

## Biosynthesis of Conjugated Linoleic Acid and Its Incorporation into Ruminant's Products\*\*

Man K. Song\* and John J. Kennelly<sup>1</sup>

Department of Animal Science, Chungbuk National University, Cheongju 361-763, Korea

**ABSTRACT :** Bio-hydrogenation of C<sub>18</sub>-unsaturated fatty acids released from the hydrolysis of dietary lipids in the rumen, in general, occurs rapidly but the range of hydrogenation is quite large, depending on the degree of unsaturation of fatty acids, the configuration of unsaturated fatty acids, microbial type and the experimental condition. Conjugated linoleic acid (CLA) is incompletely hydrogenated products by rumen microorganisms in ruminant animals. It has been shown to have numerous potential benefits for human health and the richest dietary sources of CLA are bovine milk and milk products. The *cis*-9, *trans*-11 is the predominant CLA isomer in bovine products and other isomers can be formed with double bonds in positions 8/10, 10/12, or 11/13. The term CLA refers to this whole group of 18 carbon conjugated fatty acids. Alpha-linolenic acid goes through a similar bio-hydrogenation process producing *trans*-11 C<sub>18:1</sub> and C<sub>18:0</sub>, but may not appear to produce CLA as an intermediate. Although the CLA has been mostly derived from the dietary C<sub>18:2</sub> alternative pathway may be existed due to the extreme microbial diversity in the reticulo-rumen. Regardless of the origin of CLA, manipulation of the bio-hydrogenation process remains the key to increasing CLA in milk and beef by dietary means, by increasing rumen production of CLA. Although the effect of oil supplementation on changes in fatty acid composition in milk seems to be clear its effect on beef is still controversial. Thus further studies are required to enrich the CLA in beef under various dietary and feeding conditions. (*Asian-Aust. J. Anim. Sci. 2003. Vol. 16, No. 2 : 306-314*)

**Key Words :** Bio-hydrogenation, Rumen Bacteria, Unsaturated Fatty Acids, Conjugated Linoleic Acid (CLA), Oil Source, Dietary Manipulation

### INTRODUCTION

Conjugated linoleic acid (CLA) is a component that has been shown in recent years to have numerous potential benefits for human health, including potent cancer-fighting properties (Ha et al., 1987; Shultz et al., 1992; Ip et al., 1999). Other effects include a role in reducing atherosclerosis (Lee et al., 1994; Nicolosi et al., 1997) and a benefit for diabetes treatment (Houseknecht et al., 1998). CLA is found almost exclusively in animal products. Since CLA is a incompletely hydrogenated products by rumen microorganisms in ruminant animals, bovine milk and milk products are among the richest dietary sources. The topic of CLA as it relates to ruminant production has been reviewed previously (Grinari and Bauman, 1999; Dhiman, 2000; Chilliard et al., 2001). The objective of this paper is not to provide an extensive review of the literature but rather to provide an overview of the key factors relating to the bio-hydrogenation of unsaturated fatty acids released from the dietary lipid hydrolysis by rumen microorganisms and subsequent biosynthesis of CLA in the cow, and to discuss the feasibility and potential of producing CLA enriched milk and beef.

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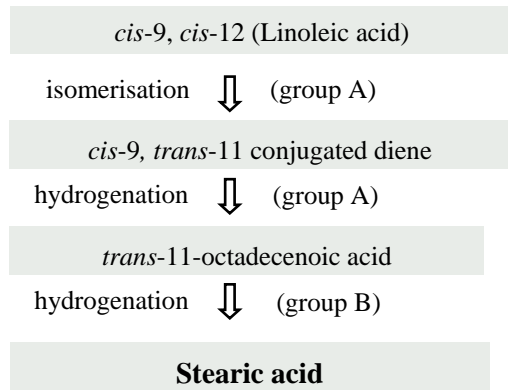
\* Corresponding Author: Man K. Song. Tel: +82-43-261-2545, Fax: +82-43-269-2549. E-mail: mksong@cbucc.chungbuk.ac.kr

<sup>1</sup> Dairy Research and Technology Centre, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5.

