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Predicting Δ^9-desaturase activity and the association with conjugated linoleic acid (CLA) concentration in bovine milk

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ABSTRACT

The major source of *cis*-9, *trans*-11 conjugated linoleic acid (CLA) in bovine milk is from the post-ruminal conversion of *trans*-11 $C_{18:1}$ (TVA) by Δ^9-desaturase. Activity of Δ^9-desaturase is predicted from the product/substrate ratios of fatty acids dependant on the enzyme. Data from three experiments was investigated. Cows selected for having soft or hard milk fat were fed ruminally protected oilseed in spring 1999, and autumn 2000 or crushed full-fat rapeseed in autumn 2001. Cows with harder milk fat had higher concentration of milk fat ($P<0.001$), and lower concentrations ($P<0.001$) of TVA and CLA. Feeding ruminally protected oilseed in spring had no effect on milk fat, TVA or CLA concentrations but in autumn increased ($P<0.001$) milk fat concentration. Feeding crushed rapeseed depressed ($P<0.001$) TVA and CLA concentrations. Ratios of fatty acid pairs dependent on Δ^9-desaturase ($C_{10:1}/C_{10:0}$, $C_{16:1}/C_{16:0}$ cis-9 $C_{18:1}/C_{18:0}$ and CLA/TVA) were lower in milk from cows with harder milk fat and the $C_{12:1}/C_{12:0}$, $C_{14:1}/C_{14:0}$ ratios were similar for soft and hard milk fat cows. Feeding ruminally protected oilseed decreased ($P<0.001$) all ratios except for $C_{12:1}/C_{12:0}$ in autumn 2000. Feeding rapeseed had no effect on the product/substraight ratios except the cis-9 $C_{18:1}/C_{18:0}$ ratio. None of these ratios were associated with milk fat CLA concentrations in spring however in autumn $C_{14:1}/C_{14:0}$ and $C_{16:1}/C_{16:0}$ were positively associated ($P<0.05$). Significant associations ($P<0.001$) between the milk fat cis-9 $C_{18:1}/C_{18:0}$ ratio and CLA concentration and between TVA and CLA concentrations were also evident. This suggests that milk fat CLA concentrations may be influenced more by the availability of TVA than by the variation in the activity of Δ^9-desaturase.

Keywords: bovine milk fat; Δ^9-desaturase; conjugated linoleic acid (CLA); product/substrate ratios

INTRODUCTION

Conjugated linoleic acid (CLA), a compound found in ruminant fat, has in recent years received much attention because of its range of benefits to human health and wellbeing (Belury, 2002). Such benefits are: reducing body fat accretion, delaying the onset of type II diabetes, retarding the development of atherosclerosis, improving the mineralisation of bone and modulating the immune system. Parodi (1999) also identified that CLA has anti-carcinogenic properties.

CLA is a mixture of positional and geometric isomers of linoleic acid of which, in milk fat, the *cis*-9, *trans*-11 isomer is dominant, making up 70-90% of total CLA. Specific functional properties have been identified for a few of these isomers, for example, the *cis*-9, *trans*-11 CLA isomer has been identified as having anti-carcinogenic properties (Ip *et al*., 1999) and the *trans*-10 *cis*-12 CLA isomer specifically inhibits milk fat synthesis (Baumguard *et al.,* 2000). Initially the belief was that *cis*-9, *trans*-11 CLA was formed in the rumen as a by-product in the biohydrogenation of linoleic to stearic acid (Hartfoot & Hazelwood, 1997). However, Griinari *et al*. (2000), Corl *et al.* (2001) and Kay *et al*. (2002) identified that *cis*-9, *trans*-11 CLA was also synthesised post-ruminally from *trans*-11 $C_{18:1}$ (*trans* vaccenic acid, TVA), by Δ^9-desaturase. Kay *et al.* (2002) estimated that at least 90% of *cis*-9, *trans*-11 CLA in milk fat from pasture-fed cows was synthesised by this pathway.

