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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Factors Affecting Conjugated Linoleic Acid (CLA) Content in Milk, Meat, and Egg: A Review

R.C. Khanal and K.C. Olson
Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT 84322, USA

Abstract: An increasing interest in enhancing the conjugated linoleic acids (CLA) content in food products is attributed to its potential anti-carcinogenic, anti-diabetic, anti-obesity, anti-atherogenic, and immunomodulatory functions in experimental animal models. It is synthesized in the rumen from linoleic acid or from the endogenous conversion of *t*-11 $C_{18:1}$ in the mammary gland by Δ^{-9} desaturase. More than a dozen isomers of CLA have been detected in foods of ruminant origin, of which *c*-9, *t*-11 comprising 80 to 90%, and *t*-10, *c*-12 comprising 3-5% of total CLA are the isomers with known physiological importance. Although food products from ruminants are the richest source of CLA for humans, it is possible to enhance the CLA content of foods from non-ruminants by supplementing CLA in the diet. The CLA content in milk, meat, or egg varies greatly from a low 0.1% or less to a high 2% or more of the milk, tissue, or egg yolk lipids, with milk lipids from ruminants having the highest concentrations. A host of factors appear to affect the CLA content in milk, meat, and other food products from various species of animals, which could be broadly classified into diet, animal, and post-harvest related factors. Of all these factors, animal diet is the primary one and could be manipulated to a great extent for enhancing the concentration of CLA in food products, both from ruminants and non-ruminants. While animal-to-animal variation is also of great significance, post-harvest related factors appear to be of minor importance. In this context, the CLA content of milk, meat, and egg, and the factors affecting its concentration have been reviewed. Understanding the various factors affecting the CLA content in food products will have practical implications to the dairy, meat, or egg producers for its enrichment in food products so we can derive the potential health benefits associated with CLA.

Key words: CLA, milk, meat, egg, food products

Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (*c*-9, *c*-12 $C_{18:2}$, LA) with two conjugated unsaturated double bonds at various carbon positions. It is formed as an intermediate during the biohydrogenation of LA to stearic acid by *Butyrivibrio fibrisolvens* (Kepler *et al*., 1966) and other rumen bacteria (Kritchevsky, 2000) or from the endogenous conversion of transvaccenic acid (*t*-11 $C_{18:1}$, TVA) by Δ^9-desaturase in the mammary gland (Corl *et al*., 2001; Griinari and Bauman, 1999). An increasing interest on CLA is attributed to its potential health benefits such as anticarcinogenic, antiatherogenic, antidiabetic, and antiadipogenic effects (Banni *et al*., 2003; Belury, 2003; Kritchevsky, 2003; Pariza, 1999).

Food products from ruminants are the richest source of CLA for humans. The CLA content in milk or meat varies greatly from a low 0.2% or less to a high 2% or more of the milk or tissue fat, with milk having higher concentrations than meat. It is affected by a host of factors, which could be broadly classified into diet, animal, and post-harvest related factors. Of all these factors, animal diet appears to affect it the most and has been given its due importance in enhancing the CLA content of food products. This is second of the two reviews related to the synthesis and content of CLA in milk, meat, and egg wherever possible, and the factors affecting it. While the first one reviewed the history and biosynthesis of CLA in ruminants, the objective of this review is to critically evaluate the importance of each of the factors affecting the concentration of CLA in milk, meat, and egg, whenever pertinent, and how such factors can be utilized to enhance the CLA isomers in these foods so humans can derive the potential health benefits associated with it.

CLA content in milk, meat, and egg: In food, CLA is found in milk fat, tissue fat, and to a lesser extent in egg yolk as well as the products derived from them. Its content is much higher in foods derived from ruminants than those from non-ruminants because of the ability of ruminants to biohydrogenate dietary unsaturated fatty acids (FA) with the help of bacteria present in the rumen. As a result, food products from ruminants are the principal dietary source of CLA. More than a dozen isomers of CLA have been detected in milk and meat fat from ruminants (Bauman *et al*., 2003), which are presented in Table 1 together with their proportion. The principle isomer present in both milk and meat fat is the *c*-9, *t*-11 CLA that accounts for 80 to 90% of the total CLA present (Chin *et al*., 1993). In fact, very few investigators have reported CLA isomers other than *c*-9, *t*-11 until recently, since the techniques used did not allow them to detect other isomers (Jensen, 2002). As a result the

Table 1: The mean positional and geometric isomer composition (% of total isomers) and the CLA content of samples of milk, butter, cheese, and beef fat[1]

CLA isomer	Milk[2]	Butter	Cheese	Beef
cis, trans-isomers				
7, 9	5.5	6.7	3.6	7.0
8, 10	1.5	0.3	1.0	2.6
9, 11	72.6	76.5	83.5	72.0
10, 12	0.4	1.1	-	2.6
11, 13	7.0	0.4	4.7	1.1
11, 13	-	-	-	2.2
12, 14	0.7	0.8	0.4	0.7
Total *cis,trans* (*trans,cis*)	87.7	85.8	93.2	88.2
trans, trans isomers				
6, 8	-	0.1	0.7	
7, 9	2.4	-	0.6	1.5
8, 10	0.4	-	0.3	0.7
9, 11	2.0	-	1.5	3.7
10, 12	0.6	-	0.5	1.9
11, 13	4.2	-	2.3	1.9
12, 14	2.8	-	0.9	1.9
13, 15	-	-	0.1	-
total *trans, trans*	12.3	9.4	6.3	12.3
cis, cis isomers				
8, 10	-	-	<0.1	-
9, 11	-	-	0.3	-
10, 12	-	-	<0.1	-
11, 13	-	-	0.3	-
total *cis, cis*	-	4.8	0.7	-
Total CLA (% of fat)		0.5	0.93	0.27