### BIO-HYDROGENATION IN THE RUMEN

An active bio-hydrogenation of unsaturated fatty acids by ruminal microbes has been well documented (Wu et al., 1991; Wang and Song, 2001; Sasaki et al., 2001, Wang et al., 2002). Earlier studies ostulated that there could be various purposes of hydrogenation in the rumen. The main function of hydrogenation was the disposal of reducing power which is essential to bacteria living in a reduced environment (Lennarz, 1966). Hydrogenation also has an essential role in the utilization of dietary fatty acids by fatty acid-auxotrophic bacteria (Hazlewood and Dawson, 1979). An alternative suggestion is to detoxify the unsaturated fatty acids (Kemp and Lander, 1984; Kemp et al., 1984).

Diverse bacteria relating to hydrogenation of the unsaturated fatty acids have been isolated. Kemp et al. (1975) isolated 5 hydrogenating strains, and estimated that strains were each present in the rumen at 10<sup>7-8</sup> per ml. The *Treponema* strain is reported to occur in about the similar numbers (Yokoyama and Davis, 1971). Verhulst et al. (1985) also isolated 7 hydrogenating strains from the rumen fluid. *Butyrivibrio fibrisolvens* was proved to be major hydrogenating bacteria (Polan et al., 1964; Kepler and Tove, 1967). Hazlewood et al. (1976) divided the hydrogenating bacteria into three groups based on the pattern of end-products of hydrogenation and on the isomerisations carried out. Bacteria are largely responsible for hydrogenation in the rumen while the protozoa are of only very minor importance.



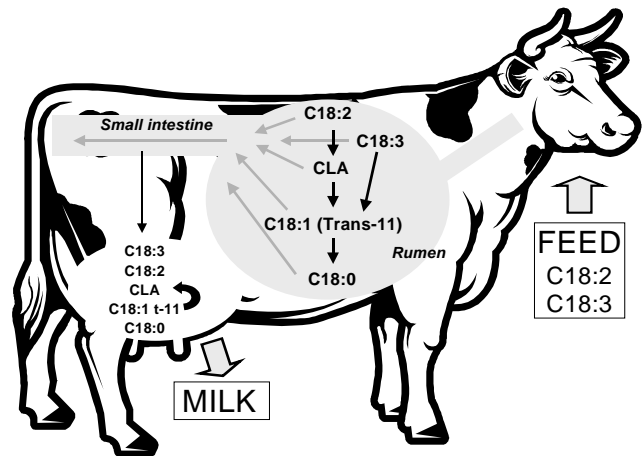
**Figure 1.** Scheme for the bio-hydrogenation of linoleic acid. Group A or B refer to the two classes of hydrogenating Bacteria (Kemp and Lander, 1984).

Figure 1 is the typical bio-hydrogenating scheme of linoleic acid (Harfoot and Hazlewood, 1988).

The pathway involves an initial isomerisation step resulting in the formation of a conjugated *cis-9, trans-11* acid which then undergoes hydrogenation of its *cis* double bond leaving *trans-11* octadecenoic acid. Finally this is hydrogenated to stearic acid (Dawson and Kemp, 1970; Verhulst et al., 1985). The conversion of *trans-11* C18:1 to C18:0 appears to involve a different group of organisms and occurs at a slower rate (Grinari et al., 1997). According to Kemp and Lander (1984), group A bacteria mostly hydrogenate linoleic acid to *trans-11*-octadecenoic acid while group B bacteria are capable of hydrogenating a wide range of octadecenoic acids, including *trans-11* (*trans-vaccenic*) acids to stearic acid.

In most studies it appeared that bio-hydrogenation in the rumen occurs rapidly but the range of hydrogenation was quite large, depending on the degree of unsaturation of fatty acids, the configuration of unsaturated fatty acids, microbial type and the experimental condition. Song and Sohn (1997) found the rapid bio-hydrogenation of linoleic and linolenic acid when incubated with oils *in vitro* as shown in Table 1. Linolenic acid content in flaxseed oil was higher as 54.9% than the other 13 fatty acids, but most of the linoleic as well as linolenic acid was disappeared. Similar results were observed from the *in vitro* study by Song and Choi (1998). Wu et al. (1991) reported that 44-68% of oleic acid, 63-79% of linoleic acid and 78-90% of linolenic acid were hydrogenated in the rumen. Fellner et al. (1995) reported based on *in vitro* study that bio-hydrogenation of infused linoleic acid averaged 77%. Palmquist and Jenkins (1980) suggested that end product from microbial hydrogenation of C<sub>18</sub>-unsaturated fatty acids was stearic acid, and this was proved by Jenkins (1993).