Large and consistent between-cow variations in milk fat CLA concentrations have been reported (Mackle *et al*., 1997; Lock & Garnsworthy, 2002; Peterson *et al*., 2002). Lock & Garnsworthy (2003) suggested that the activity of Δ^9-desaturase could explain this variability. The enzyme, Δ^9-desaturase is responsible for adding a *cis* double bond on the 9th carbon of fatty acids with chain lengths of 10 to 18. Concentrations of $C_{16:0}$ and $C_{18:0}$ are partly influenced by factors such as diet and adipose tissue metabolism, but the occurrence in milk fat of $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$ fatty acids is due, almost entirely, to *de novo* synthesis. Therefore, the presence of *cis*-9 $C_{10:1}$, *cis*-9 $C_{12:1}$ and *cis*-9 $C_{14:1}$ in milk fat is almost solely dependent on the activity of Δ^9-desaturase (Kay *et al*., 2002). Lock & Garnsworthy (2002) suggested that the *cis*-9 $C_{14:1}/C_{14:0}$ ratio was the best estimate of Δ^9-desaturase activity due to all $C_{14:0}$ being produced *de novo* in the mammary gland, consequently Δ^9-desaturation is the only source of *cis*-9 $C_{14:1}$ in milk fat. Kay *et al*. (2002) used a similar argument to include *cis*-9 $C_{10:1}/C_{10:1}$ and *cis*-9 $C_{12:1}/C_{12:1}$ ratios which occur in significant amounts in milk fat from pasture-fed cows. Pollard *et al*. (1980) suggested that Δ^9-desaturase preferentially desaturates longer chain saturated fatty acids, e.g., $C_{16:0}$ and $C_{18:0}$, and *trans* monene fatty acids, e.g., TVA. This then suggests that the presence of increased concentrations of $C_{18:0}$ or TVA will preferentially utilise Δ^9-desaturase activity to such an extent that it may limit enzyme activity for the desaturation of the shorter chain fatty acids, i.e., $C_{10:0} - C_{14:0}$. Then, in situations when the mammary gland is exposed to increased supply of C18 fatty acids, as would occure with oilseed supplementation, the $C_{14:1}/C_{14:0}$ ratio may not reliably estimate Δ^9-desaturase activity. To test this hypothesis, the fatty acid compositions of milk fat from two phenotypes: hard milk fat (HMF) and soft milk fat (SMF) cows grazing pasture only or pasture supplemeted with a lipid supplement were examined.

MATERIALS AND METHODS

In the first trial (Trial 1), 20 multiparous lactating

Friesian cows (56 ± 23 DIM) selected with harder (HMF) (n=10) or softer (SMF) milk fat (n = 10) were fed during spring 1999 either grazed pasture only or grazed pasture supplemented with 2 kg/cow/day of a ruminally protected lipid supplement (70% canola:30% soy meal, Rumentek Industries, Australia). Trial 2 was run in autumn 2000 (cows 203 ± 23 DIM), using the same cows, experimental design, and treatments as Trial 1. The third trial (Trial 3), conducted during autumn 2001 (cows 225 ± 19 DIM), again used a similar type of cow (HMF n=8; SMF n=8) and trial management as the two previous trials but the trial was a partial cross-over design and the ruminally protected canola:soy supplement was replaced with 2 kg/cow/day of full-fat rapeseed. The rapeseed was crushed by one pass through a Bisley M-3 hammer mill fitted with a 4-mm screen.

The SMF and HMF cows were selected in the following manner. In autumn 1998, 300 cows were screened for solid fat content (SFC) using the method described by MacGibbon & McLennan, (1987). From the 300 cows screened, a group of 10 cows with hard milk fat (SFC at 10°C >64%) and a group of 10 cows described as soft (SFC at 10°C <54%) were selected and used in Trials 1 & 2. In autumn 2000, a further 170 cows were screened for SFC and two groups of cows with similar milkfat characteristics as used in Trials 1 & 2 were selected.

The three trials were run as cross-over designed experiments with two, two-week experimental periods separated by a 1-week washout period. In the three trials, 2 kg/cow/d of the lipid supplements was fed to cows in individual stalls, once a day, after morning milking at 0730 h. The cows remained in their respective stalls until all supplement was consumed (approx. 30 min each day) then returned to pasture. The concentration of long-chain fatty acids (LCFA) in the oilseed supplements used in each experiment (Table 1) differed. This resulted in variable inputs of LCFA of 540, 445, and 800 g/cow/day for Trials 1, 2, 3 respectively.