[1]Adapted from Parodi (2003). [2]Shingfield *et al.* (2003).

reported values are tilted slightly in favor of *c*-9, *t*-11 CLA, because some other isomers, e.g., *t*-7, *c*-9, and $C_{20:1}$ and $C_{20:2}$ co-elute with it (Yurawecz *et al.*, 1998; Jensen, 2002). Although active biologically, *t*-10, *c*-12 isomer is present in amounts less than 5% of the total CLA and is not reported in many cases. Another isomer of quantitative importance for which the biological roles have not been reported is *t*-7, *c*-9, which comprises 3 to 16% of the total CLA present in ruminant products (Yurawecz *et al.*, 1998). Although *c*-9, *c*-11 has been shown to be the most effective isomer against breast cancer cells recently (Tanmahasamut *et al.*, 2004), its presence in milk and meat has rarely been reported. With the advancement in analytical techniques, it could be expected that each of the isomers will be quantified and their proportion in milk, meat, or egg determined more accurately.

The CLA content of milk and other dairy products from different species of animals produced under a variety of dietary conditions is given in Table 2 and that of meat, meat products, and chicken eggs is given in Table 3. The CLA reported is almost exclusively *c*-9, *t*-11 isomer, which will be the isomer referred to as CLA throughout this review unless otherwise noted. Although the CLA content of both dairy and meat products may appear to be unique to the specific products, it is not the case and depends largely on the CLA content of the original milk and meat fats. For meat products, it also depends on the sampling site, e.g., lean versus fatty steak and muscle versus tissue fat. A closer look at both the tables indicates clearly that cows' milk is the richest source of CLA, which has been found to vary from as low as 0.2% to as high as 3.7% of total milk fat. Only small amounts of CLA have been found in the milk, meat, or egg from non-ruminants (Bee, 2000a; Chin *et al.*, 1992, 1993). The CLA content in human milk seems to be around 0.1% (Park *et al.*, 1999). In egg yolk lipids, it was not even detected when laying hens were fed a normal concentrate diet (Raes *et al.*, 2002; Yang *et al.*, 2002). Similar was the case when lactating sows were fed a concentrate diet supplemented with LA (Bee *et al.*, 2000a). In tissue fat of chicken or swine fed normal concentrate diets, the CLA content was only around 0.1% of the total reported fat (Chin *et al.*, 1993). However, feeding CLA as low as 1% of the diet may boost its content in the food products of non-ruminant origin by several folds (Raes *et al.*, 2002; Ramsay *et al.*, 2001; Thiel-Cooper *et al.*, 2001). This would make it comparable to the levels observed in milk and meat from ruminants produced under the best dietary

Table 2: The CLA content (% of fat) in dairy products from ruminants

Products	Breed/Species	Diet	Content	Reference
Milk	Holstein	TMR	0.44	Kelsey *et al.* (2003)
Milk	Holstein	All pasture	2.5	Khanal *et al.* (2003a)
Milk	Holstein	All pasture	1.7	Khanal *et al.* (2002)
Milk	Holstein	Pasture + extruded soybean	1.7	Khanal *et al.* (2002)
Milk	Holstein	Pasture + extruded rapeseed	2.5	Lawless *et al.* (1998)
Milk	Holstein	TMR + canola seed	1.4	Ward *et al.* (2002)
Milk	Holstein	TMR + flax seed	1.2	Ward *et al* (2002)
Milk	Holstein	Pasture + grain mix	0.72	White *et al.* (2001)
Milk	Holstein	TMR + 1% Fish oil	0.73	AbuGhazaleh *et al.* (2003)
Milk	Holstein	Pasture + 150 g fish oil	3.3	Kay *et al.* (2003)
Milk	Holstein	TMR + 3.6% soy oil	2.1	Dhiman *et al.* (2000)
Milk	Holstein	TMR + 5.3% linseed oil	1.67	Kelly *et al.* (1998a)
Milk	Holstein	TMR + 5.3% sunflower oil	2.44	Kelly *et al.* (1998a)
Milk	Jersey	TMR	0.32	White *et al.* (2001)
Milk	Jersey	Pasture + 5.5 kg concentrate	0.59	White *et al.* (2001)
Milk	Brown Swiss	TMR	0.41	Kelsey *et al.* (2003)
Milk	Normande	All pasture	1.7	Lawless *et al.* (1998)
Milk	Water buffalo	-	0.84	Lal and Narayanan (1984)
Milk	Goat	Various	0.58-1.1	Parodi (2003)
Milk	Sheep	Various	1.2-3.0	Parodi (2003)
Milk	Human	-	0.09-0.49	Park *et al.* (1999)
Cheese	Holstein	All pasture	1.5	Khanal *et al.* (2003a)
Cheese	Holstein	Pasture + extruded soybean	1.4	Khanal *et al.* (2002)
Cheese	Holstein	TMR	0.34	Dhiman *et al.* (1999b)
Cheese	Holstein	TMR + extruded soybean	0.73	Dhiman *et al.* (1999b)
Cheese	Holstein	TMR + extruded cottonseed	0.60	Dhiman *et al.* (1999b)
Cheese	Sheep	-	0.8-2.0	Prandini *et al.* (2001)
Cheese	Goat	-	0.27-0.69	Wolff (1995)
Cheese	Mozzarella	-	0.43	Lin *et al.* (1995)
Cheese	Cheddar	-	0.40-0.47	Lin *et al.* (1995)
Cheese	Swiss	-	0.55	Lin *et al.* (1995)
Yogurt	-	-	0.44	Ma *et al.* (1999)
Yogurt	-	-	0.38	Lin *et al.* (1995)
Butter	-	-	0.61	Chin *et al.* (1993)
Butter	-	-	0.47	Ma *et al.* (1999)
Ghee	Buffalo	TMR	0.50	Aneja and Murti (1990)
Ghee	Cattle	-	0.60	Aneja and Murti (1990)
Sour cream	Cattle	-	0.41	Lin *et al.* (1995)
Buttermilk	Cattle	-	0.47	Lin *et al.* (1995)
Evaporated milk	Cattle	-	0.34-0.64	Lin *et al.* (1995)

conditions conducive for higher CLA concentrations. Economics of such an approach and the effect on other aspects of swine or poultry production need to be investigated in further detail.

