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Effects of conjugated linoleic acid on bioenergetic and milk production parameters in grazing dairy cows offered *ad libitum* or restricted pasture

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ABSTRACT

Conjugated linoleic acids (CLA) reduce milk fat synthesis in grazing dairy cows and often improve net energy balance (EBAL). Study objectives were to determine if CLA-induced milk fat depression (MFD) could be utilised during times of feed shortage (i.e drought) to improve bioenergetic and milk production parameters. Twelve multiparous mid-lactation rumen-fistulated Holstein cows were offered *ad libitum* (AL) or restricted (R) pasture and abomasally infused twice daily with 0 (0) or 50 (50) g/d CLA in a cross over design. Treatment periods lasted 10 d and were separated by a 10-d washout period. Milk and plasma samples were averaged from d 9 and 10, and EBAL was calculated from d 6-10, of the infusion periods. Pasture restriction reduced ($P<0.05$) the yield of milk and milk components. CLA reduced ($P<0.01$) milk fat yield by 45 and 46 % in AL and R, respectively. There was no effect of CLA on milk yield nor milk lactose content or yield, however milk protein content increased ($P<0.05$) in both AL and R, resulting in an increased ($P<0.05$) protein yield of 6 and 9% respectively. The CLA-induced changes to milk fat and protein increased ($P<0.01$) protein:fat ratio by approximately 2-fold in both AL and R. Calculated EBAL improved following CLA infusion (-1.84 vs. 11.22 and 1.59 vs. 13.77 MJ/cow/d for AL and R respectively; $P<0.05$), however CLA did not alter plasma bioenergetic markers (including insulin, non-esterified fatty acids, β-hydroxybutyrate, urea and glucose). Data indicate that during short periods of nutrient restriction, CLA may provide an alternative management tool to improve milk protein:fat ratio and calculated EBAL, however further studies are required to determine if CLA is effective at improving bioenergetic parameters during more severe nutrient restriction.

Keywords: conjugated linoleic acid; pasture; milk fat; energy balance.

INTRODUCTION

Abomasally infusing conjugated linoleic acids (CLA) and supplementing rumen inert-(RI) CLA reduces milk fat synthesis in lactating dairy cows and can improve calculated energy balance (EBAL) during periods of stress, i.e. immediately postpartum (Kay *et al.,* 2006) or during heat stress (Moore *et al*., 2005). In a pasture-based dairy system (as dominates in NZ), another period of transitory stress can occur during adverse weather conditions that reduce forage growth and/or quality. During these periods of reduced nutrient availability, animals typically enter into a negative energy balance (NEBAL), which can result in condition loss, metabolic stress, reproductive failure and decreased milk production (Bauman, 2000; Collier *et al*., 2005). Previous management strategies to alleviate NEBAL and maintain animal condition and milk production during feed restriction, involve increasing dietary energy via dietary supplements, or decreasing energy expenditure by reducing milking frequency. An alternative approach is to utilise *trans*-10, *cis*-12 CLA; a potent inhibiter of milk fat synthesis (Baumgard *et al*., 2005). In addition, to alleviating calculated NEBAL, CLA-induced milk fat depression (MFD) can increase milk and milk protein yield in grazing dairy cows (Mackle *et al.,* 2003; Back & Lopez-Villalobos, 2004) and early lactating cows (Odens *et al.,* 2005; Kay *et al.,* 2006), two situations where energy is probably limiting milk synthesis. Currently, the NZ milk payment system encourages an increased milk protein:fat ratio and many products such as caseinate, skim milk powder and even trim or low fat milk, have a reduced requirement for milk fat. In NZ, surplus milk fat is generally made into butter, which as a commodity, is subject to cyclical variation in returns on the international market (MAF, 2003). Back & Lopez-Villalobos (2004) used a simulation model to calculate dairy product yields and values, and reported increased value of milk (~$0.27/kg/MS) from cows fed CLA for 16 wks compared to controls. Thus there are potential economic gains in producing milk with increased protein:fat ratio during periods of nutrient restriction. Study objectives were to determine if abomasal CLA infusion inhibits milk fat synthesis in nutrient restricted grazing dairy cows and subsequently improves production and bioenergetic variables.