Throughout each of the experimental and washout periods, the experimental cows were managed as one herd and grazed pasture at an allowance of approximately 35 kg DM/cow/day throughout the experimental and washout periods.

Individual composite milk samples were collected from four consecutive milkings at the end of each experimental period using in-line milk meters (TrueTest, Palmerston North, New Zealand). Each composite sample was mixed, sub-sampled and, either analysed immediately for fat concentration (MilkoScan FT120, FOSS, Denmark) or, the cream extracted by centrifugation and stored at –20°C for later analysis of fatty acid composition.

For all Trials, fat was extracted from the cream samples using the Rˆese-Gottlieb fat extraction procedure (IDF, 1987) and stored at -20°C. Fatty acid methyl esters were quantified by gas chromatography after methylation using sodium methoxide as described by MacGibbon (1988). Fatty acid analyses were performed on a Gas Chromatogram (Shimadzu Corporation, Kyoto, Japan GC 17A-FID). During the period the fatty acid data was collected the analytical technique was modified and improved to get clearer separation of the $C_{18:1}$ and CLA isomers. The 30-m, DB-Wax column used to analyse the milk fat for Trials 1 & 2 gave no clear dissociation of $C_{18:1}$ and CLA isomers and for these Trials only total $C_{18:1}$ and total CLA were determined. However, with the development of the methodology and the adoption of a 120-m BPX-70 column, clear dissociation of the isomers of $C_{18:1}$ and CLA became possible and are reported for Trial 3.

Pastures were sampled before grazing on three occasions each week of the experimental periods. The sampling procedure was to take approximately 30 sub-samples/paddock using hand shears and cutting to an estimated grazing height formed by assessing the paddock previously grazed by the trial herd. Each pasture sample was immediately frozen and at the end of each experimental period samples were freeze dried, ground to pass through a 1 mm sieve and bulked. Fat from pasture and supplement lipids was extracted and methylated by the one-step procedure of Garces & Mancha (1993). Fatty acid analyses were performed on a Hewlett Packard 5890 Series II Gas Chromatograph equipped with a 30 m RTX-2330 column. The average fatty acid composition of the pasture and lipid supplements for each trial is presented.

TABLE 1: Total fat (% of DM) and fatty acid composition of pasture and oilseed supplements used in the three Trials used to provide data to determine the activity of Δ^9-desaturase: Trial 1 spring 1999, Trial 2 autumn 2000, Trial 3 autumn 2001.

	Trial 1		Trial 2		Trial 3	
	Pasture	**Canola[1]**	**Pasture**	**Canola[1]**	**Pasture**	**Rapeseed[2]**
LCFA[3](% of DM)	4.4	29.8	2.6	24.7	3.5	40.0
Fatty acid			**(% of LCFA)**			
$C_{16:0}$	14.7	7.9	22.8	7.0	17.3	4.7
$C_{18:0}$	4.4	2.9	7.3	3.3	2.0	1.7
$C_{18:1}$	2.3	51.0	5.8	47.4	2.9	57.9
$C_{18:2}$	12.0	28.0	19.0	29.6	15.3	21.4
$C_{18:3}$	61.3	8.6	36.5	10.6	55.4	12.4

[1]Ruminally protected 70% canola:30% soy meal

[2] Crushed rapeseed

[3]LCFA Long-chain fatty acids (C14:0 to C24:0)

STATISTICAL ANALYSES

Ratios of product/substrate were calculated for C_{10}, C_{12}, C_{14}, and C_{16} fatty acid pairs for each trial and additionally for *cis*-9 $C_{18:1}/C_{18:0}$ and *cis*-9, *trans*-11 $C_{18:2}$/TVA pairs in Trial 3. For Trials 1 & 2 the effect of treatment (oilseed supplementation and phenotype effects) on dairy cow performance (milk yield, fat % and fat yield) and fatty acid composition was determined using ANOVA with phenotype being tested against between-cow variation and oilseed supplementation against within-cow variation. Because a partial crossover design was used in Trial 3, mixed models with period, oilseed supplementation and phenotype as fixed effects, and cow as a random effect, were fitted using REML. Residual plots were used to check for departures from normality. The relationship between each of the product/substrate ratios, TVA, and the concentration of total CLA in milk fat was investigated by within treatment group regression using REML with period, oilseed supplementation and phenotype as fixed effects, CLA as a covariant and cow as a random effect. All statistical analyses were carried out using GenStat (2002).