Factors affecting CLA content: A host of factors appear to affect the CLA content in milk and meat from ruminants. As a result, a large variation occurs among the milk and meat samples collected from a host of individual animals fed the same diet and raised under similar conditions. Such factors could be divided into three broad categories: a. diet related, b. animal related, and c. post-harvest related. A short description of the effect of these factors on CLA content of milk and meat is given in Table 4.

Diet related factors: Various researchers have shown an increased CLA content in milk and meat from ruminants by grazing cows on pasture (Kay *et al.*, 2004; Khanal *et al.*, 2003a; Loor *et al.*, 2002b; Dhiman *et al.*, 1999a), supplementing total mixed rations (TMR) containing 50% forage and 50% concentrate with plant oils or oil seeds (Khanal *et al.*, 2003b; 2002, Lock and Garnsworthy, 2002; Madron *et al.*, 2002), and supplementing TMR with fish oil (Abu-Ghazaleh *et al.*, 2003; Donovan *et al.*, 2000). Manipulation of animal diet

Table 3: The CLA content (% of fat) in various meats and processed meats from ruminants and non-ruminants

Products	Species/breed	Diet	Content	Reference
Beef	Heifers	Concentrate + soy oil	0.34	Beaulieu *et al.* (2002)
Beef	Cattle	Concentrate + extruded soybean	0.73	Madron *et al.* (2002)
Beef	Cattle	All concentrate	0.12	Mir *et al.* (2000)
Beef	Cattle	All pasture finished	1.5	Poulson (2001)
Beef	Cattle	Grass based + concentrate	1.1	French *et al.* (2000)
Ground beef	Cattle	-	0.16	Ma *et al.* (1999)
Ground beef	cooked beef		0.18	Ma *et al.* (1999)
Rib roast	beef	-	0.30	Ma *et al.* (1999)
Rib roast	Cooked beef	-	0.29	Ma *et al.* (1999)
Sirloin	Beef	-	0.12	Ma *et al.* (1999)
Sirloin	Cooked beef	-	0.28	Ma *et al.* (1999)
Beef	Charolais	Concentrate based + linseed	0.80	Enser *et al.* (1999)
Beef	Charolais	Concentrate based + fish oil	0.57	Enser *et al.* (1999)
Beef	Angus × Hereford	Finishing diet + soy oil	0.28	Griswold *et al.* (2003)
Veal	Cattle	-	0.27	Chin *et al.* (1992)
Lamb	Sheep	-	0.35-0.90	Ivan *et al.* (2001)
Adipose tissue	Sheep	Browsed	1.7	Banni *et al.* (1996)
Lamb	Sheep	-	0.06-0.31	Mir *et al.* (2000)
Lamb	Sheep	Browsing	1.5	Fogerty *et al.* (1988)
Lamb	Sheep	Beet pulp + safflower	0.65-0.98	Bolte *et al.* (2002)
Lamb	Sheep	-	0.56	Chin *et al.* (1992)
Ground turkey	Turkey	-	0.25	Chin *et al.* (1993)
Chicken	Chicken	-	0.09-0.2	Chin *et al.* (1992)
Pork	Swine	-	0.12	Chin *et al.* (1993)
I/M[1] fat	Swine	2.5% CLA in diet	1.0	Joo *et al.* (2002)
S/C[2] fat	Swine	1.0% CLA in diet	2.16	Thiel-Cooper *et al.* (2001)
Lean tissue	Swine	1.0% CLA in diet	0.37	Thiel-Cooper *et al* (2001)
S/C[2] fat	Swine	1.0% CLA in diet	4.0	Ramsay *et al.* (2001)
Back fat tissue	Swine	2.0% CLA in diet	2.0	Bee (2000b)
Omental fat	Swine	2.0% CLA in diet	2.2	Bee (2000b)
L. dorsi	Swine	2.0% CLA in diet	0.98	Bee (2000b)
Breast muscle	Broiler	1.0% CLA in diet	5.2	Szymczyk *et al.* (2001)
Belly fat	Swine	1% CLA oil in diet	0.76	Eggart *et al.* (2001)
L. muscle	Swine	1% CLA oil in diet	0.28	Eggart *et al.* (2001)
Egg yolk	Chicken	1.0% CLA in diet	0.30	Jones *et al.* (2000)
Egg yolk	Chicken	1.0% CLA in diet	1.4-3.2	Raes *et al.* (2002)
Egg yolk	Chicken	Concentrate	ND[3]	Raes *et al.* (2002)
Egg yolk	Chicken	Concentrate	ND[3]	Yang *et al.* (2002)

[1] Intramuscular. [2] Subcutaneous. [3] Not detected.

