these acids in milk fat, from 4·6% to 8·5%, when cows grazing perennial pastures in mid lactation consumed maize grain at 3·8 kg/cow per day. When cows are in negative energy balance, the synthesis of short and medium-chain fatty acids by the mammary gland declines, while the mobilization of fatty acids from adipose tissue increases (Palmquist et al. 1993). Auldist et al. (1998) found that the 10 : 0–12 : 0 proportion in milk fat was lowest during winter when DM intake was at its lowest. The lack of significant dietary effects on 4 : 0–8 : 0 acids in milk fat is not surprising given that short-chain fatty acids are synthesized entirely within the mammary gland from acetate and β-hydroxybutyrate (Kennelly, 1996).

In Victoria, cereal grain supplements are commonly fed during summer and autumn to compensate for the low quantity and quality of pastures available (Robaina et al. 1998). Based on the results of the current work, one would expect the proportion of 10 : 0–14 : 0 acids to be higher

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press

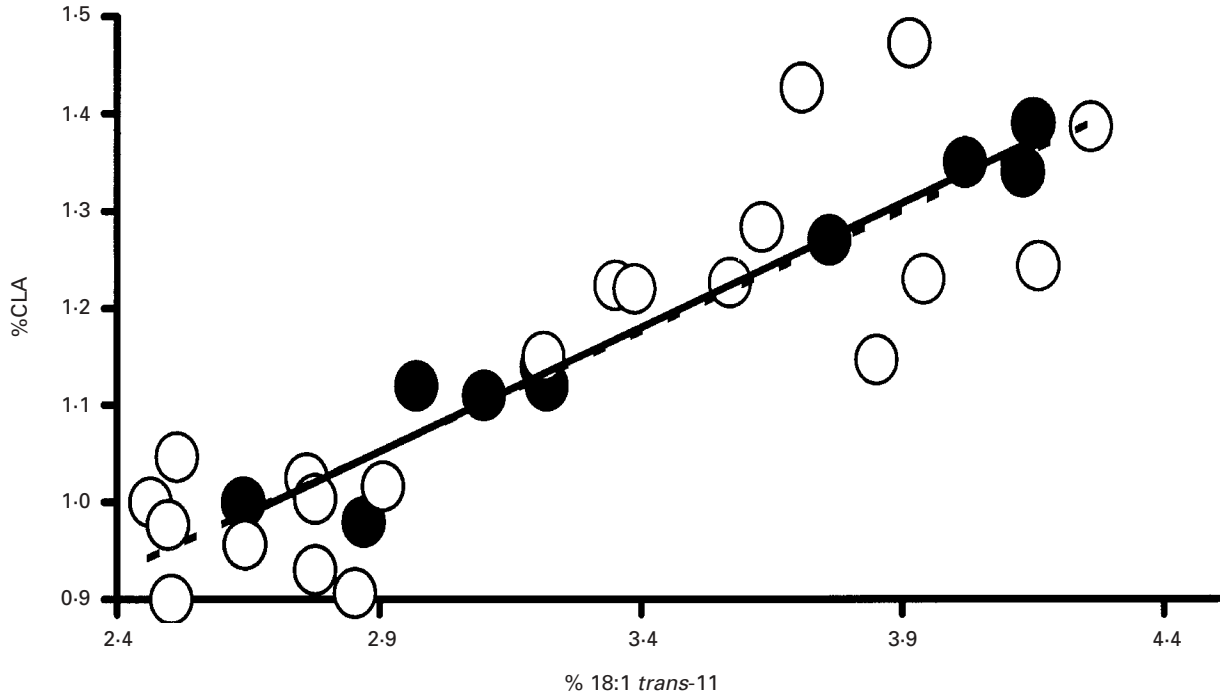

Fig. 1. Relationship between conjugated linoleic acid (CLA) and 18 : 1 *trans*-11 in milk fat.

○ Kyabram experiment, y = 0·25x + 0·32; ● Ellinbank experiment, y = 0·25x + 0·31, R^2 = 0·956.

during summer and autumn than in spring. However, this does not agree with the seasonal trends observed for milk fat in Australia, where 10 : 0–14 : 0 acids peaked in mid-spring (Thomas & Rowney, 1996). While grain intake during this period is likely to be low, mid-spring is the period when cows have reached maximum DM intake and are grazing pasture equivalent in digestibility to cereal grain. Consequently the survey results probably reflect high energy intake during that period.

In the present experiments, the proportion of 16 : 0 was also increased by grain supplementation relative to pasture-only diets. As the digestible lipid content of most cereal grain supplements is low, it follows that the increase in the proportion of 16 : 0 would have arisen from synthesis in the mammary gland in the same way as the 10 : 0–14 : 0 acids are synthesized.

