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Effects of dietary conjugated linoleic acid on production and metabolic parameters in transition dairy cows grazing fresh pasture

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Supplementation with a high dose (600 g/d) of rumen inert conjugated linoleic acids (RI-CLA) inhibits milk fat synthesis in total mixed ration (TMR)-fed dairy cows immediately *post partum*. However, effects of RI-CLA on milk fat and bioenergetic parameters during the transition period in grazing cows have not been investigated. Multiparous Holstein cows (n=39) grazing pasture were randomly assigned to one of three treatments: (1) pasture (PAS), (2) PAS+540 g/d Hyprofat (palm oil; HYPRO) and (3) PAS+600 g/d RI-CLA. HYPRO and RI-CLA supplements were isoenergetic, fed twice daily at 7.00 and 16.00 and provided 0 and 125 g CLA/d, respectively. Treatments began 27±10 d *prepartum* and continued until 36±1 days in milk (DIM). There was little or no overall effect of RI-CLA on content or yield of milk protein and lactose. RI-CLA supplementation decreased overall milk fat content and yield with RI-CLA-induced milk fat depression (MFD) becoming significant by day 3 when compared with PAS and by day 6 when compared with HYPRO. MFD continued to increase in severity during the first 24 d *post partum* after which MFD reached a plateau (~40%; RI-CLA *v.* HYPRO). Pasture-fed cows produced less milk (19·4 kg/d) than the lipid-supplemented groups and although there were no overall differences in milk yield between RI-CLA and HYPRO (22·3 kg/d) a curvilinear relationship (R^2=0·57) existed between the RI-CLA-induced milk yield response and extent of MFD. RI-CLA tended to increase milk yield (1·8 kg/d) compared with HYPRO until MFD exceeded 35% (~day 21), after which point the positive milk yield response was eliminated. Milk fat *trans*-10, *cis*-12 CLA content averaged 0·25 g/100 g in the RI-CLA treatment, was temporally independent, and was undetectable in PAS and HYPRO treatments. Based on the milk fat 14:1/14:0 ratio, RI-CLA decreased the overall Δ^9-desaturase system compared with PAS and HYPRO. Compared with HYPRO, RI-CLA had no effect on plasma glucose, insulin, leptin, or NEFA concentrations. Results indicate that a high RI-CLA dose decreases milk fat synthesis and tends to increase milk yield immediately *post partum* in pasture-fed cows; however, excessive MFD (>35%) appears to be associated with a diminished milk yield response.

Keywords: conjugated linoleic acid, milk fat depression, pasture, transition period.

Cows in early lactation typically cannot consume enough calories to meet the energetic requirements of maintenance and copious milk secretion, and consequently enter into negative energy balance (NEBAL). The severity, magnitude and day of NEBAL nadir (~4–9 days in milk (DIM) are closely associated with metabolic disorders and reproductive failures (Canfield & Butler, 1990; Drackley, 1999; Buckley et al. 2003). The impact of NEBAL on reproductive parameters is even more critical in strict pasture-based systems as calving patterns must coincide with forage availability to maintain sustainability (Rhodes et al. 2003). Attempts to improve or alleviate NEBAL traditionally involve increasing dietary energy density by the addition of concentrates or fats (Schingoethe & Casper, 1991; Hayirli & Grummer, 2004). However, the effectiveness of these dietary strategies is frequently inconsistent and is associated with potential drawbacks (*i.e.* acidosis

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and reduced dry matter intake (DMI), Hayirli & Grummer, 2004). An alternative is to decrease energy expenditure/output, and two approaches recently evaluated are once a day milking and reduced milk fat synthesis. Milk fat is the most energetically expensive component to synthesize (Bauman & Davis, 1974) and the most easily manipulated. Thus, governing it offers a unique opportunity to improve calculated net energy balance (EBAL). For conjugated linoleic acids (CLA) to improve transition success and reproductive indices it must be able to induce milk fat depression (MFD) immediately *post partum*.

Abomasally infusing CLA and supplementing rumen-inert (RI-CLA) reduces milk fat synthesis in cows consuming a total mixed ration (TMR) (Loor & Herbein, 1998; Chouinard et al. 1999b; Giesy et al. 2002; Perfield et al. 2002) or pasture (Medeiros et al. 2000; Mackle et al. 2003). However, CLA doses that are effective in established lactation (125–295 g RI-CLA/d) fail to induce MFD until 3–4 wks *post partum* (~15–20 d post NEBAL nadir; Giesy et al. 1999; Bernal-Santos et al. 2003; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; Gervais et al. 2005). Recently, it was demonstrated that CLA can inhibit milk fat synthesis immediately *post partum* in TMR-fed cows, but the amount of CLA required is markedly greater (600 g RI-CLA/d) than necessary in established lactation (Moore et al. 2004).

Study objectives were to determine whether a high dietary RI-CLA dose (600 g/d) could evoke MFD immediately *post partum* in rotationally grazed dairy cows and to evaluate the effects of CLA-induced MFD on bioenergetic parameters and production variables in early lactation.

Materials and Methods

Experimental design, animals and treatments

All procedures involving animals were approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand. Thirty-nine multiparous, dry and gestating Holstein-Friesian dairy cows were allocated to one of three treatments (13 cows/treatment) balanced for milk production and composition from the previous lactation, parity and predicted calving date. Treatments were (1) pasture only (PAS); (2) pasture+540 g/d Hyprofat (HYPRO; a palm fatty acid distillate; Bonimex BV, Rotterdam, The Netherlands); (3) pasture+600 g/d RI-CLA (RI-CLA; Bioproducts Inc., Fairlawn OH, USA). Lipid doses provided 520 g fatty acids/d and RI-CLA and HYPRO treatments were isoenergetic throughout the experiment. As cows were offered the same pasture allowance postcalving, the lipid-supplemented groups were provided with additional energy compared with the PAS treatment during lactation. Fatty acid composition of RI-CLA and Hyprofat are presented in Table 1. The RI-CLA supplement contained 24% CLA including *cis*-9, *trans*-11; *trans*-8, *cis*-10; *cis*-11, *trans*-13 CLA (isomers that do not alter mammary lipid metabolism, Baumgard et al. 2000; Perfield et al. 2004), and *trans*-10, *cis*-12 (20·9 g/d; an isomer which markedly reduces milk fat synthesis, Baumgard et al. 2000, 2002a) and other unidentified CLA isomers. To allow time for adaptation to the lipid supplement and the chance of early calving, treatments were initiated 27±10 d *prepartum* and continued for 36±1 d *post partum*. Lipid supplements were fed to both dry and lactating cows twice daily in the milking parlor at 7.00 and 16.00. To improve palatability and ensure complete consumption, 600 g RI-CLA and 540 g HYPRO were mixed with 300 g dried molasses and 300 g copra (a coconut extract) prior to feeding.

