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Endogenous synthesis and enhancement of conjugated linoleic acid in pasture-fed dairy cows

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ABSTRACT

The primary conjugated linoleic acid (CLA) isomer in milkfat, *cis*-9, *trans*-11 CLA, is a proven anticarcinogen in rodents and can be derived directly from ruminal biohydrogenation of linoleic acid. The major fatty acid in pasture, however, is α–linolenic acid; the biohydrogenation of which does not produce *cis*-9, *trans*-11 CLA as an intermediate. Therefore, in grazing cows, *cis*-9, *trans*-11 CLA must be produced by other sources, possibly via Δ^9-desaturation of *trans*-11 vaccenic acid (TVA), a biohydrogenation product of both α–linolenic acid and linoleic acid. Studies were conducted to i) determine the importance of endogenous synthesis of *cis*-9, *trans*-11 CLA in pasture-fed cows and ii) increase the level of *cis*-9, *trans*-11 CLA. The first study involved abomasal infusion of sterculic oil (SO) to inhibit the activity of the Δ^9-desaturase enzyme. Abomasal infusion of SO decreased the concentration of *cis*-9, *trans*-11 CLA in milk fat by 71% ($P<0.01$; 3.6 c.f. 12.1 ± 0.15 mg/g fatty acid). The presence of *cis*-9, $C_{10:1}$, *cis*-9, $C_{12:1}$ and *cis*-9, $C_{14:1}$ in the milk fat of cows following SO treatment indicated Δ^9-desaturase was not completely inhibited. Using the changes in these fatty acids to evaluate the extent of Δ^9-desaturase inhibition with SO treatment, an estimated 87-100% of *cis*-9, *trans*-11 CLA in milk fat was of endogenous origin. In the second study, sunflower oil at 4% DMI/cow/day invoked a 38% increase ($P<0.01$) in milk fat TVA (61.3 c.f. 44.4 ± 0.51 mg/g fatty acid) and a subsequent 28% increase ($P<0.01$) in *cis*-9, *trans*-11 CLA concentrations compared to control (28.5 c.f. 22.3 ± 0.19 mg/g fatty acid). Results demonstrate that endogenous synthesis is the major source of *cis*-9, *trans*-11 CLA in pasture-fed dairy cows and levels can be enhanced by increasing the supply of the ruminally-derived precursor, TVA.

Keywords: conjugated linoleic acid; *trans*-11 vaccenic acid; Δ^9-desaturase; milkfat; pasture.

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term for the positional and geometric isomers of octadecadienoic acids ($C_{18:2}$) with conjugated double bonds. The primary CLA isomer in dairy products, *cis*-9, *trans*-11 CLA, is a proven anticarcinogen in laboratory animals (Ip *et al.*, 1999). In addition, CLA can reduce diabetes and atherosclerosis, stimulate the immune system, alleviate cachexia and stimulate bone mineralistaion (Whigham *et al.*, 2000). These potential health benefits have led to a world-wide interest in increasing the concentration of CLA in dairy products.

In ruminants, polyunsaturated fatty acids undergo biohydrogenation in the rumen. *Cis*-9, *trans*-11 CLA is an intermediate in the biohydrogenation of linoleic acid (*cis*-9, *cis*-12 $C_{18:2}$) and *cis*-9, *trans*-11 CLA in milk fat was thought to originate solely from ruminal production. It was recently found, however, that in cows fed a typical total mixed ration (TMR) the majority of *cis*-9, *trans*-11 CLA was produced endogenously (Griinari *et al.*, 2000; Corl *et al.*, 2001). During endogenous synthesis, *trans*-11 vaccenic acid (*trans*-11 $C_{18:1}$; TVA), another intermediate in the biohydrogenation of linoleic and α-linolenic acids (*cis*-9, *cis*-12, *cis*-15 $C_{18:3}$), accumulates in the rumen and is absorbed into the mammary gland. In the mammary epithelium, TVA is converted to *cis*-9, *trans*-11 CLA by the Δ^9-desaturase enzyme.