has been the focus for increasing the CLA content of foods from ruminants (Jiang *et al.*, 1996; Jahries *et al.*, 1997; Lawless *et al.*, 1998; Dhiman *et al.*, 2000), and will continue to be the mainstay of animal nutrition research intended for enhancing CLA content in milk, meat, or even egg (Kay *et al.*, 2004; Griswold *et al.*, 2003; Khanal *et al.*, 2003b; Sackman *et al.*, 2003; Watkins *et al.*, 2003). Manipulation of animal diet primarily involves supplying LA or linolenic acid (*c*-9, *c*-12, *c*-15 $C_{18:3}$, LNA) as substrates for rumen biohydrogenation. Depending on the type, plant oils or oil seeds contain LA or LNA as the major FA, whereas pasture predominates LNA, and fish oil predominates FA of 20 or 22 carbons as the major FA. During the process, CLA or its precursor TVA escapes further biohydrogenation. The TVA is then endogenously converted to CLA in the mammary gland (Griinari and Bauman, 1999) and probably in the adipocytes (Gillis *et al.*, 2003) using Δ^9-desaturase. Dietary effect is related probably more to the FA composition (Table 5) of the material used than anything else. Supplementation of synthetic CLA isomers has also been investigated both in ruminants and non-ruminants to enhance the CLA content in milk, meat or egg. It supplies the CLA isomers to be incorporated directly into milk, meat, and egg. In case of ruminants, it is usually provided in rumen-protected form.

Fats, oils, and oilseeds: Fats, oils, and oilseeds can be quite different in their FA composition (Table 5), and accordingly could be expected to have varying effects on milk fat CLA content. A closer look at the results observed thus far indicates that fats, oils, and oilseeds that are rich in LA are very effective in increasing milk fat CLA content of cows fed total mixed rations (TMR) containing 50% forage and 50% concentrate (Whitlock *et al.*, 2003; Ward *et al.*, 2003; Dhiman *et al.*, 2000). Therefore, it could be anticipated that oils or the seeds of soybean, sunflower, safflower, solin, and cottonseed would increase the CLA content of cows' milk fat when

Table 4: Factors affecting CLA content in milk and meat

Factors	Effect on CLA	Relevant reference
A. Diet related		
I. Pasture related		
Fresh/lush green pasture	Highly positive	Dhiman *et al.* (1999a)
Pasture + full fat extruded soybean	No effect	Khanal *et al.* (2003b)
Pasture + soy oil	No effect	Kay *et al.* (2002)
Pasture + fish oil	Positive	Kay *et al.* (2003)
Maturity of pasture	Negative	Loor *et al.* (2002a)
Diversity in plant species	Positive	Collomb *et al.* (2002b)
Elevation of pasture	Highland>mountain>lowland	Collomb *et al.* (2002a)
Fresh cut pasture	Fresh>conserved	-
II. High forage diet	Positive	Jiang *et al.* (1996)
III. High grain diet	Negative	Jiang *et al.* (1996)
IV. Raw oil seeds	Minimal	Dhiman *et al.* (2000)
V. Roasted oil seeds/meals	Positive	Several
VI. Extruded oilseeds	Positive, better than roasted oil seeds	Several
VII. Plant oils	Positive, better than processed seeds	Several
VIII. Fish meal	Positive, efficient than plant seeds	Several
VIII. Fish oil	Positive, efficient than plant oils	Several
IX. Ca salts of fatty acids	Positive	Chouinard *et al.* (2001)
X. Marine algae	Positive	Franklin *et al.* (1999)
XI. Rumen pH	>6.0 pH positive	Martin and Jenkins (2002)
XII. CLA supplementation	Positive	Several
XIII. Trans fats	Positive	Porter (2003)
XIV. Ionophores	Probably positive	Fellner *et al.* (1999)
XV. Low energy diet	Probably positive	Timmen and Patton (1988)
B. Animal related		
I. Species	Ruminants>non-ruminants	Several
II. Breed	Holstein>Brown Swiss>Normandes>Jersey	Lawless *et al* (1999)
III. Stage of lactation	Minimal	Kelsey *et al.* (2003)
IV. Parity	Minimal	Kelsey *et al.* (2003)
V. Parity	Some	Lal and Narayanan (1984)
VI. Age	?	-
VII. Animal to animal	Positive with higher Ä9-desaturase activity	Kelsey *et al.* (2003)
C. Processing, overall	Debatable	Review by Parodi (2003)
I. Milk into cheese	Minimal	Dhiman *et al.* (1999b)
II. Milk into cheese	Minimal	Khanal *et al.* (2003b)
IV. Milk into yogurt	Probably positive	Review by Parodi (2003)
V. Milk into butter	Minimal	Baer *et al.* (2001)
VI. Cooking	Probably minimal	Ma *et al.* (1999)
III. Aging of cheese	Minimal	Allred (2004)
VII. Aging of cheese	Probably minimal	Lin *et al.* (1995)
VIII. Heat treatment of milk	Probably minimal	Lin *et al.* (1995)

fed in TMR. Similarly the oil or seeds from linseed, which is rich in LNA, and rapeseed, canola, peanut, and olive, which are rich in oleic acid (*c*-9, $C_{18:1}$; OLA) but also have some LA and LNA, have also been shown to increase milk fat CLA (Whitlock *et al.*, 2002; Loor *et al.* 2002a; Dhiman *et al.*, 2000). When the effect of different oil treatments (peanut oil, sunflower oil, and linseed oil, which are high in OLA, LA, and LNA, respectively) on milk fat CLA were compared, sunflower oil resulted in highest CLA concentration in milk fat (Kelly *et al.*, 1998a). Similarly, Dhiman *et al.* (2000) demonstrated that soybean oil is more effective than linseed oil in increasing the milk fat CLA content.