Table 1. Fatty acid composition of lipid supplements

Fatty acid	Hyprofat	RI-CLA
	(% of fatty acids)	
12 : 0	7·55	1·38
14 : 0	5·17	1·10
16 : 0	45·65	23·18
16 : 1 *cis*-9		0·21
18 : 0	4·02	3·19
18 : 1 *cis*-9	27·55	37·48
18 : 2 *cis*-9, *cis*-12	9·85	4·45
18 : 2 *trans*-8, *cis*-10 CLA		2·58
18 : 2 *cis*-9, *trans*-11 CLA		3·06
18 : 2 *trans*-10, *cis*-12 CLA		4·02
18 : 2 *cis*-11, *trans*-13 CLA		4·07
Other CLA		10·25
18 : 3 *cis*-9, *cis*-12, *cis*-15		0·15
20 : 0		0·13
Unknown	0·21	4·75

All cows freshened within a 21 d window and calves were weighed within 12 h of parturition and dam body weight and body condition score (BCS; 1–10 scale; Roche et al. 2004) were determined on each animal at calving and on day 36 by the same experienced assessor.

Grazing management and pasture measurements

All cows were rotationally grazed and offered a fresh allocation of pasture twice daily. Precalving, all cows grazed within the same paddock with treatments separated by a double-stranded electric fence to control pasture allowances. To maintain isoenergetic diets precalving (in an effort to ensure that the observed effects *post partum* were not confounded with precalving energetic states) different sized areas and consequently different pasture allocations were offered by multiplying pregrazing pasture mass/m^2 by desired allowance per cow (kg/d) by number of cows in each group. Pasture mass was calculated as described by Roche et al. (2005) and herbage disappearance rate (pasture offered less pasture remaining) was used to estimate average DMI as previously described (Roche et al. 2002). As a consequence pasture allowance was managed to provide 10, 8, and 8 kg DM/cow per d

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for PAS, HYPRO and RI-CLA treatments, respectively, and this resulted in precalving energy intakes of ~63 MJ/d (NE_L).

Postcalving, all cows grazed together in the same paddock and were offered an unrestricted pasture allowance of 45±8·3 kg DM/d. Individual DMI was estimated during week 4 of lactation, using the *n*-alkane technique (Mayes et al. 1986) as modified by Kennedy et al. (2003).

Throughout the entire experiment, representative pasture samples were collected daily by hand-plucking pasture to grazing height from paddocks immediately prior to grazing. Samples were bulked every 2 weeks, and triplicate samples were either frozen immediately (−20 °C) for fatty acid analysis, dried at 100 °C for DM analysis or dried at 60 °C for nutrient composition analysis. Nutrient composition samples were dried for 48 h, ground to pass through a 1·0-mm sieve (Christy Lab Mill, Suffolk, UK) and analysed for CP, NDF, ADF, soluble sugars, ash and organic matter digestibility (OMD) by near infra-red spectroscopy (Corson et al. 1999). Metabolizable energy (ME) was derived directly from predicted OMD, on the basis of an assay of cellulase digestibility *in vitro* (Roughan & Hollan, 1977; Dowman & Collins, 1982) which had been calibrated against *in vivo* standards (Corson et al. 1999).

Frozen feed samples for fatty acid analysis were freeze-dried (Cuddon instrument model 0610, Blenheim, NZ) and ground to pass through a 0·5-mm sieve. Pasture lipids were extracted and methylated according to the one-step method of Garces & Mancha (1993) as outlined in Kay et al. (2004). Fatty acid analysis was performed on a Hewlett Packard 5890 Series II GC equipped with a 30-m RTX-2330 column (30 m × 0·32 mm i.d. and 0·2 μm film thickness; Restek Corp) and GC conditions were as previously described (Kay et al. 2004).

Pasture offered precalving consisted of 82% perennial ryegrass (*Lolium perenne* L.), 3% white clover (*Trifolium repens* L.), 1% weeds and other grasses (*Dactylus glomerata*, *Holcus lanatus* and some *Poa* species) and 14% dead material on a DM basis. Postcalving pasture sward consisted of 81% perennial ryegrass (*Lolium perenne* L.), 5% white clover (*Trifolium repens* L.), 6% weeds and other grasses (*Dactylus glomerata*, *Holcus lanatus* and some *Poa* species) and 8% dead material on a DM basis. Pre and postcalving pasture nutritive and fatty acid composition is presented in Table 2.

Table 2. Chemical and fatty acid composition of pasture offered

Chemical analysis, % of DM unless stated otherwise	*Prepartum*	*Post partum*
CP	19·2	25·3
NDF	43·6	36·1
ADF	23·9	18·2
Ash	10·3	10·3
Soluble sugars	9·2	11·6
Total fatty acids	3·6	4·1
ME (MJ/kg of DM)	10·5	11·7
Fatty acids (g/100 g total fatty acids)		
16 : 0	13·0	10·3
16 : 1 *cis*-9	2·5	2·5
18 : 0	2·7	2·0
18 : 1 *cis*-9	3·5	3·9
18 : 2 *cis*-9, *cis*-12	12·5	9·2
18 : 3 *cis*-9, *cis*-12, *cis*-15	55·5	57·3
Unknown	10·3	14·8

Milk and plasma collection and analyses

Cows were milked twice daily at 6.00 and 17.00. Individual yields were recorded daily (Westfalia Surge, Oelde, Germany) and samples obtained from each cow on day 3 and every third day (±1) until 36 d *post partum*. One aliquot from each sampling was analysed for fat, lactose and protein using an infrared milk analyser (FT120; Foss Electric, Hillerød, Denmark). Milk fat was extracted from a second aliquot from days 3, 9, 15, 21, 27 and 36 (±1) using the Röese-Gottlieb fat extraction procedure (IDF, 1987) and stored at −20 °C until analysed for fatty acid composition.