Cows grazing pasture have higher concentrations of milk fat *cis*-9, *trans*-11 CLA than those fed TMR (Kelly *et al.*, 1998b; Auldist *et al.*, 2002). Whilst the primary fatty acid in TMR is linoleic acid, pasture is predominantly high in α-linolenic acid; the biohydrogentaion of which does not produce *cis*-9, *trans*-11 CLA as an intermediate, but does give rise to the endogenous precursor TVA. Thus, elevated concentrations of *cis*-9, *trans*-11 CLA in milk fat of pasture-fed cows may be due to an increased supply of TVA and increased endogenous synthesis.

The first of two studies described here had the aim of determining the quantitative importance of endogenous synthesis of *cis*-9, *trans*-11 CLA in pasture-fed cows, by inhibiting the activity of the Δ^9-desaturase enzyme with sterculic oil (SO). The aim of the second study was to enhance the concentration of milk fat *cis*-9, *trans*-11 CLA in pasture-fed cows by supplementing the diet with sunflower oil (SFO); a substrate for ruminal production of TVA.

MATERIALS AND METHODS

Experiment 1

Animals, design and treatments

Four rumen-fistulated Friesian cows were randomly assigned to treatments in a 4 x 4 Latin square design. Two treatments will be reported in this paper. The treatments were 1) abomasal infusion of 4-kg skim milk (SM)/d (control), 2) abomasal infusion of 9-g SO in a 4-kg SM emulsion/d (SO).

Each experimental period consisted of a 2-d uniformity interval, a 4-d treatment interval, and an 8-d 'washout' interval. During the uniformity and treatment intervals the animals were constrained individually in metabolism stalls. Cows were offered a consistent pasture diet *ad libitum*, cut fresh twice daily. Daily samples of pasture were bulked within each experimental interval for analysis of fatty acid concentrations. During the 8-d washout interval the cows grazed similar pasture for *ad libitum* intake.

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Abomasal Infusions

Sterculic oil was extracted from the seeds of the *sterculia foetida* tree. A SO emulsion in SM was prepared 2-d prior to each experimental interval as described by Mackle *et al*. (2002). The final concentration of SO in the emulsion was 2.25% (w/w).

The SO emulsion and SM (control) were infused into the abomasum to avoid alterations by rumen bacteria and to allow a slow and continuous administration of the SO.

To access the abomasum, a polyvinyl chloride tube (Nalgene 180 PVC; 0.47 cm i.c x 0.78 cm o.d.; Nalge Co., Rochester, NY) was passed through the rumen fistula and *sulcus omasi* into the abomasum (Spires *et al.*, 1975) where it was secured by a 10-cm rubber flange. Peristalic pumps (STA-131900; Desage, Heidelberg, Germany) were calibrated to infuse continuously at a rate of 4-kg infusate/day.

Sampling procedures

Cows were milked at approximately 0700 and 1500 h each day. For the last two days of the washout interval, milk samples were collected using in-line milk meters (Trutest, Palmerston North, New Zealand). During the uniformity and treatment intervals milk samples were collected by ladle from test buckets following thorough mixing. Samples were bulked (morning and afternoon) to form one composite sample/cow/d, then chilled and either analysed immediately or the cream extracted and frozen for later analyses.

Milk Analysis

Fresh milk samples were analysed for fat and crude protein using an infrared milk analyser (FT120; Foss Electric, Hillerød, Denmark). Milk fat was extracted from samples collected on the last day of the uniformity interval and the four days of the treatment interval. Standard fatty acid profiles were obtained by gas chromatography according to the method of MacGibbon (1988). A 120m BPX-70 column (120m x 0.25mm I.C. and 0.25um film thickness; SGE, Australia) was used with helium as the carrier gas (2.5ml/min).

CLA isomers were determined by gas chromatography as described in Fong & MacGibbon (1997) and $C_{18:1}$ isomers were quantified based on the method described by Flett & Chand (2001).

Experiment 2

Animals, experimental design and treatments

Ten rumen-fistulated Friesian cows were randomly assigned to two treatments 1) 100% pasture (control) and 2) pasture + sunflower oil at 4% DMI/cow/d (SFO). Cows grazed together for 21 days and were offered a pasture allowance of 35kg DM/cow/day. The experimental period consisted of a seven-day uniformity interval (days 1-7) and a 14-day treatment interval (days 8–21). During the 14-day treatment interval, cows in the SFO treatment group were drenched twice-daily prior to morning and afternoon milkings, with 350 ml and 400 ml respectively of SFO.