RESULTS

The amount and composition of long-chain fatty acids (LCFA) in pasture differed between spring and autumn (Table 1) and differed between the oilseed supplements. In comparison with the oilseeds, pasture had a lower concentration of LCFA with a higher concentration of a-linolenic acid ($C_{18:3}$), whereas the dominant fatty acid in the oilseed supplements was oleic acid (*cis*-9 $C_{18:1}$). The crushed full-fat rapeseed contained more LCFA and more oleic acid than the ruminally protected oilseed.

In Trials 1 & 2 the feeding of ruminally protected oilseed resulted in an increase in milk yield, and in Trial 2, in the concentration of milk fat (Table 2). The feeding of rapeseed (Trial 3) had no effect on milk yield or milk fat concentration. In each of the three trials, the SMF cows produced higher milk yields of lower milk fat concentration than the HMF cows.

Feeding oilseed supplements decreased the milk fat concentrations of fatty acids with chain length of C_{16} or less, but increased the concentration of $C_{18:0}$ and total $C_{18:1}$ (Table 3). The feeding of ruminally protected oilseed in Trials 1 & 2 had no effect on total CLA in milk fat but in Trial 3 the feeding of rapeseed depressed total milk fat CLA and *cis*-9, *trans*-11 CLA. In Trial 1, the concentrations in milk fat of $C_{16:1}$, $C_{14:0}$, $C_{14:1}$, $C_{12:0}$, $C_{12:1}$, $C_{10:1}$, and $C_{10:0}$ were similar for SMF and HMF cows. The SMF cows in Trials 1 & 2 had less $C_{16:0}$ and more $C_{18:0}$ (Trial 2), $C_{18:1}$ (total) and CLA in milk fat than the HMF cows. A similar trend was observed in Trial 3 but the concentrations of the saturated fatty acids with chains $C_{16:0}$ or shorter were lower in milk fat from the SMF cows. Concentrations of the mono-unsaturated fatty acids of chain lengths $C_{16:0}$ or shorter were unaffected by cow phenotype. Similar to Trials 1 & 2, the milk fat concentrations recorded in Trial 3 of $C_{18:0}$, $C_{18:1}$ (total), CLA (total), and *cis*-9, *trans*-11 CLA were greater for the SMF than HMF cows, but milk fat concentration of TVA was unaffected by cow phenotype. The CLA isomer *trans*-10, *cis*-12 CLA increased in milk fat from cows supplemented with rapeseed, however, phenotype had no effect on this fatty acid.

The product/substrate ratios reported to reflect Δ^9-desaturase activity (Table 4) show less consistent treatment effects than observed for the individual fatty acids presented in Table 3. Feeding ruminally protected oilseed in Trials 1 & 2 generally depressed the product/substrate ratio for each fatty acid pair but had no effect on the $C_{12:1}/C_{12:0}$ ratio in Trial 2. For Trial 3, feeding rapeseed increased the *cis*-9 $C_{18:1}/C_{18:0}$ ratio but had no effect on the other product/substrate ratios examined.

In Trials 1, 2, & 3 no significant association between the $C_{10:1}/C_{10:0}$ and $C_{12:1}/C_{12:0}$ ratios and milk fat CLA concentration was evident. Also, in Trial 1, no significant associations between the ratio of any product/substrate ratio and milk fat CLA concentration was observed. However, in Trial 2, a positive association ($P<0.05$) occurred between the $C_{14:1}/C_{14:0}$ and $C_{16:1}/C_{16:0}$ ratios and milk fat CLA concentration. In Trial 3 no association between $C_{14:1}/C_{14:0}$ and milk fat CLA was found but a significant association ($P<0.01$) between $C_{16:1}/C_{16:0}$ was evident. A significant positive association ($P<0.001$) was also evident in Trial 3 between the *cis*-9 $C_{18:1}/C_{18:0}$ ratio, the *cis*-9, *trans*-11 CLA/TVA ratio, TVA concentration,

TABLE 2: Effect on milk yield and composition of feeding either (Trials 1 & 2) pasture alone (P) or P plus 2 kg/cow/d of a ruminally protected canola (C) or, in Trial 3; P alone or P plus 2 kg/cow/d of crushed full fat rapeseed (R) to cows selected for softer and harder milk fat.