Such effects are not limited to cows' milk fat. Feeding linseed oil, high LA sunflower seeds/oil, or high OLA sunflower oil to lactating goats also increased milk fat CLA, the greatest response being to sunflower seeds/oil, which provided the highest amount of LA in the diet (Chilliard *et al.*, 2003). However, feeding the seeds from linseed or soybean to goats fed a low forage diet (30:70 forage to concentrate ratio) did not increase goat milk fat CLA (Chilliard *et al.*, 2003). It could be

Table 5: Principal fatty acids (% of fat1) present in the dietary ingredients used for enhancing CLA content in milk and meat

Dietary ingredients	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Cotton seed/oil[2]		23.4	2.2	16.5	57.4	
Extruded soybean/ soy oil[2]		14.5	3.8	19.5	53.2	9.1
Rapeseed/oil[3]		4.9	1.5	57.2	21.4	11.4
Linseed/oil[3]		5.6	3.7	17.7	15.4	57.2
Sunflower seed/oil						
High oleic[4]	0.1	4.6	2.3	79.3	13.2	0.4
High linoleic[3]		6.3	3.7	22.8	67.0	
Safflower seed/oil[4]	0.1	5.9	2.4	15.2	76.0	0.3
Canola seed/oil[5]	4.1	1.8		58.9	22.0	13.2
Palm oil[6]	1.5	43.6	3.2	45.7	2.2	0.2
Olive oil[7]		13.0	2.5	74.0	9.0	
Peanut seed/oil[8]		12.3	3.2	51.5	33.2	
Corn oil[9]		10.6	2.0	27.3	57.5	1.0
Solin seed[10]	0.4	11.7	5.3	13.0	53.5	12.2
Fish oil[11]	14.0	26.0	2.0	2.0	3.0	2.0
Tallow[12]	3.2	26.6	20.0	44.0	3.5	
Pasture[13]						
Grass	0.6	21.0	1.8	2.1	21.1	53.4
Clover	0.5	24.1	3.5	3.8	22.0	45.4
Alfalfa hay[2]	20.7	35.8	3.3	3.4	15.3	21.5
Corn silage[2]	6.9	16.7	1.9	16.8	54.9	2.7

[1] May not add to 100 because not all fatty acids are reported. [2] Dhiman et al. (1999b).
[3] Lock and Garnsworthy (2002). [4] Loor and Herbein (2003).
[5] Delbicchi *et al.* (2001). [6] Bremmer *et al.* (1998).
[7] Palmquist (1988). [8] Kelly *et al.* (1998a).
[9] Duckett *et al.* (2002), [10] Ward *et al.* (2003).
[11] Porter (2003; fish oil contained 10% of C16:1, 17 % *trans*-C18:1, and 23% of longer chain FA with 20 or more carbons). [12] Beam *et al.* (2000). [13] Loor *et al.* (2002b).

possible that low forage in the diet reduced rumen pH below 6.0, which has a negative effect on both CLA and TVA concentrations in the rumen (Troegeler-Meynadir *et al.*, 2003; Martin and Jenkins, 2002). Supplementation of LA to cows fed conventional TMR diets appears to have an edge over supplementation of LNA, probably because it contributes to both increased CLA and TVA production in the rumen, which ultimately becomes the substrate for CLA synthesis by Δ^9-desaturase in the mammary gland, whereas LNA contributes to TVA only during its biohydrogenation (Harfoot and Hazlewood, 1988). When calcium salts of plant oils were fed to lactating dairy cows, increased concentrations of milk fat CLA and TVA were observed (Chouinard *et al.*, 2001) with effects relatively smaller compared to supplementing free oils. This could be the result of increased protection of LA and LNA from biohydrogenation in the rumen.

Supplementing roasted or extruded oil seeds seems to have a greater effect on milk fat CLA content than raw seeds (Dhiman *et al.*, 2000), possibly because of low release of oil from raw seeds in the rumen compared with heat-treated seeds. Low release of oil might lead to complete biohydrogeation of LA to stearic acid in the rumen, and thus little or no effect on milk fat CLA content. Moreover, microorganisms responsible for biohydrogenation of poly-unsaturated FA must have access to the oil, which is probably very poor with raw seeds. Chouinard *et al.* (1997) fed cows with soybeans processed by grinding, micronizing, roasting, or extrusion. It was found that milk fat TVA was highest for cows fed extruded soybeans and lowest for cows fed ground soybeans. Extruding, micronizing, or roasting of soybeans resulted in two or three fold increases in milk fat CLA contents compared with a control diet containing ground soybeans (Chouinard *et al.*, 2001). It appears that FA was accessible to rumen microbes when the oil seeds were processed and that heat treatment further increased the accessibility. These studies also indicate the importance of oilseed processing on ruminal biohydrogenation and the ensuing beneficial effects on milk fat CLA and TVA concentrations. Extruding full fat soybeans at different temperatures, however, had no significant effect on milk fat CLA concentration (Chouinard *et al.*, 2001).

Fish oil has been found equally or even more effective than plant oils or oil seeds in increasing milk fat CLA content from cows fed conventional TMR diets (Whitlock *et al.*, 2002; Chouinard *et al.*, 2001; Donovan *et al.*, 2000). The highest concentration of milk fat CLA (2.2 to 2.5% of the milk fat) with fish oil supplementation has been achieved when it was included at 2% of the diet DM (Baer *et al.*, 2001; Ramaswamy *et al.*, 2001; Donovan *et al.*, 2000) with no further increase when included at 3% of the diet DM (Donovan *et al.*, 2000). Dietary supplementation of fish oil at 200 or 400 ml/d in one feeding also increased CLA content threefold (Chouinard *et al.*, 2001). Feeding such amounts of fish oil usually reduces the milk fat content (Whitlock *et al.*, 2002; Donovan *et al.*, 2000), thus reduces the overall CLA and TVA yields in the milk. However, it was possible to minimize the reduction in milk fat by combining the fish oil with other sources of LA and LNA (AbuGhazaleh *et al.*, 2003; Whitlock *et al.*, 2002; Ramaswamy *et al.*, 2001).