Wu and Palmquist (1991) and Fellner et al. (1995) indicated that the extent of hydrogenation was similar to



**Figure 2.** Formation of CLA in the cow

those measured *in vivo*, thus *in vitro* measurements are reliable to predict the fatty acid metabolism *in vivo*.

### BIOSYNTHESIS OF CLA IN THE COW

Conjugated linoleic acid (CLA) is formed in the rumen as an intermediate product in the digestion of dietary fat. Kepler and Tove (1967) in an early study showed that *cis-9, trans-11* C18:2, the major isomer of CLA, is the first intermediate formed in the biohydrogenation of linoleic acid by the rumen bacteria as shown in Figure 2. *Trans-11* C18:1 typically accumulates in the rumen. *Trans-11* C18:1 and *cis-9, trans-11* C18:2 account for approximately 50% of the trans fatty acids found in milk fat (Grinari, 1998). Although the *cis-9, trans-11* is the predominant CLA isomer in bovine milk, other isomers can be formed with double bonds in positions 8/10, 10/12, or 11/13. Each of these double bonds can be in a *cis* or *trans* configuration, giving a range of possible CLA isomers.

The term CLA refers to this whole group of 18 carbon conjugated fatty acids. Alpha-linolenic acid goes through a similar biohydrogenation process producing *trans-11* C18:1 and C18:0, but does not appear to produce CLA as an intermediate. Choinard et al. (1998) and Kelly et al. (1998) indicated that the CLA was mostly derived from the dietary C18:2. Bessa et al. (2000), however, revealed the possibility of alternative pathway that may be existed in the production of CLA from C18:3 due to the extreme microbial diversity in the reticulo-rumen. Wang et al. (2002) also observed an increased *cis-9, trans-11* CLA proportion from linseed incubation compared to that from rapeseed under the two addition levels of concentrate in incubation solution *in vitro* (Table 2). Linseed is much higher in C18:3 than rapeseed (Table 1).

Because of the extensive bio-hydrogenation of linoleic and linolenic acid to C18:1 *trans-11*, several studies have suggested that there may be little accumulation of CLA in

**Table 1.** Changes in C18-fatty acids when incubated with oils *in vitro* for 24 hour

C18-fatty acids	Rapeseed oil		Linseed oil	
	Prior to incubation	Post incubation	Prior to incubation	Post incubation
C18:0	1.93	62.20	2.72	61.24
C18:1	53.41	7.96	18.24	6.92
C18:2	25.78	0.31	15.56	0.40
C18:3	9.64	0.30	54.91	0.22

**Table 2.** Composition (%) of C18-fatty acids of culture solution when incubated with oilseeds

C18-Fatty acids	6 h incubation		12 h incubation	
	Linseed	Rapeseed	Linseed	Rapeseed
C18:0	28.96	30.41	35.56	32.65
C18:1	15.00	19.55	13.68	19.19
<i>Trans-11</i> C18:1	8.07	7.42	9.77	9.20
<i>Cis-9, trans-11</i> CLA	1.38	0.43	1.89	0.60
C18:2	9.00	12.71	6.60	10.98
C18:3	20.87	10.12	15.63	9.22

the rumen. Although it is accepted that CLA is formed in the rumen, there is good evidence that much of the *cis-9, trans-11* CLA found in bovine milk is actually synthesized within the mammary gland from C18:1 *trans-11* (Griinari and Bauman, 1999). This is possible through the action of stearoyl-CoA desaturase (SCD), an enzyme capable of adding a *cis-9* double bond to C18:1 *trans-11* to give *cis-9, trans-11* CLA. Corl et al. (2000) showed that the level of *cis-9, trans-11* in milk was reduced by over 60% after abomasal infusion of sterculic oil, a potent inhibitor of  $\Delta^9$ -desaturase. Regardless of the origin of CLA, manipulation of the bio-hydrogenation process remains the key to increasing CLA in milk by dietary means, either by increasing rumen production of CLA, or by increasing the rumen production of C18:1 *trans-11*.