	Hard		Soft		s.e.d & significance	
	P	**P + C**	**P**	**P + C**	**Cow***	**Feed**
Trial 1						
Milk yield (kg/cow/d)	21.6	23.3	23.9	25.2	1.94^{NS}	0.41^{***}
Fat %	4.81	4.86	3.83	3.94	0.15^{***}	0.10^{NS}
Fat yield (kg/cow/d)	1.04	1.13	0.91	0.98	0.07^{*}	0.03^{**}
Trial 2						
Milk yield (kg/cow/d)	8.71	10.08	8.96	10.5	0.57^{NS}	0.25***
Fat %	5.02	5.38	4.17	4.65	0.21^{**}	0.09***
Fat yield (kg/cow/d)	0.43	0.53	0.37	0.49	0.02^{*}	0.01***
Trial 3	P	P + R	P	P + R		
Milk yield (kg/cow/d)	9.2	8.7	9.6	10.1	0.85^{NS}	0.62^{NS}
Fat %	5.51	5.27	4.52	4.86	0.22^{***}	0.13^{NS}
Fat yield (kg/cow/d)	0.50	0.46	0.43	0.48	0.04^{NS}	0.03^{NS}

* Cow = SMF/HMF

TABLE 3: The proportion of selected fatty acids in milk fat from cows selected for softer and harder milk fat and fed either (Trials 1 & 2) pasture alone (P) or P plus 2 kg/cow/d of a ruminally protected oilseed (C) or in Trial 3, P alone or P plus 2 kg/cow/d crushed full fat rapeseed (R).

	Hard	Soft	s.e.d. & Significance[1]			
Fatty acid	**P**	**P + C**	**P**	**P + C**	**Cow**	**Feed**
			Trial 1			
$C_{10:0}$	3.81	3.6	3.73	3.22	0.20^{NS}	0.11^{***}
$C_{10:1}$	0.3	0.24	0.34	0.26	0.02^{NS}	0.01^{***}
$C_{12:0}$	4.34	3.75	4.2	3.36	0.25^{NS}	0.13^{***}
$C_{12:1}$	0.08	0.05	0.08	0.05	0.007^{NS}	0.004^{***}
$C_{14:0}$	12.26	10.24	11.97	9.4	0.46^{NS}	0.29^{***}
$C_{14:1}$	0.88	0.52	0.9	0.56	0.08^{NS}	0.04^{***}
$C_{16:0}$	30.52	23.11	25.95	20.22	0.91^{***}	0.47^{***}
$C_{16:1}$	1.57	1.06	1.63	1.2	0.07^{NS}	0.05^{***}
$C_{18:0}$	10.01	13.33	9.58	11.87	0.58^{NS}	0.32^{***}
$C_{18:1}$ (total)	18.52	24.7	22.4	28.93	1.13^{**}	0.69^{***}
CLA	0.86	0.8	1.27	1.16	0.11^{***}	0.08^{NS}
			Trial 2			
$C_{10:0}$	2.36	1.99	2.27	1.81	0.13^{NS}	0.03^{***}
$C_{10:1}$	0.26	0.19	0.31	0.22	0.02^{NS}	0.01^{***}
$C_{12:0}$	2.57	2.01	2.58	1.91	0.13^{NS}	0.04^{***}
$C_{12:1}$	0.07	0.06	0.08	0.07	0.006^{NS}	0.005^{NS}
$C_{14:0}$	9.91	7.53	10.17	7.31	0.31^{NS}	0.12^{***}
$C_{14:1}$	0.9	0.52	1.07	0.6	0.07^{NS}	0.03^{***}
$C_{16:0}$	30.3	21.0	28.5	19.4	0.73^{*}	0.31^{***}
$C_{16:1}$	1.9	1.05	2.03	1.07	0.06^{NS}	0.04^{***}
$C_{18:0}$	11.5	13.9	9.7	11.6	0.53^{**}	0.24^{***}
$C_{18:1}$ (total)	23.5	31.8	25.1	35.3	0.78^{**}	0.42^{***}
CLA	1.1	1.06	1.41	1.25	0.09^{**}	0.07^{NS}
			Trial 3			
	P	**P + R**	**P**	**P + R**		
$C_{10:0}$	2.28	1.50	1.81	1.36	0.12^{**}	0.09^{***}
$C_{10:1}$	0.24	0.18	0.23	0.16	0.02^{NS}	0.02^{***}
$C_{12:0}$	2.42	1.56	1.97	1.47	0.12^{*}	0.10^{***}
$C_{12:1}$	0.08	0.04	0.07	0.03	0.02^{NS}	0.02^{***}
$C_{14:0}$	8.94	6.69	8.19	6.34	0.33^{*}	0.28^{***}
$C_{14:1}$	0.76	0.62	0.80	0.58	0.08^{NS}	0.07^{***}
$C_{16:0}$	27.02	19.29	23.88	17.91	0.83^{***}	0.70^{***}
$C_{16:1}$	1.27	0.98	1.34	0.99	0.09^{NS}	0.08^{***}
$C_{18:0}$	11.84	17.72	11.99	15.75	0.80^{*}	0.64^{***}
c-9 $C_{18:1}$	18.25	28.09	22.89	31.11	1.17^{***}	0.92^{***}
TVA	5.59	3.96	5.15	3.96	0.44^{NS}	0.43^{***}
$C_{18:1}$ (total)	26.3	36.8	30.7	39.7	1.26^{***}	1.11^{***}
c-9 t-11 CLA	1.71	1.30	2.12	1.60	0.15^{**}	0.15^{***}
t-10 c-12 CLA	0.11	0.16	0.10	0.20	0.02^{NS}	0.02^{***}
CLA (total)	1.90	1.60	2.37	1.94	0.16^{***}	0.15^{**}