Sampling procedures

Cows were milked at approximately 0700 and 1500 h each day. On days 2, 7, 13, 17 and 20 of the experiment, samples were collected from morning and afternoon milkings to form one composite sample/cow/d using in-line milk meters as described for experiment one.

Milk Analysis

All milk samples were analysed for fat and crude protein as described in experiment one. Individual samples collected on days 7, 13 and 21 were analysed for fatty acid composition as in experiment one.

RESULTS

Experiment 1: Following SO infusion, DMI, milk yield and the concentration and yield of milk components were unaltered, but distinct changes in the milk fatty acid composition occurred (Table 1). Data from day four of the treatment interval were used for analysis. Infusion of SO reduced (P<0.01) milk fat *cis*-9, *trans*-11 CLA concentration by 71% (Table 1). Concentrations of all milk fatty acids containing a *cis*-9 double bond decreased (P<0.01) following SO treatment (Table 1). Consequently, product:substrate ratios dependent on Δ^9-desaturase decreased (P<0.05; Table 1). Total CLA levels in milk fat of control cows measured 15.1mg/g fatty acids, with the *cis*-9, *trans*-11 isomer accounting for 80% (Table 1).

Experiment 2: Fourteen days of SFO administration did not affect milk yield or milk protein concentration but resulted in a 25% reduction (P<0.01) in milk fat concentration. Administration of SFO for seven days caused no significant change in *cis*-9, *trans*-11 CLA or TVA concentration in milk fat. After 14 days of SFO administration, TVA levels increased by 38% (P<0.01; Table 2) and *cis*-9, *trans*-11 CLA levels increased by 28% (P<0.01; Table 2). Amongst control and treatment cows there was a linear relationship (P<0.05) between TVA and

TABLE 1: Effect on milk fatty acid composition (mg/g fatty acid) and product:substrate ratios dependent on Δ^9-desaturase following 4 days of abomasal infusion of sterculic oil (SO).

Fatty acids	control	SO	s.e.d	P values
10:0	32.8	31.5	3.4	0.71
10:1, *cis*-9	3.7	0.9	0.5	<0.00
12:0	34.5	33.4	5.0	0.83
12:1, *cis*-9	1.7	0.3	0.3	0.01
14:0	107.2	116.6	8.4	0.30
14:1, *cis*-9	12.7	4.9	1.1	<0.01
16:0	260.0	274.5	13.4	0.32
16:1,*cis*-9	12.6	5.0	0.9	<0.01
18:0	115.6	209.2	15.5	<0.01
18:1, *trans*-11	35.6	39.9	1.4	0.02
18:1, *cis*-9	186.9	84.2	13.6	<0.01
18:2, *cis*-9, *cis*-12	5.5	5.1	0.8	0.59
CLA, total	15.1	6.6	1.3	<0.01
CLA, *cis*-9, *trans*-11	12.1	3.6	1.5	<0.01
Product:substrate ratios				
10:1, *cis*-9 to 10:0	0.11	0.03	0.01	<0.01
12:1, *cis*-9 to 12:0	0.05	0.01	0.01	0.02
14:1, *cis*-9 to 14:0	0.12	0.04	0.01	<0.01
16:1, *cis*-9 to 16:0	0.05	0.02	0.00	<0.01
18:1, *cis*-9 to 18:0	1.62	0.41	0.09	<0.01
CLA, *cis*-9, *trans*-11 to 18:1 *trans*-11	0.34	0.09	0.03	<0.01

TABLE 2: Effect on milk fatty acid composition (mg/g fatty acid) following 14 days of dietary sunflower oil (SFO) supplementation (4% DMI/cow/d).

Fatty acids	Control	SFO	s.e.d	P values
10:0	26.3	15.8	1.2	<0.01
10:1, *cis*-9	3.7	1.8	0.2	<0.01
12:0	29.6	18.8	1.2	<0.01
12:1, *cis*-9	0.9	0.4	0.1	<0.01
14:0	102.2	79.2	3.0	<0.01
14:1, *cis*-9	11.9	9.8	0.9	0.05
16:0	276.3	194.8	8.3	<0.01
16:1, *cis*-9	16.6	14.1	1.6	0.15
18:0	93.9	117.9	9.4	0.03
18:1, *trans*-11	44.4	61.3	5.1	0.01
18:1, *cis*-9	180.8	252.8	11.4	<0.01
18:2, *cis*-9, *cis*-12	8.4	14.7	2.0	0.01
CLA, total	25.3	31.5	2.1	0.01
CLA, *cis*-9, *trans*-11	22.3	28.5	1.9	0.01

cis-9, *trans*-11 CLA (Figure 1). Stearic (G8:0) and linoleic acids in milk fat increased by 25% (P<0.05) and 75% (P<0.01) respectively following 14 days of SFO treatment (Table 2).