Milk and meat from ruminants, therefore, contains more CLA than that of non-ruminants (Table 3). The amount of CLA found in whole milk is generally about 4.5 to 5.5 mg/g fat (approximately 0.45 to 0.55%), although variation of as much as 2.5 to 18 mg/g fat has been reported. Breed, age of the dairy cow, and stage of lactation may influence the milk CLA content to some degree but the effect of these parameters has not been well characterized. The CLA contents of meat and dairy products are altered little by processing, storage, or cooking and hence, the concentration in food depends primarily on the concentration in the raw material.

That CLA is produced in the rumen during the biohydrogenation process has been known for a long time. The unexpected effects of these fatty acids on health have only been discovered in more recent years.

### MANIPULATION OF THE DIET

The concentration of CLA in bovine milk fat can vary quite substantially depending on the feeding strategy adopted. For instance, pasture feeding has been found to

result in a much higher milk fat CLA concentration than that achieved with typical total mixed rations (TMR) based on conserved forage and grain. Dhiman et al. (1999) reported the CLA concentration of milk to be 22.1 mg/g fat with pasture feeding compared to 3.8 mg/g fat with TMR feeding. The exact reason for the effect of pasture on CLA levels is not certain. Similar enrichment of CLA has often been achieved when the TMR is supplemented with unsaturated fat from oilseeds. Kelly et al. (1998) supplemented the basal diet with 53 g/kg dry matter (DM) of peanut oil (high oleic acid), sunflower oil (high linoleic acid), or linseed oil (high linolenic acid). CLA concentrations were 13.3, 24.4, and 16.7 mg/g milk fat, respectively. The increase in CLA levels observed with the sunflower oil treatment represented levels approximately 500% greater than those typically seen in traditional diets. Chouinard et al. (1998) fed diets supplemented with 4% DM of calcium salts of fatty acids from canola oil, soybean oil, or linseed oil. The resulting milk CLA concentrations were 13.0, 22.0, 19.0 mg/g fat for canola oil, soybean oil,

**Table 3.** CLA content of various foods

Foodstuff	Total CLA content (mg/g fat)
Dairy products :	
Homogenized milk	5.5
Butter fat	4.7
Mozzarella cheese	4.9
Plain yogurt	4.8
Ice cream	3.6
Meats :	
Ground beef	4.3
Lamb	5.6
Pork	0.6
Chicken	0.9
Salmon	0.3
Ground turkey	2.5

(Chin et al., 1992)

and linseed oil respectively, and 3.5 mg/g fat for control. Soybean oil, which is high in linoleic acid, was most effective at increasing the CLA. The level and type of CLA isomers obtained using supplemental fat varies to a large extent depending on the ruminal conditions. In creased CLA production in the rumen, however, is pronounced when the oil was supplemented compared to oilseeds since the degree of hydrogenation depends upon the extent of degradation of oil seeds (Wang et al., 2002).

A study by Kelly and Bauman (1996) at Cornell University using supplemental fat found that the CLA levels in milk were halved when the forage:concentrate ratio of the diet was changed from 50:50 to 20:80. Furthermore, Griinari et al. (1998) showed that high concentrate diets could alter the products of rumen biohydrogenation of polyunsaturated fatty acids resulting in an increase in the proportion of *trans*-10 C<sub>18:1</sub> and *trans*-10, *cis*-12 CLA isomers.

Bell and Kennelly (2000) carried out a feeding trial with 28 lactating Holstein cows to determine if they could manipulate the animal's diet. The cows were fed one of four diets for a 15 day treatment period: (1) Control (CTL); (2) Low fat diet (LF); (3) High fat diet A (HFA); (4) High fat diet B (HFB). Both the low fat (LF) and high fat (HFA and HFB) treatments produced milk with a lower milk fat percentage than CTL, the greater reduction being seen for the high fat diets (Table 4). This is not surprising as a

reduction in milk fat is often observed when supplemental fat is added to the dairy ration.