The C18 fatty acids of bovine milk fat originate from the circulation, having been released by lipolysis from adipose tissue, or absorbed from the gut (Kaufmann & Hagemeister, 1987). Both routes may contribute to the decrease in the proportions of 18 : 0 and 18 : 1 9*c* acids in the milk fat with grain supplementation. Firstly, with increasing energy intake as a result of grain supplementation, reduced mobilization of fatty acids from the adipose tissue may occur. Secondly, as pasture is a rich source of the polyunsaturated acid 18 : 3 (Palmquist, 2001), a decrease in pasture intake with grain supplementation may lead to lower amounts of polyunsaturated C18 acids being available for biohydrogenation to 18 : 1 and 18 : 0 in the rumen. Although grain supplementation significantly decreased pasture intake in the Ellinbank experiment, it had little effect in the Kyabram experiment. Therefore, it is likely that reduced mobilization of fatty acids from the adipose tissue had a greater effect on the proportion of C18 fatty acids than had pasture intake itself.

In contrast with the other C18 fatty acids, the proportion of 18 : 2 increased with grain supplementation in both experiments. Although this result is somewhat surprising in the light of the above discussion, it agrees with earlier experiments (Santos et al. 1997; Crocker et al. 1997) which found 18 : 2 to be increased in response to diets that were higher in ruminally available carbohydrate. Latham et al. (1972) related the high 18 : 2 content of milk fat in cows fed fat-depressing rations to the inhibition of ruminal lipolysis caused by low ruminal pH, which allows 18 : 2 in cereals to escape ruminal biohydrogenation.

For all the supplements investigated in this study, there was a positive linear relationship between CLA and 18 : 1 *trans*-11 (Fig. 1), which agrees with observations made by other workers (e.g., Jahreis et al. 1999). This is further evidence for a common origin for these fatty acids. Generally, the milk fat of cows grazing pasture and receiving no supplemental feed contains more CLA than that of cows given typical dairy diets (Dhiman et al. 1999; Jahreis et al. 1997), except when pasture is supplemented with full-fat soybean or rapeseed (Lawless et al. 1998). Although one experiment in the present investigation strongly supported this, with pasture-plus-grain diets giving significantly

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press

less CLA and *trans*-18 : 1 acids than pasture-only diets, the second experiment, conducted in a different dairy region, gave the opposite result.

The opposite results for the effect of grain supplementation on CLA in the two experiments are difficult to explain. Walker et al. (2000) showed that at high levels of grain feeding, sufficient to reduce milk fat concentration significantly, CLA proportion increased. This response was most obvious when cows showed milk fat depression, attributable to grain-induced ruminal acidosis. A similar response could have occurred in the Ellinbank experiment as grain supplements in the Ellinbank experiment reduced the milk fat concentration by 4 g/kg compared with only 2·3 g/kg in the Kyabram experiment (Table 2). The different CLA proportions might also be attributable to nutritional differences between the Kyabram and Ellinbank experiments. Cows in the Ellinbank experiment consumed about 2 kg DM per day more than cows on the corresponding treatments at Kyabram. This higher DM intake combined with marginally better quality pasture at Ellinbank resulted in the cows at Ellinbank consuming more metabolizable energy on a daily basis. Furthermore, high variations in the concentration of milk fat CLA between individual cows have been reported (Lawless et al. 1999), and such differences could have contributed to the opposite CLA results for the Kyabram and Ellinbank experiments. CLA proportions in the current work were similar to those reported by Auldist et al. (1998) but significantly higher than those reported by Mackle et al. (1997).

CLA has recently been the focus of much interest because of its anticarcinogenic properties (Cook & Pariza, 1998; O'Shea et al. 1998; Katan, 2000; MacDonald, 2000). Until recently, ruminal biohydrogenation of polyunsaturated C18 fatty acids was considered to be the main source of CLA in milk fat. However, newer evidence suggests that rumen production from polyunsaturated C18 acids is not the only route to CLA. It can also be produced by desaturation of 18 : 1 *trans*-11 (Corl et al. 1998; Griinari et al. 2000; Palmquist 2001). More interestingly, there is evidence to suggest that man also has the ability to convert dietary 18 : 1 *trans*-11 to CLA (Salminen et al. 1998). Thus 18 : 1 *trans*-11 in milk fat might make a further contribution over and above the beneficial effects of preformed CLA. The 18 : 1 *trans*-11 fatty acid is the predominant *trans* 18 : 1 isomer in milk fat (Parodi, 1970; Wolf et al. 1998). On-farm strategies to increase the content of CLA in milk fat should therefore consider the effect of such strategies on both CLA and 18 : 1 *trans*-11.