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MATERIALS AND METHODS

Experimental design, animals and treatments

Twelve mulitparous, mid-lactation Holstein-Friesian cows were allocated to one of two dietary treatments; *ad libitum* (AL) or restricted (R) pasture allowance, balanced for milk production, milk composition and parity. Cows were maintained on their designated pasture allowance throughout the entire experiment and were abomasally infused with 0 (0) or 50 (50) g/d CLA (Bioproducts Inc., Fairlawn, OH) in a crossover design. Abomasal infusions began 5 d after dietary allocation, and continued for two 10-d periods separated by a 10-d washout period. Daily infusate volumes were loaded into two 60-ml syringes (25 ml/syringe) and infused prior to a.m. and p.m. milkings as described by Mackle *et al.* (2003). To reduce viscosity and ensure all lipid was infused, CLA was warmed to ~35^{o}C prior to dosing and chased with 180 ml of warm water. Cows receiving 0 g/d CLA were infused with an additional 50 ml warm water. Fatty acid composition of the CLA is presented in Table 1. The 50 g/d CLA treatment provided 9.25 g *trans*-10, *cis*-12 CLA and 17 g/d *cis*-9, *trans*-11; *trans*-8, *cis*-10; *cis*-11, *trans*-13 CLA (CLA isomers that do not alter mammary lipid metabolism; Baumgard *et al.,* 2000; Perfield *et al*., 2004).

All cows were rotationally grazed and offered fresh pasture twice daily. Different pasture allocations for AL (42 ± 6.3 kg DM/cow/d) and R (22 ± 8.5 kg DM/cow/d) treatments were offered by multiplying pregrazing pasture mass per m^2 by desired allowance per cow (kg/cow/d) by number of cows in each group (n = 6). All cows grazed within the same paddock and different sized areas and allocations were separated by double stranded electric fences.

Dry matter intake (DMI) and pasture analysis

Individual DMI was estimated during the last 5 d of each infusion period using the *n*-alkane technique as described by Roche *et al*. (2005). Throughout the experiment, representative pasture samples were collected daily by hand-plucking pasture to grazing height from paddocks immediately prior to grazing. Samples were bulked every 5 d, and triplicate samples were either frozen immediately (-20^{o}C) for fatty acid analysis (Kay *et al*., 2004; Table 1), dried at 100^{o}C for DM analysis or dried at 60^{o}C for nutrient composition (Table 1). Metabolizable energy (ME) was derived directly from predicted organic matter digestibility (Kay *et al.,* 2006; Table 1).

TABLE 1: Fatty acid and nutrient composition of pasture and conjugated linoleic acid (CLA) supplement[1]

Variable	Pasture	CLA supplement
Fatty acid, % of total fatty acids		
14:0	0.8	0.0
16:0	15.4	4.2
16:1 *cis*-9	1.8	0.0
18:0	6.7	2.2
18:1 *cis*-9	3.1	21.1
18:2 *cis*-9, *cis*-12	12.5	6.5
CLA (total)	0.0	61.6
CLA *cis*-8, *trans*-10	0.0	9.2
CLA *cis*-9, *trans*-11	0.0	14.9
CLA *trans*-10, *cis*-12	0.0	18.5
CLA *cis*-11, *trans*-13	0.0	10.5
Other CLA	0.0	8.5
18:3 *cis*-9, *cis*-12, *cis*-15	48.9	0.0
20:0	1.9	0.0
Unknown[2]	8.9	4.4
Nutrient composition, % of DM [3]		
Crude protein	16.2	
Lipid	4.1	
Ash	10.4	
Neutral detergent fibre	49.8	
Acid detergent fibre	25.4	
Soluble sugars	9.1	
ME (MJ/kg DM)	10.4	

[1]Data represent average from *ad libitum* (42 ± 6.3 kg DM/cow/d) and restricted (22 ± 8.5 kg DM/cow/d) pasture for entire experimental period
[2]Represents unidentified fatty acids
[3]Dietary DM averaged 16.7 %

Milk sampling and analysis

Milk yield and milk composition were determined daily. On d 9 and 10 of the infusion periods, milk fat was extracted from the composite samples and analysed for fatty acid content as described by Kay *et al*. (2004).

Blood sampling and analysis

Plasma was collected on d 9 and 10 of the infusion periods and analysed for non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), glucose, urea and insulin as described by Roche *et al.* (2005) and Kay *et al.* (2006).