Fatty acid methyl esters were prepared by the transmethylation procedure described by Christie (1982) with modifications (Chouinard et al. 1999a). Fatty acid methyl esters were quantified using a GC (Hewlett Packard GC system 6890; Wilmington DE, USA) equipped with a flame ionization detector and a CP-7489 fused silica capillary column (100 m × 0·25 mm i.d. with 0·2 μm film thickness; Varian, Walnut Creek CA, USA). GC oven parameters, gas variables, and fatty acid peak identification were as previously described (Kay et al. 2004; Moore et al. 2004).

One evacuated blood tube containing a sodium heparin pellet (100 i.u. sodium heparin/ml blood) to prevent coagulation was collected from each cow by coccygeal venipuncture prior to treatment initiation (~day 27±10 precalving), ~day 17±10 precalving, day of calving and days 1, 2, 3, 4, 7, 14, 28 and 35 *post partum*. Plasma was harvested following centrifugation at 2800 ***g*** at 4 °C for 12 min and analysed for NEFA, glucose, leptin and insulin. NEFA (colorimetric method) and glucose (hexakinase method) analyses were conducted by Alpha Scientific, Hamilton, NZ with inter-assay and intra-assay CV <2% for all assays. Insulin and leptin were measured in duplicate by double-antibody RIA as previously described by Hales & Randle (1963) and Blache et al. (2000), respectively.

Calculations

Net energy balance (EBAL) was calculated during week 4 of lactation (days 21–28) using the following equation; EBAL = net energy intake − (net energy for maintenance + net energy for lactation). Net energy intake was calculated by multiplying the *n*-alkane estimated DMI by the net energy of pasture (6·45 MJ/kg DM) plus the net energy value of the lipid supplement (21·01 MJ/kg DM; NRC, 2001); molasses

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Table 3. Effect of supplementing Hyprofat and rumen inert-conjugated linoleic acid for 27±10 d precalving to 36±1 d postcalving on milk production parameters of pasture-fed dairy cows†

	Treatments‡				*P*		
	PAS	HYPRO	RI-CLA	SEM	TRT	DAY	TRT × DAY
Milk yield							
kg/d	19·4[a]	22·5[b]	22·1[b]	0·7	0·01	<0·01	0·02
Milk fat							
%	5·12[a]	4·76[b]	3·35[c]	0·15	<0·01	<0·01	<0·01
g/d	998[a]	1045[a]	738[b]	37	<0·01	0·06	0·04
Milk protein							
%	3·68	3·54	3·60	0·05	0·08	<0·01	0·03
g/d	720	790	794	37	0·30	0·04	0·49
Milk lactose							
%	4·89[a]	4·85[a]	5·00[b]	0·03	0·01	<0·01	0·03
g/d	962	1102	1115	56	0·12	<0·01	0·28

† Average over the 36-d *post partum* period
‡ Treatments were pasture only (PAS); pasture+540 g/d Hyprofat (HYPRO); and pasture+600 g/d RI-CLA (RI-CLA)
[a,b,c] Values within rows with different superscripts indicate $P<0·05$

(7·37 MJ/kg DM; NRC, 2001) and copra (7·75 MJ/kg DM; Miller, 1979). Digestibility and absorbability was assumed to be similar between the two fat supplements. Net energy for maintenance was calculated according to NRC (2001) using the following equation: net energy for maintenance = 0·06 × body weight$^{0·75}$ (Holmes et al. 2002). Net energy for lactation was calculated according to NRC (2001) by the following equation: net energy for lactation = ((0·0929 × fat %)+(0·0547 × crude protein %)+(0·0395 × lactose %)) × milk production.

Statistical Analysis

Milk and plasma data were analysed by repeated measures using the PROC MIXED procedure of SAS (2001) with an autoregressive covariance structure and day of lactation as the repeated effect. The model contained day of lactation, treatment and day of lactation × treatment interactions. Cows were the random effect, and day of lactation, treatment and day of lactation × treatment interaction were the fixed effects. DMI and EBAL were analysed using the PROC MIXED procedure of SAS (2001), with treatment as the dependent variable and did not contain a time or repeated component. The milk yield *v.* MFD relationship was analysed using the PROC CORR procedure of SAS (2001) using RI-CLA and HYPRO treatment means from days 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33 and 36 *post partum*. The model contained percent change in milk yield and percent decrease in milk fat content. SEM are reported and differences considered significant when $P<0·05$ unless otherwise stated.

Results

RI-CLA supplementation decreased ($P<0·01$) overall milk fat content and yield, with RI-CLA-induced MFD becoming

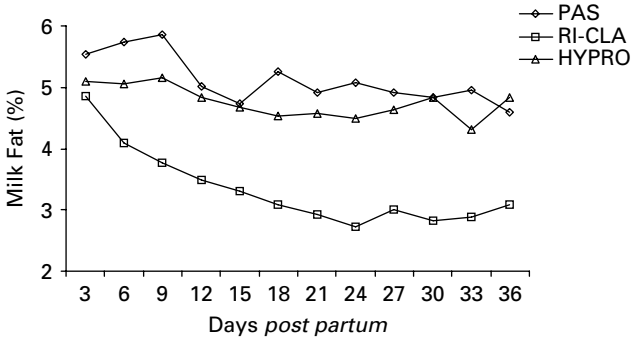

Fig. 1. Effects of supplementing Hyprofat and rumen inert-conjugated linoleic acid for 27±10 d precalving to 36±1 d postcalving on temporal pattern of milk fat content. Treatments were pasture only (PAS); pasture+540 g/d Hyprofat (HYPRO); and pasture+600 g/d rumen inert-conjugated linoleic acid (RI-CLA). Values represent least squares means (n=13/treatment); SEM averaged 0·25 and ranged from 0·23 to 0·35