The mechanism by which fish oil supplementation increases concentration of milk fat CLA and TVA is not clear. Fish oil is rich in longer chain poly-unsaturated FA of 20 and 22 carbons, which are not the likely candidates to yield CLA and TVA directly during biohydrogenation in the rumen. It has been proposed that the longer chain poly-unsaturated FA from fish oil inhibit the complete biohydrogenation of LA in the rumen by inhibiting the growth of bacteria responsible for hydrogenating TVA or through the inhibition of their hydrogenases (Griinari and Bauman, 1999) leading to an increased escape of TVA from the rumen. Franklin *et al.* (1999) reported a six or seven fold increase in milk fat CLA content when a control diet was supplemented with marine algae (*Schizochytrium sp.*). Further research is needed to describe the pathways for rumen biohydrogenation of longer chain poly-unsaturated FA from fish oils and other FA sources of marine origin.

Another form of supplementing FA is tallow, which is rich in long chain saturated FA and can be supplemented in protected or unprotected forms. Its effect on milk fat CLA is minimal (Chouinard *et al.*, 1998), because it is the 18-carbon unsaturated FA that yield CLA or TVA upon biohydrogenation in the rumen and not the saturated FA that are present in higher amounts in tallow. There was no increase in bacterial TVA content, and the milk fat TVA content was low (1.6%) for cows supplemented with tallow at 4 or 5% in the diet (Pantoja *et al.*, 1996).

Pasture: Changes in FA composition and the presence of conjugated dienes in milk fat were noticed as early as 1935 when cows were turned out to pasture during summer (Booth *et al.*, 1935). The total conjugated dienes in milk fat increased from 0.4 to 0.8% during winter when cows were kept indoors to 1.3 to 2.5% during summer when cows were grazed on pasture (Kuzdal-Savoie and Kuzdal, 1961). Riel (1963) studied the seasonal variation of conjugated dienes in Canadian milk and showed 2 to 3-folds increase in milk fat conjugated dienes during summer when cows were turned out to pasture. He observed lowest concentration of milk fat conjugated dienes at 0.6% of the total fat in March, which is higher than what we observe in present day milk (0.4 to 0.5%) produced from cows fed TMR. It is possible that the cows then were fed a diet with a higher proportion of forage that would increase CLA content in milk fat compared with present day TMR. The highest concentration at 1.65% of the total milk fat was observed in June and September. The dramatic increase in milk fat CLA contents after turning cows out to pasture were established later in a series of experiments (Jahries *et al.*, 1997; Stanton *et al.*, 1997, Kelly *et al.*, 1998b; Lawless *et al.*, 1998; Dhiman *et al.*, 1999a) and continue to be the mainstay of research for enhancing CLA content in cows' milk (Kay *et al.*, 2004; Schroeder *et al.*, 2003; Stockdale *et al.*, 2003; Ward *et al.*, 2003).

Initially it was shown that the abrupt change from indoor feeding (grass silage, hay, and beets) to grazing cows on pasture increased conjugated dienes sharply until a maximal effect was reached after 5 days (Kuzdal-Savoie and Kuzdal, 1961). However, Khanal *et al.* (2003a) showed that milk fat CLA content continued to increase until d 23 after turning cows out to pasture and stabilized at 2.54% of milk fat, which was 550% of the original level, until d 29, after which cows were withdrawn from pasture. Both milk fat and milk fat TVA contents followed a similar trend. This may be the time required for adaptation of the rumen microbes to the changing diet as well as the physiology of milk fat synthesis on the basis of type and quantity of FA supplied through the diet. It was also shown that only 4 d were needed to bring the milk fat CLA and TVA contents back to their original level once the cows were withdrawn from pasture and put back on a similar TMR diet (Khanal *et al.*, 2003a).

Cows grazing lush green pastures seem to produce milk fat with highest concentrations of CLA. Supplementation of cows grazing on pasture with feeds rich in LA provides an extra source of substrates for CLA and TVA in addition to LNA provided through pasture. In an effort to study whether such a strategy would have an added effect on milk fat CLA contents, Khanal *et al.* (2002) supplemented cows grazing lush green pasture with full fat extruded soybeans (FFES) or soy oil and observed no significant increase ($P > 0.05$) in CLA contents of milk, blood serum, or rumen digesta nor was there a significant increase ($P > 0.05$) in milk fat CLA yields. Later, Khanal *et al.* (2003b) supplemented cows grazing on pasture with a grain mix containing 75, 10, 10, and 5% of FFES, ground corn, beet pulp, and molasses, respectively and compared the milk fat CLA contents with cows grazing on pasture alone. Once again there was no increase in milk fat CLA content for cows grazing on pasture supplemented with feeds high in LA compared with pasture alone cows. Similarly, Kay *et al.* (2002) drenched cows grazing on pastures with 500 ml of sunflower oil for four days and found no difference in milk fat CLA contents compared with pasture only cows. However, Kay *et al.* (2003) recently showed that milk fat CLA content of cows grazing on pastures could be increased with supplementation of fish oil or fish oil plus sunflower oil, but not with sunflower oil alone. These results suggested that not an extra supply of the substrates of LA or LNA but the feed sources that inhibit further hydrogenation of TVA to stearic acid would enhance milk fat CLA content. Effect of supplementation of cows grazing on pasture with other sources of LA and LNA, or other sources of FA that inhibit further biohydrogenation of TVA to stearic acid on milk fat CLA content has not been investigated.