[1] All cow x feed interactions were non significant (NS)

TABLE 4: Product/substrate ratios for fatty acid pairs in milk fat dependent on Δ^9-desaturase and the regression of the ratio with total CLA. The trial cows were selected with softer and harder milk fat and fed either (Trials 1& 2) pasture alone (P) or P plus 2 kg/cow/d of a ruminally protected oilseed (C) or, in Trial 3; P alone or P plus 2 kg/cow/d crushed full fat rapeseed (R).

	Hard		Soft		s.e.d.& Significance		RegressionRatio/CLA[1]		
Variant	**P**	**P + C**	**P**	**P + C**	**Cow**	**Feed**	**Slope**	**s.e.**	**Sig.**
Trial 1									
$C_{10:1}/C_{10:0}$	0.080	0.068	0.094	0.082	0.005^{*}	0.006^{***}	0.012	0.008	NS
$C_{12:1}/C_{12:0}$	0.018	0.014	0.019	0.016	0.001^{NS}	0.001^{***}	0.002	0.002	NS
$C_{14:1}/C_{14:0}$	0.077	0.051	0.076	0.060	0.006^{NS}	0.002^{***}	0.008	0.008	NS
$C_{16:1}/C_{16:0}$	0.052	0.046	0.063	0.060	0.002^{**}	0.003^{*}	0.009	0.007	NS
Trial 2									
$C_{10:1}/C_{10:0}$	0.113	0.098	0.139	0.123	0.009^{*}	0.002^{***}	0.007	0.007	NS
$C_{12:1}/C_{12:0}$	0.033	0.032	0.032	0.039	0.004^{NS}	0.004^{NS}	0.010	0.008	NS
$C_{14:1}/C_{14:0}$	0.091	0.069	0.106	0.083	0.008^{NS}	0.002^{***}	0.010	0.005	*
$C_{16:1}/C_{16:0}$	0.063	0.050	0.071	0.055	0.003^{*}	0.001^{***}	0.009	0.004	*
Trial 3	**P**	**P + R**	**P**	**P + R**					
$C_{10:1}/C_{10:0}$	0.106	0.117	0.131	0.116	0.01^{NS}	0.008^{NS}	0.007	0.007	NS
$C_{12:1}/C_{12:0}$	0.036	0.025	0.030	0.021	0.01^{NS}	0.008^{NS}	0.003	0.008	NS
$C_{14:1}/C_{14:0}$	0.088	0.091	0.098	0.089	0.01^{NS}	0.007^{NS}	0.013	0.007	*
$C_{16:1}/C_{16:0}$	0.047	0.051	0.056	0.055	0.004^{**}	0.003^{NS}	0.008	0.003	**
c-9 $C_{18:1}$:$C_{18:0}$	1.54	1.61	1.92	2.01	0.109^{***}	0.092^{**}	0.290	0.075	***
c-9 *t*-11$C_{18:2}$/*t*-11C $_{18:1}$	0.314	0.333	0.420	0.412	0.029^{***}	0.027^{NS}	0.049	0.023	*
t-11$C_{18:1}$	5.59	3.96	5.15	3.96	0.44^{NS}	0.43^{**}	1.694	0.291	***