significant ($P<0·05$; 13%) by day 3 when compared with PAS and by day 6 ($P<0·01$; 19%) when compared with HYPRO (Table 3; Fig. 1). There was no overall RI-CLA treatment effect on milk protein content, nor protein or lactose yield. Compared with both PAS and HYPRO treatments, RI-CLA slightly increased ($P<0·01$; 0·13 percentage units) lactose content. There was no overall difference in milk yield between HYPRO and RI-CLA treatments (22·3 kg/d); however, cows from both lipid supplement treatments produced more ($P<0·01$) milk than the PAS treatment (19·4 kg/d) during the first 36 DIM (Table 3). There was a milk yield, treatment × day of lactation interaction ($P<0·02$; Table 3) with RI-CLA tending ($P<0·13$) to produce more milk (1·8 kg/d) than HYPRO for the first 20 d *post partum*, at which point RI-CLA milk yield declined and HYPRO cows tended ($P<0·12$) to produce more milk

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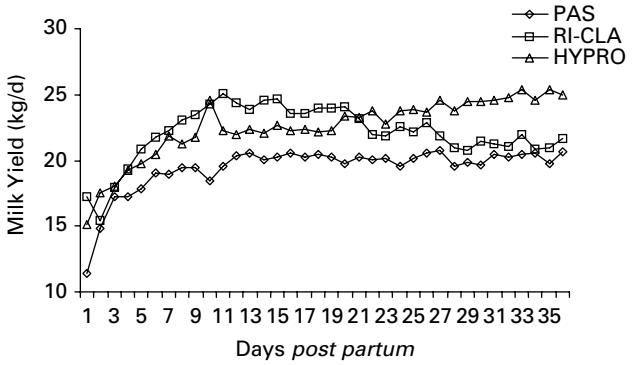

Fig. 2. Effects of supplementing Hyprofat and rumen inert-conjugated linoleic acid for 27±10 d precalving to 36±1 d postcalving on temporal pattern of milk yield during first 36 d *post partum*. Treatments were pasture only (PAS); pasture+540 g/d Hyprofat (HYPRO); and pasture+600 g/d rumen inert conjugated linoleic acid (RI-CLA). Values represent least squares means (n=13/treatment); SEM averaged 1·31 and ranged from 1·27 to 1·40

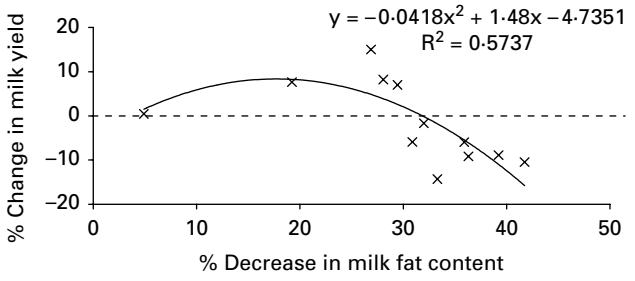

Fig. 3. Relationship between milk yield and milk fat content in cows supplemented with Hyprofat compared with cows supplemented with rumen inert-conjugated linoleic acid for 27±10 d precalving to 36±1 d postcalving. Data points represent differences in treatment means from samples collected on days 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33 and 36 *post partum*. Treatments were pasture+540 g/d Hyprofat (HYPRO); and pasture+600 g/d rumen inert conjugated linoleic acid (RI-CLA).

(2·5 kg/d) for the remainder of the 36-d experimental period (Fig. 2).

RI-CLA-induced MFD continued to become more severe during the first 24 d *post partum* at which point it reached a plateau (~40%; RI-CLA *v.* HYPRO; Fig. 1). A trend for a curvilinear relationship (R^2=0·57; P<0·07) existed between the milk fat content and milk yield response in RI-CLA *v.* HYPRO cows (Fig. 3). Milk yield tended to increase (1·8 kg/d; P<0·09) when MFD was moderate (<35%); however, as MFD exceeded 35% (~21 DIM) the positive milk yield response declined.

Post-calving pasture DMI, estimated by the *n*-alkane method during week 4 of lactation (days 21–28), was not affected by treatment (11·4±0·8 kg/d; Table 4); thus cows supplemented with lipid (HYPRO and RI-CLA) had a higher net energy intake (88 MJ/d; NE_L) than PAS cows (75 MJ/d; NE_L). During week 4, dietary RI-CLA

Table 4. Effects of supplementing Hyprofat and rumen inert-conjugated linoleic acid for 27±10 d precalving to 36±1 d postcalving on energetic variables of pasture-fed dairy cows

	Treatments†				
	PAS	HYPRO	RI-CLA	SEM	*P*
DMI‡ (kg)	11·3	11·0	12·0	0·8	0·77
Body weight (kg)					
d 0	456	454	452	16	0·99
d 36	398	409	391	15	0·72
BCS§ (1–10)					
d 0	4·7	4·5	4·7	0·2	0·70
d 36	3·9	4·0	3·9	0·2	0·94
Net EBAL¶ (MJ)	−20·47[a]	−7·29[b]	3·89[b]	4·19	<0·01
Calf body weight (kg)	36	37	37	1	0·63

†Treatments were pasture only (PAS); pasture+540 g/d Hyprofat (HYPRO); and pasture+600 g/d RI-CLA (RI-CLA)
‡Mean dry matter intake calculated *via n*-alkane technique from 21–28 DIM
§Body condition score
¶Mean net EBAL=net energy intake−(net energy for maintenance+net energy for lactation): calculated from 21–28 DIM
[a,b,c]Values within rows with different superscripts indicate P<0·05

supplementation improved calculated net EBAL (Table 4) when compared with PAS (P<0·01; 3·89 *v.* −20·47 MJ/d) and tended to improve EBAL compared with HYPRO treatment (P<0·09; 3·89 *v.* −7·29 MJ/d). Treatment had no effect (Table 4: P>0·60) on calf body weight (37 kg) nor on BCS or body weight at calving (4·6 and 454 kg, respectively) or at 36 DIM (3·9 and 399 kg, respectively).