Calculations

Net EBAL was calculated during the last 5 d of infusion periods (d 6-10) as described by Kay *et al.* (2006) using the following equation;

EBAL = net energy intake – (net energy for maintenance + net energy for lactation)

Statistical Analysis

All data were analysed using the PROC MIXED procedure of SAS (2001). The model contained period, pasture allowance, CLA dose and all possible interactions. Standard errors of the mean are reported and differences considered significant when P<0.05, unless otherwise stated.

RESULTS

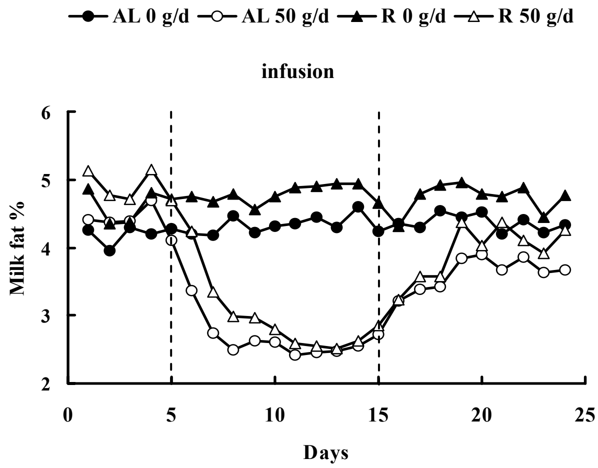
Pasture restriction reduced (P<0.01) DMI and the yield of milk and milk components (Table 2). Abomasal infusion with 50 g/d CLA reduced milk fat content and yield by 43 and 45%, and 48 and 46% in the AL and R groups, respectively (Table 2). There was no effect of CLA infusion on milk yield nor milk lactose content or yield, however milk protein content increased (P<0.05) by 8 and 5% in AL and R treatments, resulting in an increased (P<0.05) protein yield of 6 and 9%, respectively. The CLA-induced changes to milk fat and protein resulted in an increased (P<0.01) protein:fat ratio of 1.54 vs. 0.82 for AL and 1.44 vs. 0.72 for R (Table 2).

Pasture allowance did not alter the content of most individual milk fatty acids, with the exception of *cis*-9, *cis*-12 18:2 (linoleic acid) and *cis*-9, *cis*-12, *cis*-15 18:3 (linolenic acid) which decreased (P<0.05) following pasture restriction (Table 3). Abomasal infusion with 50 g/d CLA increased (P<0.01) milk fat content of *trans*-8, *cis*-10 CLA, *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA, *cis*-11, *trans*-13 CLA and other unidentified CLA isomers independent of pasture allowance (Table 3). There was also an increase in *cis*-9 18:1 (oleic acid) and linoleic acid following CLA infusion in both R and AL treatments. Abomasal infusion with 50 g/d CLA decreased the content of fatty acids from *de novo* origin and increased the content of fatty acids derived from preformed sources in both pasture treatments (Table 3). Calculated EBAL (d 6 - 10 of the infusion periods) improved following CLA infusion (-1.84 vs. 11.22 and 1.59 vs. 13.77 MJ/cow/d for AL and R treatments, respectively; P<0.01; Table 2). However, there was no effect of CLA infusion on insulin, NEFA, BHBA, urea, and glucose (data not presented). Similarly, there was no effect of pasture allowance on these blood metabolites and hormones (data not presented).

DISCUSSION

Twice daily abomasal infusion with CLA reduced milk fat synthesis in dairy cows offered *ad libitum* or restricted pasture. Irrespective of pasture allowance, MFD became significant by d 2 of infusion and reached nadir by d 4, with milk fat content returning to pre-infusion levels by 5 d post infusion (Figure 1).

FIGURE 1: Temporal pattern of milk fat content. Treatments were *ad libitum* (AL; 42 ± 6.3 kg DM/cow/d;) or restricted (R; 22 ± 8.5 kg DM/cow/d) pasture allowance with abomasal infusion of 0 (0) or 50 (50) g/d CLA for 10 d. Values represent least squares means and SEM averaged 0.25.



The CLA-induced inhibition of milk fat synthesis was primarily due to a decrease in *de novo* synthesized fatty acids (Table 3), which agrees with previous CLA research (Mackle *et al*., 2003; Kay *et al.,* 2006). Milk fat content of CLA isomers, linoleic acid and oleic acid increased following CLA infusion, reflecting the fatty acid composition of the CLA supplement (Table 1). Pasture restriction decreased milk fat linoleic and linolenic acid content probably due to decreased DMI and subsequent reduced intake of these individual fatty acids.

