

# Technical Note: Production of Butter with Enhanced Conjugated Linoleic Acid for Use in Biomedical Studies with Animal Models<sup>1,2</sup>

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## ABSTRACT

Cancer models utilize massive doses of carcinogen so that investigations of anticarcinogenic effects require equally large doses. Conjugated linoleic acids (CLA), predominately consumed in dairy products, are thought to be anticarcinogenic. Our objective was to naturally produce a CLA-enhanced butter for use in biomedical studies with animal models. To do this, we fed cows a low forage diet supplemented with sunflower oil. This resulted in increases in content of CLA of milk fat, but the markedly elevated concentrations were transient and declined over a 3-wk period. By collecting milk fat over the first few days on the diet (d 7 to 10) and selecting cows with the greatest CLA concentrations, we were able to produce a butter in which CLA content was enhanced sevenfold over control butter (41 vs. 5 mg/g of fatty acids) and the *cis*-9, *trans*-11 isomer predominated (91%). Thus, butter produced by this method can be used to investigate the preventive role of CLA in natural foods with biomedical models of different types of cancer. Furthermore, the butter allows examination of the other beneficial health effects of CLA reported with animal models.

**(Key words:** conjugated linoleic acid, milk fat, fatty acids)

**Abbreviation key:** CLA = conjugated linoleic acid.

The term “functional foods” is used as a generic description for foods or food ingredients that may provide a health benefit beyond the traditional nutrients it contains (7). Research with animal models has demonstrated a wide range of beneficial health effects for conjugated linoleic acids (CLA) (8). Foods derived from ruminant animals are the major source of CLA in human diets. The concentration of CLA in dairy products is essentially a function of the concentration in raw milk fat, and this can vary widely with different nutritional schemes (2). The predominant isomer of CLA in dairy products is *cis*-9, *trans*-11. However, the CLA used in animal model studies are typically commercial sources that consist of a mixture of positional and geometric isomers.

In animal studies with biomedical models of cancer, a massive dose of carcinogen is given so that over a short interval the number of animals with tumors and the number of tumors will be maximized (9). Likewise, to examine anticancer effects in these models, large doses of anticarcinogen will be required. Our objective was to naturally produce a butter with a concentration of CLA great enough that it could be used in experimental animal studies of carcinogenesis. Herein we report the results of these efforts and some of the characteristics of the product.

Based on our earlier work of diet effects on milk fat CLA (6), we fed a low forage diet supplemented with sunflower oil (69% linoleic acid) at 5.2% of dietary dry matter (Table 1). Procedures were approved by the Cornell Institutional Animal Care and Use Committee. Our design was to sample milk after 1 wk on the sunflower oil diet and determine CLA. Cows that had concentrations of CLA over 20 mg/g of fatty acids would continue on the diet with milk collections commencing from cows with the greatest concentration of CLA at wk 2.

For fatty acid analysis, fat was extracted and trans-methylated, and methyl esters of fatty acids were separated by gas chromatography using a fused silica capillary column (Supelcowax-10; 60 m × 0.32 mm i.d. with

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**Table 1.** Ingredients and chemical composition of the sunflower oil diet.

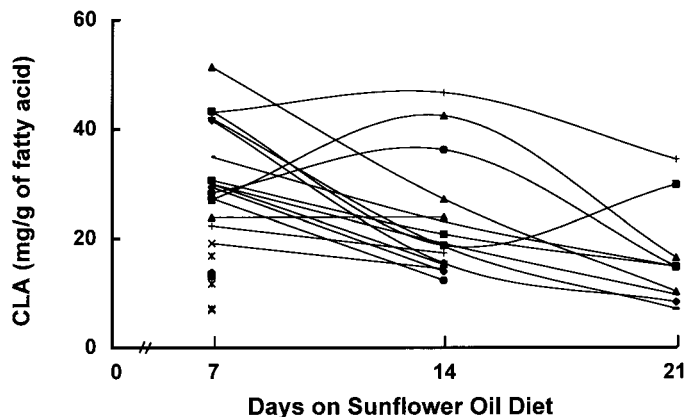
Composition	Sunflower oil diet
Ingredients, % of DM	
Corn silage	31.3
Hay crop silage	14.6
Corn grain, high-moisture	27.2
Sunflower oil	5.2
Soybean meal	14.6
Protein supplement <sup>1</sup>	4.2
Limestone	0.3
Sodium bicarbonate	0.5
Calcium sulfate	0.5
Mineral-vitamin mix <sup>2</sup>	1.6
Chemical analysis, % of DM	
CP	18.8
Crude fat	8.2
NDF	27.9
ADF	17.3
Dry matter, %	36.9
NE <sub>L</sub> , mcal/kg of DM	1.94