Milk fat concentration of most short- and medium-chain fatty acids (6:0–16:1) were reduced by RI-CLA supplementation (Table 5). There was an overall decrease (P<0·01) in content of fatty acids synthesized *de novo* (sum 4:0–15:0) and a corresponding increase (P<0·01) in preformed fatty acids (sum 17:0–20:0). On a molar basis, the decrease in fatty acid synthesis was primarily (>65%; day 36) due to a decrease in fatty acids synthesized *de novo* (Fig. 4). In the control treatments (PAS and HYPRO) the contribution of fatty acids divided into origin; *de novo* and preformed (sum 16:0+16:1) and preformed were temporally independent during the 36-d period (Fig. 4).

Milk fat concentration of all measured CLA isomers, except *cis*-9, *trans*-11 CLA, increased (P<0·01) with RI-CLA supplementation (Table 5). Milk fat *trans*-10, *cis*-12 CLA content averaged 0·25 g/100 g in the RI-CLA treatment, was temporally independent, and was undetectable in PAS and HYPRO treatments (Fig. 5). Based on the overall milk fat 14:1/14:0 ratio, RI-CLA supplementation decreased the Δ^9-desaturase system compared with PAS and HYPRO (Table 5) but the extent of inhibition did not change over time (results not presented).

There were no overall treatment effects on *prepartum* (results not presented) or *post partum* plasma glucose, insulin or leptin levels (Table 6). Irrespective of treatment there was a day effect (P<0·01) on glucose, insulin, leptin

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Table 5. Effect of supplementing Hyprofat and rumen inert-conjugated linoleic acid for 27 ± 10 d precalving to 36 ± 1 d post calving on milk fatty acid composition of pasture-fed dairy cows†

	Treatments‡				*P*		
Fatty acids	PAS	HYPRO	RI-CLA	SEM	TRT	DAY	TRT × DAY
	g/100 g of fatty acids						
4 : 0	2·91	2·98	2·80	0·13	0·64	<0·01	<0·01
6 : 0	2·72[a]	2·63[a]	2·02[b]	0·10	<0·01	<0·01	<0·01
8 : 0	1·33[a]	1·26[a]	0·87[b]	0·05	<0·01	<0·01	<0·01
10 : 0	2·68[a]	2·52[a]	1·70[b]	0·14	<0·01	<0·01	<0·01
12 : 0	2·85[a]	2·78[a]	2·00[b]	0·14	<0·01	<0·01	<0·01
14 : 0	8·87[a]	8·17[a]	6·74[b]	0·31	<0·01	<0·01	<0·01
14 : 1 *cis*-9	0·56[a]	0·45[c]	0·23[b]	0·02	<0·01	<0·01	<0·01
15 : 0	0·80[a]	0·73[ab]	0·70[b]	0·03	0·05	0·18	0·08
16 : 0	25·73[a]	27·84[c]	23·28[b]	0·35	<0·01	<0·01	0·04
16 : 1 *cis*-9	1·48[a]	1·44[a]	1·06[b]	0·66	<0·01	0·02	0·31
17 : 0	0·67[a]	0·58[b]	0·60[b]	0·02	<0·01	<0·01	0·31
18 : 0	13·31[a]	12·66[a]	15·09[b]	0·43	<0·01	0·24	0·30
18 : 1 *cis*-9	24·93	24·23	26·05	0·84	0·32	<0·01	<0·01
18 : 1 *trans*-6-8	0·25[a]	0·29[c]	0·46[b]	0·01	<0·01	<0·01	<0·01
18 : 1 *trans*-9	0·19[a]	0·23[c]	0·42[b]	0·01	<0·01	<0·01	<0·01
18 : 1 *trans*-10	0·25[a]	0·30[a]	0·58[b]	0·02	<0·01	<0·01	0·29
18 : 1 *trans*-11	2·95	3·38	3·70	0·21	0·06	<0·01	0·11
18 : 1 *trans*-12	0·31[a]	0·33[a]	0·60[b]	0·02	<0·01	<0·01	0·15
18 : 2 *cis*-9, *cis*-12	1·08[a]	1·23[a]	1·79[b]	0·05	<0·01	<0·01	<0·01
18 : 2 *trans*-7, *cis*-9	<0·01[a]	<0·01[a]	0·04[b]	0·01	<0·01	0·50	0·56
18 : 2 *trans*-8, *cis*-10 CLA	0·03[a]	<0·01[a]	0·16[b]	0·01	<0·01	<0·01	<0·01
18 : 2 *cis*-9, *trans*-11 CLA	0·77	0·89	0·89	0·06	0·31	<0·01	0·09
18 : 2 *trans*-10, *cis*-12 CLA	<0·01[a]	<0·01[a]	0·25[b]	0·01	<0·01	0·19	0·13
18 : 2 *cis*-11, *trans*-13 CLA	<0·01[a]	<0·01[a]	0·36[b]	0·02	<0·01	<0·01	<0·01
Other CLA	0·18[a]	0·17[a]	2·12[b]	0·09	<0·01	0·06	0·06
18 : 3 *cis*-9, *cis*-12, *cis*-15	1·01[ab]	0·87[b]	1·13[a]	0·04	<0·01	0·31	0·14
20 : 0	0·08[a]	0·09[a]	0·11[b]	0·01	<0·01	<0·01	0·16
Unknown§	4·11	4·00	4·29	0·15	0·39	<0·01	<0·01
Fatty acid origin							
de novo¶	22·75[a]	21·50[a]	17·04[b]	0·77	<0·01	<0·01	<0·01
16 : 0 & 16 : 1	31·27[a]	33·27[c]	28·62[b]	0·34	<0·01	<0·01	<0·01
Preformed††	45·99[a]	45·24[a]	54·36[b]	0·90	<0·01	<0·01	<0·01
Δ^9-desaturase ratio							
14 : 1/14 : 0	0·06[a]	0·06[a]	0·04[b]	<0·01	<0·01	<0·01	0·50

† Average over the 36-d *post partum* period
‡ Treatments were pasture only (PAS); pasture+540 g/d Hyprofat (HYPRO); and pasture+600 g/d RI-CLA (RI-CLA)
§ Represents unidentified fatty acids
¶ Sum 4 : 0–15 : 0
†† Sum 17 : 0–20 : 0
[a,b,c] Values within a row without a common superscript indicate $P<0·05$

and NEFA levels as these variables demonstrated the expected temporal patterns of transitioning cows (results not presented). *Post partum* plasma NEFA levels were higher ($P<0·05$) in the PAS cows compared with HYPRO and RI-CLA treatments but did not differ between the two lipid treatments (Table 6).