The CTL diet resulted in milk fat with a *cis*-9, *trans*-11 CLA concentration of 0.49%, similar to that typically reported for whole milk (Table 5). Cows fed the HFB diet produced milk fat with 5.63% *cis*-9, *trans*-11 CLA, approximately 12 times greater than the CTL diet. Although the yield of fat was lower in the HFB treatment, the yield of CLA was still approximately nine times greater than the yield of CLA for the CTL treatment (Table 5).

The HFA and HFB diets also resulted in a significant increase in the amount of *trans* fatty acids (especially C18:1 *trans*-11) in the milk. In the past decade there has been an accumulation of evidence that suggests that *trans* fatty acids may contribute to the development of coronary heart disease (CHD, Willett et al., 1993). However, the study reported by Willett et al. (1993) showed that the association between *trans* fatty acids and CHD was specific for *trans* fatty acids from industrial hydrogenated fats, whereas *trans* fatty acids of animal origin were not correlated with CHD. The primary *trans* fatty acids in bovine milk are C18:1 *trans*-11 and CLA, whereas partially hydrogenated vegetable oils are characterized by a range of *trans* fatty acids such as C18:1 *trans*-8, *trans*-9, *trans*-10, *trans*-11, *trans*-12 and *trans*-13. As noted earlier, CLA has been found to inhibit cholesterol-induced atherosclerosis in rabbits and hamsters. Furthermore, there is evidence that

**Table 4.** Influence of dietary fat level on the yield and composition of milk

Items	CTL <sup>1</sup>	LF	HFA	HFB
Milk yield kg/day	26.87 <sup>a</sup>	27.58 <sup>a</sup>	26.78 <sup>a</sup>	27.82 <sup>a</sup>
Fat %	4.01 <sup>a</sup>	3.57 <sup>b</sup>	2.83 <sup>c</sup>	2.95 <sup>c</sup>
Protein %	3.33 <sup>a</sup>	3.37 <sup>a</sup>	3.11 <sup>a</sup>	3.23 <sup>a</sup>
Lactose %	4.3 <sup>ab</sup>	4.54 <sup>a</sup>	4.26 <sup>b</sup>	4.50 <sup>ab</sup>

Within a row, values with different superscripts are significantly different (p<0.05).

<sup>1</sup> CTL is Control; LF is low fat diet; HFA is high fat diet A; HFB is high fat diet B.

**Table 5.** Fatty acid composition of milk fat from cows fed different diets

Fatty acids <sup>1</sup>	CTL <sup>2</sup>	LF	HFA	HFB
C18:0	6.25 <sup>a</sup>	5.68 <sup>a</sup>	9.92 <sup>b</sup>	8.75 <sup>c</sup>
C18:1 <i>trans</i> -11	1.52 <sup>a</sup>	1.68 <sup>a</sup>	10.55 <sup>b</sup>	14.77 <sup>c</sup>
C18:1 <i>cis</i> -9	12.65 <sup>a</sup>	13.16 <sup>a</sup>	20.41 <sup>b</sup>	18.25 <sup>c</sup>
C18:1 (n12)	0.77 <sup>a</sup>	1.05 <sup>a</sup>	2.08 <sup>b</sup>	1.64 <sup>c</sup>
C18:1 (n7)	0.67 <sup>a</sup>	0.69 <sup>a</sup>	0.95 <sup>b</sup>	0.91 <sup>b</sup>
C18:1 (n6)	0.45 <sup>a</sup>	0.52 <sup>a</sup>	3.05 <sup>b</sup>	2.30 <sup>c</sup>
C18:2	1.51 <sup>a</sup>	1.62 <sup>a</sup>	2.97 <sup>b</sup>	2.82 <sup>b</sup>
Cis-9, <i>trans</i> -11 CLA	0.49 <sup>a</sup>	0.56 <sup>a</sup>	3.70 <sup>b</sup>	5.63 <sup>c</sup>
<i>Trans</i> -10, <i>cis</i> -12 CLA	ND <sup>a</sup>	ND <sup>a</sup>	0.054 <sup>b</sup>	0.054 <sup>b</sup>
<i>Trans/trans</i> CLA	0.033 <sup>a</sup>	0.046 <sup>a</sup>	0.15 <sup>b</sup>	0.17 <sup>b</sup>
Total CLA yield g/day	5.1 <sup>a</sup>	5.4 <sup>a</sup>	28.5 <sup>b</sup>	45.8 <sup>c</sup>

Within a row, values with different superscripts are significantly different (p<0.05).