The lipid supplement used in the Ellinbank experiment contained predominantly the long-chain saturated fatty acids, 16 : 0 (32%) and 18 : 0 (47%), which were expected to increase the proportion of these acids in the milk fat. However, only the proportion of 16 : 0 was significantly increased by lipid supplementation. The absence of a significant effect of the lipid supplement on 18 : 0 may have been the result of desaturation of the 18 : 0 to produce 18 : 1 in the milk fat. The relatively small effects of lipid supplement could also have been due to the fact that 250 g of lipid supplement probably represented a relatively small amount of the total lipid entering the small intestine when compared with the total amount consumed in the diet and synthesized in the rumen.

In conclusion, this study showed that feeding barley grain to dairy cows grazing pasture in early lactation increases the proportion of the component fatty acids of milk fat that are synthesized endogenously in the mammary gland. As these are saturated fatty acids, the hardness of milk fat would be expected to increase. Where functional properties of milk fat are important to the dairy manufacturer, feeding strategies used by the farmer should take into account possible effects of these supplements on hardness of the milk fat. Barley-based cereal grain supplements alter the proportion of CLA and other *trans* fatty acids in milk fat. Whether this is an increase or decrease appears to depend on the quality of pasture on offer and the physiological state of the cow. Hay or straw fibre supplements do not affect the fatty acid composition of milk fat of grazing dairy cows in early lactation.

We thank John Reynolds for statistical analyses, Richard Stockdale and Glen Walker for reviewing the manuscript, and the Department of Natural Resources and Environment and Food Science Australia for financial support.

References

Armstrong D, Knee J, Doyle P, Pritchard K & Gyles O 1998 *A survey of water-use efficiency on irrigated dairy farms in northern Victoria and southern New South Wales*. Institute of Sustainable Irrigated Agriculture, Kyabram, Australia: Department of Natural Resources and Environment

Auldist, MJ, Walsh BJ & Thomson NA 1998 Seasonal and lactational influences on bovine milk composition in New Zealand. *Journal of Dairy Research* **65** 401–411

Bannon CD, Craske JD & Hilliker AE 1985 Analysis of fatty acid methyl esters with high accuracy and reliability. IV Fats with fatty acids containing four or more carbon atoms. *Journal of the American Oil Chemists Society* **62** 1501–1507

Cook ME & Pariza M 1998 The role of conjugated linoleic acid (CLA) in health. *International Dairy Journal* **8** 459–462

Corl BA, Chouinard PY, Bauman DE, Dwyer DA, Griinari JM & Nurmela KV 1998 Conjugated linoleic acid in milk fat of dairy cows originates in part by endogenous synthesis from *trans*-11 octadecenoic acid. *Journal of Dairy Science* **81** (Suppl. 1) 233

Crocker LM, De Peters EJ, Fadel JG, Perez-Monti H, Taylor SJ, Wyckoff JA & Zinn RA 1997 Influence of processed corn grain in diets of dairy cows on digestion of nutrients and milk composition. *Journal of Dairy Science* **81** 2394–2497

Dhiman TR, Ananad GR, Satter LD & Pariza MW 1999 Conjugated linoleic acid content of milk from cows fed different diets. *Journal of Dairy Science* **82** 2146–2156

Doyle PT, Lawson AR, Stockdale CR & Cohen DC 2000 *Pasture for Dairy Production in Victoria*, 2nd Edition. Kyabram, Australia: Department of Natural Resources and Environment

Grainger C, Cowan T & Dellow D 1996 Dairy Cow Nutrition. In *Australian Dairy – The Comprehensive Reference to the Australian Dairy Industry*, pp. 161–190 (Ed. S Schelling). East Hawthorn, Victoria, Australia: Morescope Publishing Pty Ltd

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press

Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV & Bauman DE 2000 Conjugated linoleic acid is synthesised endogenously in lactating dairy cows by Δ9-desaturase. *Journal of Nutrition* **130** 2285–2291

Grummer RR 1991 Effect of feed on the composition of milk fat. *Journal of Dairy Science* **74** 3244–3257

Hawke JC & Taylor MW 1995 Influence of nutritional factors on the yield, composition and physical properties of milk fat. In *Advanced Dairy Chemistry*, Volume 2, pp. 37–87 (Ed. PF Fox). London: Chapman & Hall

Jahreis G, Fritsche J & Steinhart H 1997 Conjugated linoleic acid in milk fat: High variation depending on production system. *Nutrition Research* **17** 1479–1484