[1] All significant regressions had positive slopes

and total CLA concentration.

DISCUSSION

The feeding of either ruminally protected oilseed or rapeseed, lowered concentrations of the fatty acids synthesised *de novo* ($C_{10} - C_{14}$), and C_{16}, which is partially synthesised *de novo* (Palmquist *et al*., 1993). Concentrations of the unsaturated derivatives of Δ^9-desaturase ($C_{10:1}$, $C_{12:1}$, $C_{14:1}$ $C_{16:1}$, *cis*-9 $C_{18:1}$, CLA and cis-9, trans-11 CLA) also declined. The only exceptions were CLA in Trial 1 and $C_{12:1}$ in Trial 2 but the trend (P=0.08) was also for $C_{12:1}$ to decline in concentration as a result of feeding oilseed. This gives a clear indication that *de novo* synthesis was inhibited by an increased supply to the mammary gland of long-chain fatty acids ($C_{18:0}$ and $C_{18:1}$) originating from the oilseed supplements (Palmquist *et al*., 1993). Ashes *et al*. (1992) reported a reduction of *de novo* synthesised fatty acids from feeding ruminally protected oilseed and Murphy *et al*. (1995) reported a similar result from feeding rapeseed. All product/substrate ratios, in Trials 1 & 2, dependent on Δ^9- desaturase, with the exception of $C_{12:1}/C_{12:0}$ in Trial 2, declined with feeding ruminally-protected oilseed. The decline in the saturated and *cis*-9 monounsaturated even-chain-length fatty acids from $C_{10:0}$ to $C_{16:1}$, and the decline in the ratios of the product/substrate pairs suggests that the activity of all the enzymes responsible for *de novo* fatty acid synthesis, i.e., fatty acid synthetase, acetyl CoA carboxylase, and Δ^9-desaturase, decreased with feeding ruminally protected oilseed.

Reduced *de novo* synthesis has been attributed to the presence, post-ruminally, of the *trans*-10, *cis*-12 CLA isomer (Baumguard *et al*., 2000). The identification that this isomer increased in milk fat from cows fed rapeseed may explain the reduced *de novo* synthesis resulting from feeding non-ruminally protected oilseed supplements.

No change in milk fat CLA concentrations occurred to feeding ruminally protected oilseed but the feeding rapeseed reduced the concentrations of *cis*-9, *trans*-11 CLA, total CLA and TVA in milk fat. Previous studies, Lawless *et al*. (1998), Griinari *et al*. (2000), Kay *et al*. (2002), and Ward *et al*. (2002) have all reported a high correlation between milk fat TVA and CLA concentrations. This is similar to the significant relationship reported in our study.