It appears that grazing a cool season pasture with a C-3 carbon pathway containing more than 50% of their total FA as LNA increases milk fat CLA (Dhiman *et al.*, 1999a) more than its warm season counterparts with C-4 carbon pathways containing <40% of total FA as LNA (White *et al.*, 2001). A mature pasture, which has higher proportions of $C_{14:0}$ and $C_{16:0}$ and less LNA, is also not likely to produce as much milk fat CLA content as does the lush green pasture when grazed by lactating dairy cows (Loor *et al.*, 2002b). Similarly, cows receiving all of their diet from pasture produced milk fat with higher CLA contents compared with cows receiving either one-third or two-third of their diet from pasture (Dhiman *et al.*, 1999a). Diversity in the species of forages available on pasture also increased the milk fat CLA content (Collomb *et al.*, 2002a) and so did the increased altitude of pasture (Collomb *et al.*, 2002b). Although, effects of the variety of a grass/legume on milk fat CLA contents are not clear, it could be expected to relate more to their FA composition than anything else.

Other diet related factors: Dietary copper at physiological concentrations may affect lipid metabolism in beef animals (Engle and Spears, 2000) as well as dairy cows (Engle *et al.*, 2001). It may alter rumen biohydrogenation (Engle *et al.*, 2001) or stimulate the desaturation of $C_{18:0}$ in adipose as well as mammary tissues (Corl *et al.*, 1999). Morales *et al.* (2000) have found an increased CLA content in milk fat when dietary Cu was depleted for 2 months. It requires further investigation to define the effects of Cu on milk or tissue fat CLA in dairy or beef cows.

It has been demonstrated that cows fed less energy than required produce milk fat with substantial reductions in short and medium chain FA and an increase in $C_{18:1}$ FA compared with the milk of adequately fed cows (Timmen and Patton, 1988). It is not clear how much of such an increase in $C_{18:1}$ is actually in TVA that would eventually contribute to milk fat CLA. Whether a decreased energy intake by cows while on pasture is contributing to the increased milk fat CLA content is not known. If so, it might be interesting to find whether milk fat CLA content could be increased by decreasing the energy content of TMR.

Ionophores appear to have some positive effects on milk fat CLA content (Fellner *et al.*, 1999). This should be regarded in light of the positive effects that maintaining ruminal pH at 6.0 or above has on CLA and TVA contents in rumen cultures (Troegeler-Meynadir *et al.*, 2003; Martin and Jenkins, 2002). Ruminal pH could probably be linked to the higher milk fat CLA content of cows fed diets with a higher proportion of forage compared with less forage in the diet (Jiang *et al.*, 1996). It is of greater importance in high yielding dairy and beef cow diets where large amounts of grain is included to boost the energy concentration of the diet and thus decrease the rumen pH below 6.0. The effect of boosting the energy concentration of their diet without decreasing the rumen pH may have practical significance in enhancing the milk fat CLA concentration and yield.

Important considerations: Two aspects of the feeding strategies designed for enhancing milk fat CLA content need to be considered. First, reduction in milk yield, which is usually associated with cows grazing on pasture. Reduced milk yield may sometimes be a heavy price to pay for increasing the milk fat CLA content unless the dairy producers are given an extra incentive to do so. Second, reduction in milk fat content, which is usually associated with increased proportion of free oil such as fish or plant oils in the diet that are normally used for enhancing milk fat CLA content.

The overall goal of the feeding strategies should, therefore, be to increase milk fat CLA concentration with minimum effects on milk yield or its fat content. This is possible either by supplementing cows grazing on pasture through some feeds that not only maintain the high CLA concentration while on pasture but also minimize the reduction in milk yield (Khanal *et al.*, 2002) or by including the substrates for CLA synthesis in the form accessible to rumen microbes without compromising their growth and multiplication such as extruded oilseeds instead of free oils (AbuGhajaleh *et al.*, 2003; Whitlock *et al.*, 2002).

Under South Asian conditions information on the dietary factors affecting CLA content in milk from cows or water buffaloes is very limited. Moreover, feeding and management system here are entirely different from that in the west making the findings of the west less relevant. This is an area where research focus is warranted.

Dietary factors affecting beef fat CLA content: The ability to enhance CLA content in beef fat through the diet appears to be less effective than that on milk fat CLA. It is probably the result of the nature of finishing diets fed to beef animals in the US, which contain 80-90% grain, and which reduce the rumen pH well below the level of 6.0 required for enhanced production of CLA and TVA in the rumen. Nonetheless, increased concentration of CLA and TVA has been shown for beef obtained from animals fed various diets or dietary supplements (Porter, 2003; Madron *et al.*, 2002; Poulson, 2001; French *et al.*, 2000). Although Beaulieu *et al.* (2002) observed no increase in CLA concentration in tissue lipids of cattle fed a high-concentrate diet supplemented with soybean oil, Griswold *et al.* (2003) suggested that short term feeding strategies for enhancing CLA content in beef is possible. This was evident in another study when inclusion of FFES in the diet of finishing beef steers increased the concentrations of both CLA and TVA in intramuscular, intermuscular, and subcutaneous fat (Madron *et al.*, 2002). However, the increases were relatively small and were much less than those observed for similar diet comparisons in lactating dairy cows. Based on ratios of TVA to CLA in duodenal flow and adipose tissues, Gillis *et al.* (2003) estimated that over 86% of CLA in beef fat originated from desaturation of TVA. The increase in forage level from 12 to 36% in beef steer diets increased the duodenal flow of TVA linearly without altering the flow of CLA (Sackman *et al.*, 2003). It suggested that endogenous desaturation of TVA to CLA increases with the increased proportion of forage in the beef cow diets.