Discussion

During the periparturient period, energy intake is often insufficient to meet the energy demands of maintenance and milk synthesis, and dairy cows typically go into NEBAL (Drackley, 1999). The duration, magnitude and days to NEBAL nadir are associated with reduced milk yield, prolonged days to first ovulation, acyclicity and subsequent reproductive failures (Canfield & Butler, 1990; Beam & Butler, 1999; Buckley et al. 2003). Dairy cows grazing pasture can experience additional dietary stress during the transition period as adverse weather conditions (drought, flooding etc.) can further restrict pasture availability, thus amplifying the severity and/or duration of NEBAL. Furthermore, pasture dairy systems are primarily based

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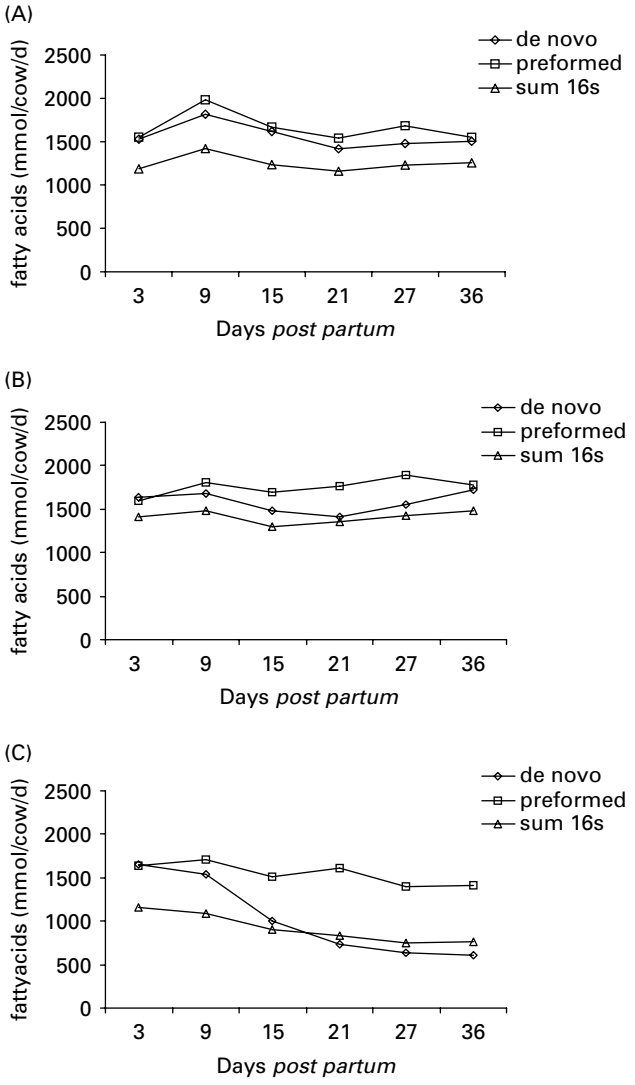

Fig. 4. Temporal pattern of fatty acid contribution on a molar basis, divided into orgin; *de novo* (sum 4:0–15:0), *de novo* and preformed (sum 16:0+16:1) and preformed (sum 17:0–20:0) for (A) pasture only (B) Hyprofat and (C) rumen-inert-conjugated linoleic acid supplemented for 27±10 d pre-calving to 36±1 d postcalving. Treatments were pasture only (PAS); pasture+540 g/d Hyprofat (HYPRO) and pature+600 g/d rumen-inert conjugated linleic acid (RI-CLA).

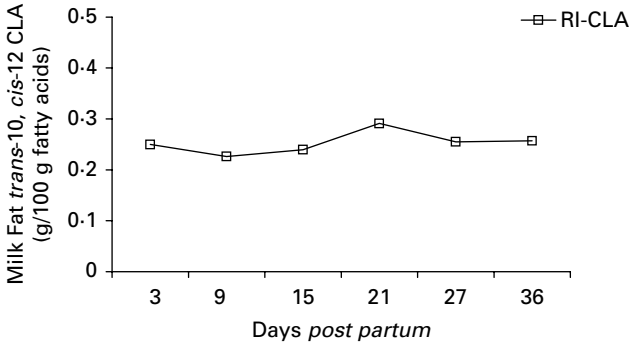

Fig. 5. Effects of supplementing rumen inert-conjugated linoleic acid and Hyprofat for 27±10 d precalving to 36±1 d post-calving on temporal pattern of milk fat *trans*-10, *cis*-12 CLA content in pasture-fed dairy cows. Treatments were pasture only (PAS); pasture+540 g/d Hyprofat (HYPRO) and pasture+600 g/d rumen-inert conjugated linoleic acid (RI-CLA). Milk fat *trans*-10, *cis*-12 CLA content was undetectable in PAS and HYPRO treatments. Values for RI-CLA treatment represent least squares means (n=13); SEM averaged 0·16 and ranged from 0·12 to 0·19.

on seasonal calving patterns and to attain a seasonal 12-month calving interval, pasture-fed cows need to conceive by 85 d *post partum* (Rhodes et al. 2003). This magnifies the importance of alleviating NEBAL as soon as possible following parturition.