DISCUSSION

Experiment one was designed to quantify the level of endogenous synthesis of *cis*-9, *trans*-11 CLA in milk fat from pasture-fed cows. This was achieved by inhibiting the activity of the Δ^9-desaturase enzyme by abomasal infusion of SO. Sterculic oil contains cyclopropene fatty acids that are specific inhibitors of the Δ^9-desaturase enzyme. Inhibition of this enzyme was reflected in the dramatic reduction in concentration of milk fatty acids containing a *cis*-9 double bond, including a 71% reduction in *cis*-9, *trans*-11 CLA, and a decrease in the fatty acid product:substrate ratios dependent on Δ^9-desaturase (Table 1).

The inhibition of Δ^9-desaturase in the mammary gland was not complete, and the 71% reduction in *cis*-9, *trans*-11 CLA is therefore a minimum estimate of endogenous synthesis. An indication of the extent of the inhibition is provided by the changes in the milk fat composition of *cis*-9, $C_{10:1}$, *cis*-9, $C_{12:1}$ and *cis*-9 $C_{14:1}$. The source of C_{10}, C_{12} and C_{14} fatty acids in milk fat is *de novo* synthesis in the mammary gland, and the introduction of a double bond by the mammary Δ^9-desaturase results in the *cis*-9 monounsaturated fatty acids derived from these. Griinari *et al.* (2000) and Corl *et al.* (2001) used changes in *cis*-9 $C_{14:1}$ to calculate a correction factor for the extent of Δ^9-desaturase inhibition by SO. In the present study, concentrations of *cis*-9, $C_{10:1}$ and *cis*-9, $C_{12:1}$ were also quantified for a similar purpose. Compared to the control, milk fat contents *cis*-9, $C_{10:1}$, *cis*-9, $C_{12:1}$ and *cis*-9 $C_{14:1}$ were decreased by 76, 82 and 61% respectively, during the SO treatment. Using these values to correct for incomplete inhibition of Δ^9-desaturase indicated that from 87-100% of the milk fat content of *cis*-9, *trans*-11 CLA was derived from endogenous synthesis in pasture-fed cows.

Using a similar approach, involving SO to inhibit Δ^9-desaturase, Griinari *et al.* (2000) and Corl *et al.* (2001) estimated endogenous synthesis accounted for 64 and 78% respectively of the *cis*-9, *trans*-11 CLA in milk fat for cows fed TMR.

The milk fat content of *cis*-9, *trans*-11 CLA in the control cows of this study was at least twice that recorded by Griinari *et al.* (2000) and Corl *et al.* (2001) in control cows fed TMR (12.1 c.f. 4.2 and 6.5 mg/g fatty acid respectively). Whilst the primary fatty acid in TMR is linoleic acid, the major fatty acid found in pasture is α–linolenic acid. Intermediates in the biohydrogenation pathway of α–linolenic acid include TVA but do not include *cis*-9, *trans*-11 CLA. These factors could be one reason that in pasture-fed cows, a higher proportion of *cis*-9, *trans*-11 CLA in milk fat arises from endogenous synthesis than in TMR fed cows.