<sup>1</sup> All values presented as percentage of fatty acid methyl esters.

<sup>2</sup> CTL is Control; LF is low fat diet; HFA is high fat diet A; HFB is high fat diet B.

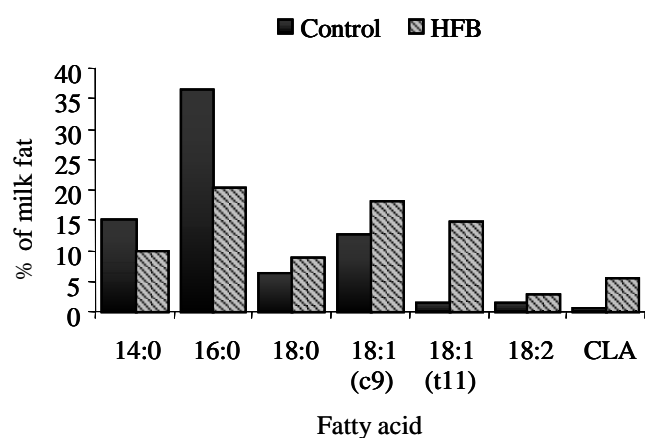
C18:1 *trans*-11 can be desaturated to *cis*-9, *trans*-11 CLA in human tissues (Salminen et al., 1998). Convincing evidence that *trans* fatty acids from milk have an adverse effect on health is lacking.

Overall, study by Bell and Kennelly (2000) showed that milk fat can be modified to give a more favorable composition (Figure 3).

Furthermore, it demonstrated the feasibility of producing CLA enriched milk using modifications to the diet of the cow. Work is underway to confirm these results using increased animal numbers per treatment.

The effect of oil supplementation on changes in fatty acid composition in beef has been controversial. Madron et al. (2002) carried out the feeding trial with 30 cross bred Angus steers to examine the effect of supplementation of extruded full fat soybean (ESB) which was high in linoleic acid (48.6%) on the CLA content in various depots. The crude fat (CF) percentage in concentrates increased by 2 to 4% by supplementation of ESB compared to that of control (3.9% CF). They found only a small increase in *cis*-9, *trans*-11, C18:2 from the high ESB supplementation (Table 6). Treatments were significantly different with the HESB diets, resulting in an average CLA concentration that was about 17% greater than that of the control. Theoretically, this would increase ruminal production of *trans*-11 C18:1 for the endogenous synthesis of CLA, but the observed increase in the CLA content in muscle lipid was relatively small.

Other studies in which steers were fed diets supplemented with soybean oil (Beaulieu et al., 2000, 2002) also observed little or no change in the CLA content of body fat. However, Enser et al. (1999) fed steers diets supplemented with 6% linseed oil and fish oil and observed a two-fold increase in the adipose tissue content of CLA. Mir et al. (2000) observed a similar increase in body fat of lambs fed a diet supplemented with 6% safflower oil.



**Figure 3.** Summary of the main changes in fatty acid composition of milk from cows fed the control diet (CTL) and high fat diet B (HFB).

Further studies are still required to confirm the supplemental effects of lipids high in linoleic acid on the CLA content in beef under various conditions.

### SYNTHETIC CLA

Conjugated linoleic acid can be synthesized in the laboratory from vegetable oils like sunflower. As noted earlier, CLA produced in this way tends to contain a mixture of CLA isomers. This type of product is already available commercially for feeding to swine because of its ability to improve lean gain in the growing animal. Synthetic CLA could be used to increase the CLA concentration in bovine milk if protected in some way from the rumen environment. Methods available to achieve this include encapsulation of the fat in formaldehyde-treated casein or feeding the fat as a calcium salt, although the extent of protection obtained with these methods can be variable.