Feeding RI-CLA during established lactation decreases milk fat content and yield (Loor & Herbein, 1998, 2003; Chouinard et al. 1999a, b; Perfield et al. 2002; Mackle et al. 2003). However, feeding similar levels of RI-CLA during the transition period or early lactation indicates CLA is ineffective at reducing milk fat synthesis until ~weeks 3–4 of lactation in both TMR and pasture-fed cows (Mederios et al. 2000; Bernal-Santos et al. 2003; Castandea-Gutierrez et al. 2005; Gervais et al. 2005). In order for supplemental CLA to be used as a management tool to improve EBAL parameters in grazing cows as we hypothesized, it must reduce milk fat synthesis immediately *post partum* (*i.e.* 1–7 DIM), when NEBAL is most severe. Recently we demonstrated that CLA inhibits milk fat synthesis immediately *post partum* in TMR-fed cows (Moore et al. 2004) but a much larger dose (~3×) is required. Results presented here confirm that this also applies to grazing dairy cows. Milk fat content was significantly reduced by RI-CLA by 3 (13%) and 6 (19%) DIM compared with cows grazing pasture only or cows supplemented with Hyprofat, respectively. MFD severity increased as lactation progressed and maximum MFD (40 and 46%, compared with PAS and HYPRO, respectively) occurred on day 24 *post partum*. Consistent with the decrease in milk fat content, overall milk fat yield was reduced by RI-CLA treatment (26 and 29% compared with PAS and HYPRO, respectively).

In agreement with previous CLA-induced MFD trials in established lactation (Loor & Herbein, 1998, 2003; Chouinard et al. 1999a, b; Baumgard et al. 2000, 2002b; Perfield et al. 2002; Mackle et al. 2003) and early lactation (Moore et al. 2004; Castandea-Gutierrez et al. 2005) inhibition of milk fat synthesis was primarily due to a decrease in production of fatty acids synthesized *de novo* (Table 5; Fig. 4). In the present study, on a molar basis, yield of all fatty acids decreased but reduction in *de novo* derived fatty acids on day 36 accounted for more than 65% of the reduction in total milk fat. In addition, the temporal pattern for the decrease in *de novo* derived fatty acids (Fig. 4c) closely mimicked the reduction in milk fat

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Table 6. Effect of supplementing Hyprofat and rumen inert-conjugated linoleic acid for 27±10 d precalving to 36±1 d postcalving on blood metabolites and hormones in pasture-fed dairy cows†

	Treatments‡				*P*		
	PAS	HYPRO	RI-CLA	SEM	TRT	DAY	TRT × DAY
Glucose (mmol/l)	3·42	3·40	3·36	0·06	0·75	<0·01	0·04
Insulin (ng/ml)	0·28	0·33	0·30	0·02	0·38	<0·01	0·14
Leptin (ng/ml)	0·98	1·00	1·05	0·05	0·62	<0·01	0·48
NEFA (mmol/l)	0·81[a]	0·62[b]	0·60[b]	0·05	0·02	<0·01	0·10

† Average over the 36-d *post partum* period
‡ Treatments were pasture only (PAS); pasture+540 g/d Hyprofat (HYPRO); and pasture+600 g/d RI-CLA (RI-CLA)
[a,b,c] Values within rows with different superscripts indicate $P<0·05$

content (Fig. 1) and further establishes the central role of fatty acid synthesis *de novo* in CLA-induced MFD.

The reason CLA is less effective at reducing milk fat synthesis immediately *post partum* is not clear, but as MFD was primarily due to a reduction in fatty acid synthesis *de novo* (especially with extensive MFD; Baumgard et al. 2001, 2002b) we speculated that the mammary gland's decreased sensitivity to CLA immediately *post partum* may be due to reduced contribution of synthesis of fatty acids *de novo* during this period. However, our results indicate that fatty acid production from different origins (*de novo v.* preformed) in the control treatments (PAS and HYPRO) did not change appreciably during the first 36 DIM (Fig. 4). Thus, this offers little support for our aforementioned hypothesis. Alternatively, due to the high plasma NEFA levels associated with NEBAL during the transition period (Table 6) we speculated that due to NEFA competition, mammary epithelial CLA uptake may be reduced, and this might explain why CLA is less effective at decreasing milk fat synthesis immediately *post partum*. However, milk fat *trans*-10, *cis*-12 CLA content (and thus mammary gland *trans*-10, *cis*-12 CLA uptake) was similar at 1 DIM to that at 36 DIM (Fig. 5) even though MFD became more severe during this time frame. Furthermore, the transfer efficiency rate (from dietary supplement to milk fat) of *trans*-10, *cis*-12 CLA was approximately 9% and this did not appreciably change (±2·8) during the first 36 DIM (results not shown). Thus, the biological reasons why CLA is less effective in early lactation remain unanswered, but it certainly is not due to a lack of mammary CLA uptake.

Treatment had no effect on pasture DMI, estimated using the *n*-alkane technique, during week 4 of lactation (11·4 kg DM/cow per d) and this agrees with previous TMR CLA transition (Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004) and pasture-based trials (Mederios et al. 2000; Mackle et al. 2003). Although both lipid-supplemented treatments markedly improved calculated EBAL compared with PAS, as a consequence of a similar pasture DMI, consuming additional energy *via* lipid supplement and severely decreasing milk fat yield, RI-CLA treated cows had a much higher (>23·0 MJ/d; Table 4) calculated EBAL than PAS cows. Compared with HYPRO, CLA-supplemented cows tended to increase (>10·5 MJ/d) calculated EBAL, which can be directly attributed to MFD as these cows were producing similar volumes of milk and consuming similar quantities of feed during this portion of the trial (21–28 DIM; Table 4, Fig. 1). The improved calculated EBAL parameters agree with previous research indicating that high dietary RI-CLA doses numerically improved calculated EBAL during weeks 2 and 3 of lactation and significantly reduced days to EBAL nadir (Moore et al. 2004).