Jahreis G, Fritsche J, Mockel P, Schone F, Moller U & Steinhart H 1999 The potential anticarcinogenic conjugated linoleic acid, *cis*-9, *trans*-11 C18:2 in milk of different species: cow, goat, ewe, sow, mare and woman. *Nutrition Research* **19** 1541–1549

Katan MB 2000 Trans fatty acids and plasma lipoproteins. *Nutrition Reviews* **58** 188–191

Kaufmann W & Hagemeister H 1987 Composition of milk. In *Dairy Cattle Production*, pp. 107–171 (Ed. HO Gravert). Amsterdam: Elsevier Science Publishers

Kennelly JJ 1996 The fatty acid composition of milk fat as influenced by feeding oilseeds. *Animal Feed Science and Technology* **60** 137–152

Latham MJ, Storry JE & Sharpe ME 1972 Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. *Applied Microbiology* **24** 871–877

Lawless F, Murphy JJ, Harrington D, Devery R & Stanton C 1998 Elevation of conjugated *cis*-9, *trans*-11-octadecadienoic acid in bovine milk because of dietary supplementation. *Journal of Dairy Science* **81** 3259–3267

Lawless F, Stanton C, L'Escop P, Devery R, Dillon P & Murphy JJ 1999 Influence of breed on bovine milk *cis*-9, *trans*-11-conjugated linoleic acid content. *Livestock Production Science* **62** 43–49

MacDonald HB 2000 Conjugated linoleic acid and disease prevention: A review of current knowledge. *Journal of the American College of Nutrition* **19** 111S–118S

Mackle TR, Petch SF, Bryant AM, Auldist MJ, Henderson HV & MacGibbon AKH 1997 Variation in the characteristics of milk fat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *New Zealand Journal of Agricultural Research* **40** 349–359

O'Shea M, Lawless C, Stanton C & Devery R 1998 Conjugated linoleic acid in bovine milk fat: a food-based approach to cancer chemoprevention. *Trends in Food Science and Technology* **9** 192–196

Palmquist DL, Beaulieu AD & Barbano DM 1993 Feed and animal factors influencing milk fat composition. *Journal of Dairy Science* **76** 1753–1771

Palmquist DL 2001 Ruminal and endogenous synthesis of CLA in cows. *Australian Journal of Dairy Technology* **56** 134–137

Parodi PW 1970 Fatty acid composition of Australian butter and milk fats. *Australian Journal of Dairy Technology* **25** 200–205

Parodi PW 1976 Distribution of isomeric octadecenoic fatty acids in milk fat. *Journal of Dairy Science* **59** 1870–1873

Ratnayake WMN 1998 Analysis of trans fatty acids. In *Trans Fatty Acids in Human Nutrition*, pp. 115–161 (Eds JL Sebedio & WW Christie). Dundee: Oily Press

Robaina AC, Grainger C, Moate P, Taylor J & Stewart J 1998 Responses to grain feeding by grazing dairy cows. *Australian Journal of Experimental Agriculture* **38** 541–549

Salminen I, Mutanen M, Jauhiainen M & Aro A 1998 Dietary *trans* fatty acids increase conjugated linoleic acid levels in human serum. *Journal of Nutritional Biochemistry* **9** 93–98

Santos FAP, Huber JT, Theurer CB, Swingle RS, Wu Z, Simas JM, Chen KH, Chan SC, Santos J & De Peters EJ 1997 Comparison of barley and sorghum grain processed at different densities for lactating dairy cows. *Journal of Dairy Science* **80** 2098–2103

Stockdale CR 1999 Effects of cereal grain, lupins-cereal grain or hay supplements on the intake and performance of grazing dairy cows. *Australian Journal of Experimental Agriculture* **39** 811–817

Thomas L & Rowney M 1996 Australian milk fat survey – fatty acid composition. *Australian Journal of Dairy Technology* **51** 112–114

Wales WJ, Williams YJ & Doyle PT 2001 Effect of grain supplementation and the provision of chemical or physical fibre on marginal milk production responses of cows grazing perennial pastures. *Australian Journal of Experimental Agriculture* **41** 465–471

Walker GP, Wales WJ, Doyle PT & Dellow DW 2000 The content of conjugated linoleic acid in milk in response to feeding a cereal grain-based supplement to dairy cows grazing pastures. *Asian-Australasian Journal of Animal Sciences* **13** Suppl. 63

Wolf RL, Precht D & Molkentin J 1998 Occurrence and distribution profiles of trans-18:1 acids in edible fats of natural origin. In *Trans Fatty Acids in Human Nutrition*, pp. 1–33 (Eds JL Sebedio & WW Christie). Dundee: Oily Press

https://doi.org/10.1017/S0022029903006241 Published online by Cambridge University Press