Fat is the most energetically expensive (>50% of total milk energy) milk component for dairy cows to synthesize and present data demonstrates that during times of nutrient restriction, CLA-induced MFD improves calculated EBAL by ~12 MJ/cow/d. Based on previous research (Mackle *et al*., 2003; Back & Lopez-Villalobos, 2004; Kay *et al*., 2006) that reported CLA (abomasal infusion or dietary supplementation) increased milk and protein yield, we speculated that increased available energy during times of nutrient restriction would be repartitioned towards milk and/or milk protein production. This is in contrast to previous studies utilizing TMR-fed cows where effects of CLA are specific to milk fat with no effects on milk yield or milk protein content or yield (Baumgard *et al*., 2000; 2002; Perfield *et al.,* 2002; 2004).

TABLE 2: Effect of abomasal infusion of 0 or 50 g/d conjugated linoleic acid (CLA) for 10 d on production parameters in cows grazing *ad libitum* or restricted pasture allowance[1]

	***Ad libitum* allowance[2]**		**Restricted allowance[3]**			***Effects[4]***		
Variable	0 g/d CLA	50 g/d CLA	0 g/d CLA	50 g/d CLA	SED	CLA	***A***	***CLAxA***
DMI, kg/d	13.41	13.37	11.57	12.23	0.52	0.56	0.01	0.51
EBAL, MJ/d[5]	-1.84	11.22	1.59	13.77	3.89	<0.01	0.46	0.91
Milk yield, kg/d	15.74	15.41	11.46	11.93	0.71	0.86	<0.01	0.41
Fat, %	4.44	2.52	4.94	2.57	0.13	<0.01	0.04	0.09
Fat, kg/d	0.69	0.39	0.56	0.30	0.02	<0.01	<0.01	0.21
Protein, %	3.62	3.89	3.50	3.68	0.08	<0.01	0.04	0.56
Protein, kg/d	0.57	0.60	0.39	0.43	0.02	0.05	<0.01	0.67
Lactose, %	4.73	4.65	4.76	4.70	0.07	0.31	0.56	0.92
Lactose, kg/d	0.74	0.72	0.55	0.57	0.04	0.69	<0.01	0.44
Protein:Fat	0.82	1.54	0.72	1.44	0.03	<0.01	<0.01	0.86

[1]Data represent average from d 9 and 10 of infusion periods
[2]Pasture allowance of 42 ± 6.3 kg DM/cow/d
[3]Pasture allowance of 22 ± 8.5 kg DM/cow/d
[4]Significance of effects for CLA dose (CLA), pasture allowance (A) and interaction between CLA dose and pasture allowance (CLAxA)
[5]Mean net EBAL = net energy intake – (net energy for maintenance + net energy for lactation): calculated from d 6-10 of the infusion periods

TABLE 3: Effect of abomasal infusion of 0 or 50 g/d conjugated linoleic acid (CLA) for 10 d on milk fatty acid content (% of total fatty acids) in cows grazing *ad libitum* or restricted pasture allowance[1]

	***Ad libitum* allowance[2]**		**Restricted allowance[3]**			***Effects[4]***		
Fatty acid, %	0 g/d CLA	50 g/d CLA	0 g/d CLA	50 g/d CLA	SED	CLA	***A***	***CLAxA***
Individual isomers								
18:0	10.61	11.72	10.54	12.02	0.69	0.08	0.87	0.79
18:1 c9	18.34	20.42	19.25	20.78	0.61	<0.01	0.08	0.23
18:1 t6-8	0.10	0.14	0.08	0.11	0.02	0.06	0.17	0.50
18:1 t9	4.78	5.25	4.05	4.79	0.03	0.04	0.04	0.61
18:1 t10	0.47	0.56	0.43	0.42	0.02	0.47	0.20	0.45
18:1 t11	4.16	4.19	4.19	4.59	0.22	0.36	0.35	0.42
18:1 t12	0.41	0.41	0.30	0.32	0.03	0.64	<0.01	0.60
18:2 c9, c12	1.28	1.70	1.17	1.50	0.05	<0.01	0.01	0.34
CLA t8, c10	0.35	0.66	0.25	0.63	0.02	<0.01	<0.01	0.08
CLA c9, t11	1.87	2.32	1.85	2.42	0.10	<0.01	0.72	0.53
CLA t10, c12	0.10	0.64	0.09	0.74	0.02	<0.01	0.08	0.06
CLA c11, t13	0.19	0.25	0.16	0.26	0.02	<0.01	0.44	0.28
Other CLA	1.13	1.56	1.10	1.62	0.04	<0.01	0.44	0.03
18:3 c9, c12, c15	1.05	1.18	0.98	1.00	0.06	0.20	0.05	0.39
Fatty acid origin								
de novo[5]	22.70	16.70	21.59	16.89	0.73	<0.01	0.54	0.39
16:0 & 16:1	28.16	28.35	289.83	28.49	0.92	0.87	0.61	0.72
Preformed[6]	40.97	47.24	417.73	47.37	1.09	<0.01	0.67	0.76