Mixtures of CLA isomers have been found to have an inhibitory effect on milk fat synthesis (Lor and Herbein, 1999; Chouinard et al., 1999). The *trans*-10, *cis*-12 CLA appears to be isomer responsible for this effect (Baumgard et al., 2000). Abomasal infusion of *trans*-10, *cis*-12 in levels up to 14 g/day for five days produced a dose response reduction in milk fat yield and concentration in dairy cows (Baumgard et al., 2001). Feeding trials using calcium salts of CLA have demonstrated that they are an effective method of reducing milk fat percentage (Giesy et al., 1999; Sippel et al., 2001). A study using goats showed that CLA could also be protected from rumen digestion by encapsulating the CLA in a formaldehyde-treated casein (Gulati et al., 2000). In view of the potential of synthetic CLA as a method of increasing the concentration of CLA in bovine milk, Bell and Kennelly (2001) carried out a study to evaluate the effect of this product on milk yield and composition. Four Holstein cows received abomasal infusion of: (1) control, no fat infusion (CTL), (2) 150g/day of synthetic CLA, 31.7% *c*-9, *t*-11; 30.4% *t*-10, *c*-12 (CLA), (3) 150g/day of safflower oil (SAFF), and (4) 150g/day of tallow (TALL). Infusion was carried out for 20-22 hours/day for 11 day periods in a 4 × 4 Latin square design. Analysis of fatty acid composition showed that the concentration of CLA isomers increased significantly as a result of CLA infusion (Table 7). The concentration of linoleic acid (C18:2) was significantly increased with infusion of safflower oil (76% linoleic acid). Since the yield of milk fat was reduced with CLA infusion, the yield of all the fatty acids (except the CLA's) was significantly reduced with the CLA treatment (Table 8).

Bell and Kennelly (2000) did not observe these types of effects in the feeding trial described above were they had a

**Table 6.** Fatty acid composition of adipose tissue depots in steers

Fatty acids	Control	LESB <sup>1</sup>	HESB <sup>1</sup>
C18:0	14.23 <sup>b</sup>	15.52 <sup>a</sup>	16.52 <sup>a</sup>
C18:1, <i>trans</i> -6 to 10	1.34 <sup>b</sup>	1.73 <sup>a</sup>	1.80 <sup>a</sup>
C18:1, <i>trans</i> -11	1.33 <sup>b</sup>	1.42 <sup>b</sup>	1.71 <sup>a</sup>
C18:1, <i>cis</i> -9	39.28 <sup>a</sup>	38.52 <sup>ab</sup>	37.75 <sup>b</sup>
C18:2, <i>cis</i> -9, <i>cis</i> -12	1.61 <sup>b</sup>	1.67 <sup>ab</sup>	1.91 <sup>a</sup>
C18:3, <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.21 <sup>c</sup>	0.23 <sup>b</sup>	0.28 <sup>a</sup>
CLA, <i>cis</i> -9, <i>trans</i> -11	0.66 <sup>b</sup>	0.69 <sup>b</sup>	0.77 <sup>a</sup>

<sup>1</sup>Diets of LESB and HESB contain 12.7 and 25.6% of extruded full-fat soybeans, respectively.

**Table 7.** Fatty acid composition of milk fat from cows receiving abomasal infusion of CLA and other fatty acids

Fatty acid	CTL <sup>1</sup>	TALL	SAFF	CLA
C18:0	11.0 <sup>a</sup>	11.0 <sup>a</sup>	11.2 <sup>a</sup>	13.5 <sup>a</sup>
C18:1 <i>cis</i> -9	24.4 <sup>ab</sup>	25.6 <sup>a</sup>	22.8 <sup>b</sup>	18.2 <sup>c</sup>
C18:2	1.8 <sup>a</sup>	2.1 <sup>a</sup>	7.6 <sup>b</sup>	2.3 <sup>a</sup>
<i>Cis</i> -9, <i>trans</i> -11 CLA	0.59 <sup>a</sup>	0.61 <sup>a</sup>	0.58 <sup>a</sup>	1.77 <sup>b</sup>
<i>Trans</i> -10, <i>cis</i> -12 CLA	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	0.85 <sup>b</sup>

Within a row, values with different superscripts are significantly different ( $p < 0.05$ ).

<sup>1</sup> CTL is control (no fat infusion); TALL is infusion of 150 g/day beef tallow; SAFF is infusion of 150 g/day of safflower oil; CLA is infusion of 150 g/day of synthetic CLA.