The improved calculated EBAL compared with PAS was corroborated by the reduction (26%) in circulating NEFA levels (Table 6), which are thought to reflect EBAL (Bauman et al. 1988). We also expected that CLA-supplemented cows would have decreased NEFA concentration compared with HYPRO, but this was not the case (Table 6). However, this agrees with previous research (Moore et al. 2004; Castandea-Gutierrez et al. 2005) and a reason for the lack of effect is not clear, as reducing energy output without altering other components of the EBAL equation should theoretically reduce the demand to mobilize adipose reserves. However, adipose-derived NEFA contribute only about ∼10% to the total milk fat preformed pool (Pullen et al. 1989) and even if this was slightly underestimated in early lactation, detecting differences in circulating NEFA due to CLA-induced MFD may be difficult.

As expected due to additional energy intake, both lipid-supplemented treatments produced more overall milk compared with PAS. Although there was no overall milk yield difference between HYPRO and RI-CLA treatments (Table 3), a curvilinear relationship existed between severity of MFD and positive milk yield response. RI-CLA cows tended to produce more milk (1·8 kg/d) during the first 20 d *post partum* when MFD was moderate (<35%); however, as MFD became more severe (>35%, ∼day 21) the response was eliminated and RI-CLA cows tended to produce less milk (2·5 kg/d) than HYPRO fed cows during the remainder of the trial (Fig. 1). The increase is similar to previous reports indicating RI-CLA cows had higher milk yields in early lactation (Geisy et al. 1999; Bernal-Santos et al. 2003). This suggests that during a time of energy deficiency (*i.e.* early lactation), moderate inhibition of milk fat synthesis may spare energy that is then partitioned for increased milk yield; however, severe MFD may adversely affect cellular mechanisms involved in milk synthesis and/or secretion. The curvilinear response in milk yield is similar to a CLA dose-response trial (Mackle et al. 2003), which demonstrated an increase in milk yield with moderate CLA-induced MFD, but no milk yield response with a high CLA dose, an amount that caused extensive MFD in

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pasture-fed dairy cows. Similarly, in a CLA dose trial using TMR-fed cows (Chouinard et al. 1999a), high CLA doses that resulted in severe MFD reduced milk yield by almost 3 kg/d. Furthermore, Bell & Kennelly (2003) reduced milk yield by almost 40% when they abomasally infused a CLA dose 4-fold higher than necessary to evoke 40% MFD (Baumgard et al. 2001). Therefore, although the CLA dose did not change during the present study, the milk yield response appeared to follow a similar pattern to the aforementioned trials with milk yield response adversely affected by severe MFD (Fig. 3). The reason why severe MFD appears to adversely affect milk yield is unclear. We had previously hypothesized that Δ^9-desaturase (responsible for adding a *cis* double bond to the 9th carbon in fatty acid chains and thus a major regulator of cell membrane fluidity; Ntambi, 1995) which is inhibited during extensive MFD (Chouinard et al. 1999a; Perfield et al. 2002; Loor & Herbein, 2003; Mackle et al. 2003) may decrease membrane fluidity to such an extent as to adversely affect cellular functions and inhibit milk secretion. However, although overall Δ^9-desaturase activity/expression (based on the 14 : 1/14 : 0 milk fat ratio) was inhibited by RI-CLA in the present study, inhibition was detected by day 3 *post partum* and the extent of inhibition did not increase as lactation progressed (results not presented) even though MFD became more severe and milk yield declined. However, the milk yield response is also confounded with DIM and days on treatment and although neither of these parameters has been associated with adverse milk yield effects in previous CLA research (Perfield et al. 2002; Bernal-Santos et al. 2003; Selberg et al. 2004; Gervais et al. 2005) we currently cannot eliminate the possibility that they influenced our results.

Although supplemental CLA has been shown to improve glucose homeostatic parameters in diabetic models, it paradoxically sometimes causes insulin resistance in non-diabetic animal models (see review by Brown & McIntosh, 2003). Supplemental RI-CLA had no effect on either circulating glucose or insulin in this trial (Table 6). The lack of a CLA effect on the aforementioned variables is similar to other transition and long-term lactation trials (Perfield et al. 2002; Castaneda-Gutierrez et al. 2005) and short-term experiments evaluating metabolic responses to homeostatic signals (Baumgard et al. 2000, 2001, 2002a). The fact that neither CLA nor HYPOR causes insulin resistance or disturbs bioenergetic set points (even during extensive MFD) suggest that they may possibly be useful and safe management tools to manipulate whole animal energy status during the transition period.

The CLA supplement in the current study provided 20·9 g of *trans*-10, *cis*-12 CLA/d (an isomer known to cause MFD; Baumgard et al. 2000, 2002b) and utilizing established equations (de Veth et al. 2004) we calculated that ~7 g of *trans*-10, *cis*-12 CLA escaped biohydrogenation and was available for intestinal absorption. This represents a transfer efficiency of ~9% which is higher (~2·5×) than previous reports on TMR-fed cows in established lactation (Perfield et al. 2002) and slightly higher than in early lactation (Moore et al. 2004). Nonetheless, it appears that (similar to TMR experiments) a majority of the RI-CLA supplement is hydrogenated and partially metabolized in the rumen and this is supported by the consistent increase in milk trans-18 : 1 monoenes (putative products of incomplete CLA hydrogenation) from cows fed RI-CLA (Table 5; Perfield et al. 2002; Moore et al. 2004).

Conclusion

The present study demonstrates that a high dietary RI-CLA dose (124·7 g CLA/d) reduces milk fat synthesis immediately *post partum* and may be useful as a management tool to alleviate NEBAL in rotationally grazed dairy cows. Moderate MFD appeared to cause a positive response in milk yield; however, as lactation progressed and MFD became more severe, the positive milk yield response diminished. As lactation progresses, the mammary gland appears to become more sensitive to CLA; however, the biological mechanism behind this remains unclear. Further research is required to determine why the mammary gland demonstrates decreased sensitivity to CLA immediately *post partum* and why severe MFD appears to adversely affect milk yield. This study also demonstrates that HYPRO supplementation (540 g/cow per d) may provide a potential management tool to alleviate NEBAL in grazing dairy cows during early lactation. Cows supplemented with HYPRO had improved calculated net EBAL and produced more milk than cows consuming pasture only. Longer-term effects of HYPRO supplementation on milk production and reproductive parameters and economic analysis require further investigation.

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