[1]Data represent average from d 9 and 10 of infusion periods
[2]Pasture allowance of 42 ± 6.3 kg DM/cow/d
[3]Pasture allowance of 22 ± 8.5 kg DM/cow/d
[4]Significance of effects for CLA infusion (CLA), pasture allowance (A) and interaction between CLA infusion and pasture allowance (CLAxA)
[5]Sum 4:0 – 15:0
[6]Sum 17:0 – 20:0

Mackle *et al*. (2003) suggested spared energy has a more profound effect on milk production in grazing cows than in cows fed a more energy dense total mixed ration (TMR; a diet that might better match energy and other nutrient requirements). Data from the present study demonstrated a CLA-induced increase in protein yield, however this differed from Mackle *et al.* (2003) and Back & Lopez-Villalobos (2004) as the increase was due to increased protein content rather than increased milk yield.

One possible reason for the lack of increase in milk yield in the present study may be due to the severity of the CLA-induced MFD (>40%). Kay *et al*. (2006) reported a curvilinear relationship between severity of MFD and increase in milk yield in dairy cows immediately postpartum. Cows supplemented with RI-CLA tended to produce more milk when MFD was moderate (<35%), however as MFD became more severe (>35%) the positive milk yield response was eliminated. This theory is supported by Mackle *et al*. (2003) who reported an 11% 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 and Back & Lopez-Villalobos (2004) who reported a 11% increase in milk yield following moderate (26%) CLA-induced MFD during the first 16 weeks of lactation. These studies suggest that during times of energy deficiency, moderate inhibition of milk fat synthesis may spare energy that is partitioned towards increased milk yield, however, severe MFD may adversely affect cellular mechanisms involved in milk synthesis and/or secretion. The reason why severe MFD appears to adversely affect milk yield remains unclear but several hypotheses are discussed by Kay *et al*. (2006).

Additionally, the lack of a positive milk yield response in the restricted treatment may be due to limitations of this particular model to mimic nutrient deprivation. During times of nutrient inadequacy, homeorheteric mechanisms alter tissue metabolism in an attempt to maintain milk production and consequently the animal enters into NEBAL (Bauman & Currie, 1993). These coordinated changes result in increased plasma NEFA, BHBA and decreased glucose and insulin content. In the present study, however, DMI only decreased by ~1.5 kg DM/cow/d with pasture restriction, and neither EBAL nor plasma hormones and metabolites were affected by pasture allowance. Therefore, nutrient deprivation in the present study may not have been extensive enough to detect/measure milk yield improvements due to increased available energy.

Although milk yield was not affected in the present experiment, the increase in milk protein and decrease in milk fat yield resulted in a two-fold increase in milk protein:fat ratio. Based on the present weighted milk payment scheme in NZ, which favors production of milk protein over fat, this could potentially increase milk value.

In conclusion, data from the present study demonstrates that abomasal infusion of CLA inhibits milk fat synthesis to the same extent in cows fed *ad libitum* or restricted pasture. CLA-induced MFD increased calculated EBAL in both AL and R treatments and available energy appeared to be partitioned towards protein synthesis, causing increased protein content, yield and protein:fat ratio. Milk yield decreased with pasture restriction, and did not improve with CLA treatment as was hypothesized. The lack of a positive CLA-induced milk yield response may be associated with the severe reduction in milk fat synthesis (>40%) or potential current model limitations. Further research, utilizing a model with a lower CLA dose and/or more severe nutrient restriction, may determine if CLA could potentially alleviate NEBAL and improve production and bioenergetic parameters during periods of feed shortage.

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