<sup>1</sup>Contained blood meal, feather meal and corn gluten meal (Taylor-By-Products, Wyalusing, PA).

<sup>2</sup>Formulated to meet or exceed mineral and vitamin requirements.

0.25- $\mu$ m film thickness; Supelco, Bellefonte, PA) (1). A combination of chromatography methods was used to separate methyl esters of *trans*-octadecenoic acid and CLA isomers. First, *trans*-monoenoic and *cis*-monoenoic acids were separated by silver ion HPLC (Waters 600 fluid unit controller; Waters Associates, Milford, MA) with an analytical silver ion modified cation-exchange column (ChromSpher 5 Lipids; 4.6 mm i.d.  $\times$  250 mm stainless steel with 5- $\mu$ m particle size; Chrompack, Middlebury, The Netherlands). The mobile phase was 0.1% acetonitrile in heptane. The *trans*-monoenoic acid isomers were further separated by gas chromatography using a fused silica capillary column (CP-Sil 88; 100 m  $\times$  0.25 mm i.d. with 0.20- $\mu$ m film thickness; Chrompack) and temperature gradients as described (4). This gas chromatography method also separated the CLA isomers into five peaks representing three peaks of *cis*, *trans*-isomers and two peaks of *cis*, *cis* and *trans*, *trans* isomers. The *cis*, *trans*-CLA isomers were further separated into six different peaks representing different positional isomers by continuing the silver ion HPLC with columns in series (tandem) as described (10).

To make butter, raw milk was first pasteurized by the high temperature-short time method (model #3919, Alfa-Laval Type-P13-RCF 1982, Kenosha, WI) at 79°C for 18 s, and then separated into cream and skim milk. Cream was vat pasteurized at 72°C for 30 min and stored in the cooler for 24 h. It was then churned (Zane Butter Churn Model #A, General Dairy Equipment, Minneapolis, MN) for 30 min at 10°C until



**Figure 1.** Temporal pattern of conjugated linoleic acids (CLA) in milk fat of cows fed the sunflower oil diet. Each symbol depicts an individual cow (n = 23).

butter was the size of popcorn kernels and the buttermilk was drained off. Butter was then rinsed and washed with 4°C water, and the unsalted butter was transferred to 0.5-kg plastic containers and stored at -20°C until used.

In our initial study, 23 cows started on the sunflower oil diet. At the end of wk 1 the concentration of CLA (mg/g of fatty acids) for all cows averaged 26, and individual cows ranged from 7 to 51 (Figure 1). Cows with the greatest concentration of CLA continued on the diet (n = 16), but by wk 2, concentrations of CLA had declined substantially for many cows; concentra-

**Table 2.** Fatty acid composition of butter made from cows fed control and sunflower oil diets.

Fatty acid	Control diet	Sunflower oil diet
— g/100 g of fatty acids —		
C4:0	3.99	5.36
C6:0	2.33	1.40
C8:0	1.38	0.71
C10:0	3.15	1.45
C12:0	3.61	1.69
C14:0	11.44	7.37
C14:1	1.22	0.94
C15:0	1.08	0.59
C16:0	30.93	17.84
C16:1	1.49	1.72
C17:0	0.48	0.34
C18:0	9.32	11.27
<i>cis</i> -9 C18:1	18.11	24.17
<i>trans</i> C18:1 (all isomers)	5.04	15.04
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.59	2.85
C18:3	0.36	0.21
CLA <sup>1</sup> (all isomers)	0.53	4.07
Others	2.95	2.98

<sup>1</sup>CLA = Conjugated linoleic acids.