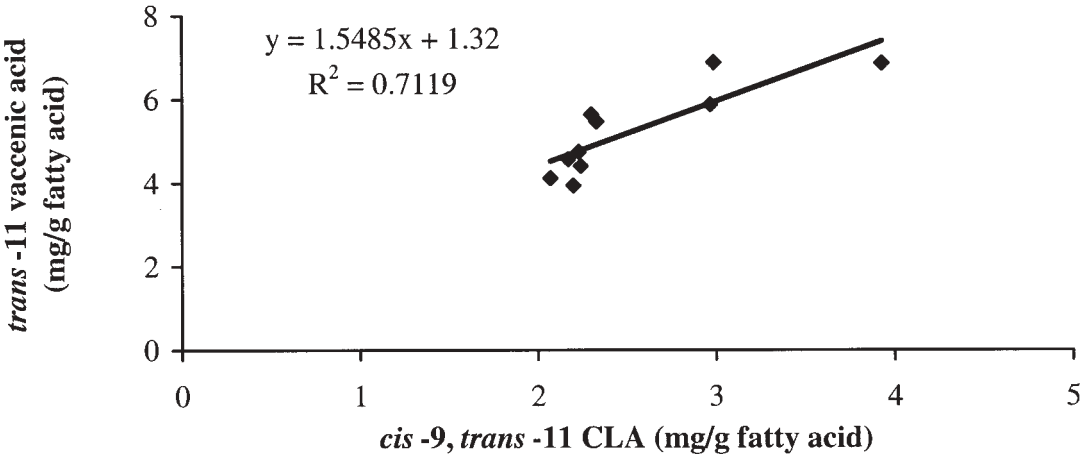
Given the importance of TVA in pasture-fed cows, experiment two involved dietary supplementation of SFO in an attempt to enhance ruminal TVA and subsequently increase the endogenous synthesis of *cis*-9, *trans*-11 CLA. After seven days of SFO administration, there was no increase in milk fat concentration of TVA or *cis*-9, *trans*-11 CLA, however, following 14 days of SFO treatment, these fatty acids had increased by 38 and 28% respectively (Table 2). The lack of response after seven days may be due to the change in the dietary fatty acid profile. Pasture contains predominantly α–linolenic acid, whilst SFO contains primarily linoleic acid. Rumen micro-organisms may not have had sufficient time in seven days to adapt from a 100% pasture diet to a pasture plus SFO diet.

Figure 1 shows the close linear relationship between TVA and *cis*-9, *trans*-11 CLA in individual milk samples collected from control and SFO cows after 14 days of treatment. These data are typical of those reported for TVA and *cis*-9, *trans*-11 CLA in milk fat from a number of studies across a wide range of diets (Grinarri & Bauman, 1999).

Several overseas studies have demonstrated that supplementation of TMR with plant oils high in linoleic acid can result in 200 – 500% increases in milk fat *cis*-9, *trans*-11 CLA concentrations (Kelly *et al*., 1998a; Dhiman *et al*., 2000; Chouinard *et al*., 2001). These increases were substantially greater than the 28% increase in *cis*-9, *trans*-11 CLA observed in our study. When supplements are added into a TMR-based system, animals have a continuous intake throughout the day. In the present study, however, oil was administered twice daily when cows were brought in for milking; this may effect the dynamics of ruminal biohydrogenation. Milk fat concentrations of linoleic and stearic acid increased by 75 and 25% respectively in this study following SFO treatment. The increase in linoleic acid, the predominant fatty acid in SFO, indicates that some of the linoleic acid did not undergo biohydrogenation in the rumen. Additionally, the increase in stearic acid suggests that the biohydrogenation that did occur was complete. Thus, the extensive biohydrogenation of linoleic acid produced stearic acid instead of the desired intermediate, TVA, and subsequently resulted in only a minor increase in milk fat *cis*-9, *trans*-11 CLA concentration.

Overall, results from these studies clearly demonstrate that endogenous synthesis is the major source of *cis*-9, *trans*-11 CLA in milk fat of pasture-fed cows.

FIGURE 1. Correlation between *trans*-11 vaccenic acid and *cis*-9, *trans*-11 CLA (mg/g fatty acids) in milk fat from cows following 14 days of control or sunflower oil treatment.



Endogenous synthesis accounted for more than 87% of the total *cis*-9, *trans*-11 CLA in milk fat and SFO administration resulted in an increase in milk fat *cis*-9, *trans*-11 CLA concentration. These factors, and the linear relationship shown between TVA and *cis*-9, *trans*-11 CLA are important when developing feeding strategies for further enhancing milk fat CLA concentrations of pasture-fed cows; such strategies should focus on increasing rumen production of TVA. As the initial level of *cis*-9, *trans*-11 CLA appears to be higher in milk fat from pasture-fed cows, the scope for enhancement of this anticarcinogen in order to produce high *cis*-9, *trans*-11 CLA dairy products shows great potential in New Zealand pasture-based dairying system.

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