The concentration of *cis*-9, *trans* 11 CLA is dependent on two factors: the availability of TVA and the activity of Δ^9-desaturase. Corl *et al*. (2002) and Lock & Garnsworthy (2003) suggested that the ratio of *cis*-9 $C_{14:1}/C_{14:0}$ is the best indicator of Δ^9-desaturase activity. Patterson *et al*. (2002) reported strong correlations between $C_{14:1}/C_{14:0}$ and the other product/substrate ratios which they suggested confirmed the $C_{14:1}/C_{14:0}$ ratio as superior for predicting Δ^9- desaturase activity. In our study this ratio was unaffected by cow phenotype or by feeding rapeseed but was affected by feeding ruminally-protected oilseed. Milk fat CLA concentrations, however, responded differently to the effects of phenotype and feed on the product/substrate ratios. This gives support to the inconsistent association reported between the $C_{14:1}/C_{14:0}$ ratio and milk fat CLA concentration in each of the three experiments. Patterson et al. (2002); Corl *et al*. (2002) and Lock & Garnsworthy (2003) did not examine the correlation between the product/substrate ratio and total CLA concentrations. The occurrence of *cis*-9 $C_{18:1}$ in milk fat originates from either synthesis in the mammary gland through Δ^9-desaturation of $C_{18:0}$ or by preformed *cis*-9 $C_{18:1}$ entering the mammary gland from blood. Pollard *et al*. (1980) suggested that Δ^9-desaturase preferentially desaturates TVA, $C_{18:0}$ and $C_{16:0}$ compared to the shorter chain acids. Griinari *et al*. (2000) and Corl *et al*. (2001) suggested a similar order of selective desaturation of Δ^9-desaturase. With the increase in $C_{18:0}$ resulting from feeding oilseed, we speculate that to maintain fluidity of intercellular triglycerides (Timmen & Patton, 1988), Δ^9-desaturase acts preferentially on desaturating $C_{18:0}$. This suggests that less activity of Δ^9-desaturase is then available to desaturate the lower concentrations of the shorter-chain acids (e.g., $C_{14:0}$, $C_{12:0}$ and $C_{10:0}$).

We hypothesise that under standard dietary conditions the $C_{14:1}/C_{14:0}$ ratio may predict Δ^9-desaturase activity and assist in explaining to some degree the between-cow variation in milk fat CLA concentrations. However, in situations where the concentrations of the more-dominant fatty acids, $C_{18:0}$ and $C_{18:1}$ are increased, the use of the $C_{14:1}/C_{14:0}$ ratio as a predictor is of less value. The results of the three experiments examined, suggest that a better predictor of Δ^9-desaturase activity may be the product/substrate ratios of the longer-chain fatty acids, $C_{16:1}/C_{16:0}$ and *cis*-9 $C_{18:1}/C_{18:0}$. The counter-argument to this hypothesis is that the fatty acids making up the $C_{16:1}/C_{16:1}$ and $C_{18:1}/C_{18:0}$ ratios are influenced by negative energy balance (Palmquist *et al*., 1993) and diet, possibly confounding a clear interpretation of Δ^9-desaturase activity. However, given the consistently reported association between milk fat TVA and CLA concentrations, also found in Trial 3, between-cow variation in milk fat CLA concentrations may be better explained by differences in ruminal biohydrogenation processes than by product/substrate ratio predicators of mammary enzyme activity.

The analysis of three experiments in which groups of cows with two different phenotypes: hard and soft milkfat, were fed oilseeds showed cow phenotype had no effect on the concentration of the *cis*-9 mono-unsaturated fatty acids of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$ but did influence *cis*-9 $C_{18:1}$ and *cis*-9, *trans*-11 CLA concentrations. The feeding of oilseeds depressed *de novo* fatty acid synthesis, but had variable effects on the product/substrate ratios reported to predict Δ^9-desaturase activity. The *cis*-9 $C_{18:1}/C_{18:0}$ ratio was significantly associated ($P<0.001$) with milk fat CLA concentrations and from the data analysed, the assumption is made that this ratio may be the better predictor of Δ^9-desaturase activity than the more commonly used $C_{14:1}/C_{14:0}$ ratio. Concentrations of $C_{18:0}$ and *cis*-9 $C_{18:1}$ fatty acids are however influenced by factors exogenous to the mammary gland and the relationship found in Trial 3 between the *cis*-9 $C_{18:1}/C_{18:0}$ ratio and CLA may have been influenced more from these than the activity of Δ^9-desaturase. From this, we conclude that between-cow variability in the ruminal biohydrogenation of TVA, may better explain the between-cow variation in milk fat CLA

concentrations than variation in the activity of Δ^9-desaturase.

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