**Table 3.** Distribution of fatty acid isomers of *trans*-C<sub>18:1</sub> and conjugated linoleic acids.

Isomer distribution	Control diet	Sunflower oil diet
<i>trans</i> -C <sub>18:1</sub> isomers, %		
<i>trans</i> -4	0.6	0.4
<i>trans</i> -5	0.6	0.3
<i>trans</i> -6/8	4.9	5.0
<i>trans</i> -9	5.6	4.8
<i>trans</i> -10	12.4	18.4
<i>trans</i> -11	24.5	48.3
<i>trans</i> -12	12.0	6.7
<i>trans</i> -13/14	23.1	9.9
<i>trans</i> -15	7.8	3.1
<i>trans</i> -16	8.5	3.1
Conjugated linoleic acid, %		
<i>cis/trans</i> <sup>1</sup>	85.8	96.9
7-9	6.7	4.4
8-10	0.3	0.6
9-11	76.5	90.8
10-12	1.1	0.8
11-13	0.4	0.1
12-14	0.8	0.1
<i>cis-cis</i>	4.8	1.2
<i>trans-trans</i>	9.4	1.9

<sup>1</sup>*cis-trans* = *cis-trans* or *trans-cis* sequence of double bonds.

tions averaged 37 and 23 mg/g of fatty acid for wk 1 and 2, respectively. This was unexpected, so rather than collect the milk fat, we continued cows on the diet; by wk 3 a further decline occurred for many cows so that the CLA averaged 16 mg/g of fatty acids (Figure 1).

The addition of plant oils to a low forage diet results in a substantial increase in the CLA concentration in milk fat reaching 4 to 5% of milk fatty acids in individual cows, as shown in the present study (Figure 1) and our earlier report (6). However, the very high concentrations observed for some cows at the initiation of the sunflower oil diet were transient, as indicated by the temporal pattern of CLA (Figure 1). Thus, rumen biohydrogenation for most cows was undergoing marked changes over the first few weeks on the sunflower oil diet.

To achieve our objective, we repeated the study and began collections at wk 1. A total of 10 cows started on the sunflower diet and milk was collected from the six cows with the greatest concentrations of CLA over the interval of d 7 to 10. Milk from herd mates fed a similar TMR that contained no sunflower oil (control diet) was collected at the same time, and milk from both groups was used to make butter. Butter from cows fed the control diet had a typical CLA concentration (5 mg/g of fatty acid), whereas butter from cows fed the sunflower oil diet averaged 41 mg/g of fatty acid (Table 2). An examination of the isomer distribution indicated that the major isomer of CLA in both butters was *cis-*

9, *trans*-11, although the proportion (90.8%) was greater in butter from the sunflower oil diet (Table 3). There were also other differences in the fatty acid composition. In general, the CLA-enriched butter had relatively less short and medium chain fatty acids and a greater content of unsaturated fatty acids (Table 2). In particular, the *trans*-C<sub>18:1</sub> content was increased almost threefold (Table 2). Examination of the *trans*-C<sub>18:1</sub> isomer distribution demonstrated that all isomers were increased, but the increase in the *trans*-11 C<sub>18:1</sub> was especially pronounced (Table 3). *Trans*-11 C<sub>18:1</sub> is produced in the rumen as an intermediate in the ruminal biohydrogenation of polyunsaturated fatty acids (2). Our recent studies have demonstrated that the major source of CLA in milk fat is endogenous synthesis by  $\Delta^9$ -desaturase with *trans*-11 C<sub>18:1</sub> as the substrate (3).

By manipulation of the diet and selection of cows at the point of greatest milk fat content of CLA, we were able to naturally produce a butter with greatly enhanced concentrations of CLA for use in biomedical studies with animal models. The first of these studies examined CLA as a cancer preventive agent using a rat model of breast cancer (5). Results demonstrated that mammary tumor incidence and number were reduced by over 50% in rats given the CLA-enhanced butter produced in the present study. Thus, the use of CLA-enhanced butter demonstrated that the *cis*-9, *trans*-11 isomer of CLA is anticarcinogenic in this animal model, and it can be an effective anticarcinogen when consumed as a natural dietary component in dairy products. The CLA-enhanced butter can also be used to extend this work to biomedical models of other types of cancer and in animal models of other reported beneficial effects of CLA (antidiabetic, antiobesity, antiatherosclerosis).

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