ND=Not detected

large enrichment of *cis*-9, *trans*-11 CLA in the milk. This may suggest that the effects observed with the synthetic product were due to the *trans*-10, *cis*-12 isomer. As discussed already, *trans*-10 isomers of CLA have a potent inhibitory effect on milk fat synthesis. In the study by Bell and Kennelly (2000), infusion of 150 g CLA/day for 11 days also resulted in reduced fat content as well as other changes not previously noted with CLA infusion. This study demonstrated that post-ruminal delivery of CLA isomers could significantly increase the concentration of these fatty acids in milk. However, it also showed that the extent of enrichment possible for *trans*-10 isomers of CLA is limited because of other unacceptable effects on milk yield and composition. This places a constraint on the degree to which bovine milk could be used as a vehicle to increase the supply of *trans*-10, *cis*-12 CLA in the human diet.

#### CLA ENRICHED MILK AND BEEF – A NEW PRODUCT OPPORTUNITY?

The preceding sections illustrated the feasibility of producing CLA enriched milk and beef in some part. An important question is whether the degree of enrichment achieved will translate into any real benefit for the person consuming the milk. Intake of CLA in North America has been estimated at 52 to 137 mg CLA/day (Ritzenthaler et al., 1998). Extrapolation from animal studies has suggested that the level of CLA intake necessary to produce anti-carcinogenic effects in humans may be about 3 g per day.

Using the CLA percentage achieved with the HFB diet (Table 5), one serving of whole milk (460 mg CLA) and a sandwich with butter (365 mg CLA) and cheddar cheese (721 mg CLA) would provide 1546 mg (1.546 g) CLA. This example illustrates how CLA enriched milk and milk products could supply dietary CLA at levels that may potentially benefit health, without the need for unrealistic changes to eating habits.

Production of the CLA enriched beef is known to be more difficult work than that of milk although we found some possibility. But the effort to produce the CLA enriched beef will be continued. The concept of enhancing the levels of health promoting fatty acids in food is not new. A good example of this has been the introduction of eggs enriched in omega-3 fatty acids. This recognizes the trend among consumers towards an increased desire to make diet choices that promote good health. Of course, consumers could increase their CLA intake by taking synthetic CLA in pill form, which is already available in health food stores. The main difference between the CLA in these products and milk CLA is the broader range of isomers in the synthetically produced CLA. The relative value for human health of this range of CLA isomers compared to the CLA found in ruminant milk fat is uncertain. Nevertheless, CLA enriched milk produced through manipulation of the dairy ration has an advantage over this type of product in that it can be promoted as a “natural” source of CLA. It may also be easier for CLA enriched milk to gain acceptance since milk already has a wide distribution and consumers are well accustomed to seeing a broad variety of dairy products in

**Table 8.** Yield of fatty acids in milk fat from cows receiving abomasal infusion of CLA and other fatty acids

Fatty acid (g/day)	CTL <sup>1</sup>	TALL	SAFF	CLA
C18:0	58.5 <sup>a</sup>	60.3 <sup>a</sup>	70.5 <sup>a</sup>	29.8 <sup>b</sup>
C18:1 cis-9	130 <sup>a</sup>	137 <sup>a</sup>	142 <sup>a</sup>	38.7 <sup>b</sup>
C18:2	9.5 <sup>a</sup>	10.9 <sup>a</sup>	46.8 <sup>b</sup>	4.7 <sup>c</sup>
Cis-9, trans-11 CLA	3.08 <sup>a</sup>	3.31 <sup>a</sup>	3.59 <sup>a</sup>	3.90 <sup>a</sup>
Trans-10, cis-12 CLA	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	1.86 <sup>b</sup>

Within a row, values with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup> CTL is control (no fat infusion); TALL is infusion of 150 g/day beef tallow; SAFF is infusion of 150 g/day of safflower oil; CLA is infusion of 150 g/day of synthetic CLA.

the shops. The challenge will be in overcoming the existing public perception regarding milk fat and health.

### CONCLUSION

That a compound exists in ruminant fat with such potent health promoting effects has been an unanticipated discovery. The ability to enhance the concentration of CLA through manipulation of the diet demonstrates the feasibility of producing CLA enriched dairy products. As consumers become more and more conscious of the link between food and health, milk and beef designed to have enhanced levels of CLA may provide new market opportunities for the ruminant products.

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