Data related to CLA content and the factors affecting it in meat fat from goat, sheep, cattle, water buffalo, and other ruminants is very limited under South Asian conditions. Once again the difference in feeding and management system between the west and South Asian countries needs to be considered while making suggestions as to how the CLA content in meat fat from these ruminants could be enhanced.

Dietary factors affecting CLA content in foods from non-ruminants: It has been mentioned above that foods from non-ruminants do not contain any appreciable amounts of CLA isomers under traditional feeding. In some cases it was not even detected (Raes *et al.*, 2002; Yang *et al.*, 2002). However, several researchers have shown a considerable enhancement in CLA content of egg yolk, broiler meat, or pork through dietary supplementation of CLA (Watkins *et al.*, 2003; Szymczyk and Pisulweski, 2003; Cherian *et al.*, 2002; Bee, 2000a). Egg yolk contents of *c*-9, *t*-11 and *t*-10 *c*-12 isomers of CLA have been shown to increase linearly with the increasing concentration of CLA isomers in the layer's diet (Cherian *et al.*, 2002; Jones *et al.*, 2000; Du *et al.*, 1999). Jones *et al.* (2000) found that incorporation of CLA in the egg yolk was highest on d 24 and d 36, whereas Chamruspollert and Sell (1999) observed it after 11 d. High concentration of CLA at 0.82, 5.82, and 11.2% of the total FA in egg yolk lipids occurred when laying hens were fed a diet with 0.5, 2.5, or 5.0% CLA (Chamruspollert and Sell, 1999), which is even higher than the concentration observed in milk or meat fat from ruminants. However, such a high dose of CLA in the diet may have other implications in growth, production, and reproduction of birds. The transfer efficiency of *c*-9, *t*-11 isomer was higher than that of *t*-10 *c*-12 isomers with an overall CLA incorporated into egg yolk being 7.95g CLA/100 g of total FA (Schafer *et al.*, 2001). Similarly, feeding CLA to broiler chickens at 0.5, 1.0, or 1.5% of the diet resulted in substantial incorporation of CLA isomers into their tissue lipids (Szymczyk *et al.*, 2001), thus providing another potential CLA-rich source of meat for humans. Sirri *et al.* (2003) have observed an increased deposition of CLA in muscles with the increasing CLA level in the diet of broiler chickens.

Concentration of CLA isomers in ham fat was higher for pigs fed CLA regardless of the level of supplementation in the diet (Corino *et al.*, 2003). The backfat, liver, and muscle lipids showed an increased concentration of CLA isomers in pigs fed 2.0% CLA in the diet with highest concentration of 5.65% of total FA in the backfat followed by liver lipids at 2.41% and muscle lipids at 1.47% (Teschendorf *et al.*, 2002). Feeding pigs with increasing levels of *trans* $C_{18:1}$ FA resulted in a linear increase in both TVA and CLA in backfat as well as neutral lipids and phospholipids of *M. Longissimus dorsii*, with backfat having higher concentration of CLA than *M. Longissimus dorsii* (Glaser *et al.*, 2002). Moreover, rate of bioconversion of TVA to CLA in pig adipose tissue was not limited up to 25 g total *trans* $C_{18:1}$ FA including 3.3 g of TVA per kg feed. Similarly, both the *c*-9, *t*-11 and *t*-10, *c*-12 isomers of CLA were increased in belly and longissimus fat depots of pigs fed CLA (Gatlin *et al.*, 2002). Transfer efficiency of dietary CLA isomers was 41 to 52% for the backfat and 55 to 69% for the mature milk with highest values for *c*-9, *t*-11 isomer when pregnant and lactating sows were fed diets with supplemental CLA (Bee, 2000a). Ostrowska *et al.* (2003), however, concluded that although feeding pigs diets supplemented with CLA increases lipid CLA, but the resultant change in the FA profile in pig fat could potentially outweigh the benefits of increasing CLA.

Since TVA is converted into CLA in humans and other monogastric animals (Loor *et al.* 2002c), it should be possible to enhance the CLA concentration in human or other monogastric milk by supplementing diets with TVA. Loor *et al.* (2002c) have also shown that dietary TVA increases the CLA content in tissues of lactating mice and suckling pups.

Although no substantial amount of CLA isomers is present in milk or meat from non-ruminants under traditional feeding (Chin *et al.*, 1992; 1993), these studies have shown that CLA content in foods from non-ruminants could be enhanced substantially through incorporation of CLA in the diet. Whether supplementation of CLA in the diet of non-ruminants has other negative effects in the economics and overall production and reproduction performance of animals has not been investigated in detail.

Animal related factors: Of all the animal related factors, animal-to-animal variation appears to be the most important one. The variation among individual cows would primarily be related to two factors, a. rumen production of TVA and CLA, and b. the activity of Δ^9-desaturase. However, rumen production of TVA and CLA are related to the biohydrogenation of substrates available from the diet, and type and number of bacteria that biohydrogenate the available substrates to produce CLA and TVA. Considering the fact that rumen output of CLA contributes only marginally to the overall CLA content in milk and possibly meat fat, the activity of Δ^9-desaturase is important to describe at least some of the animal-to-animal variation. Ruminant tissues that have substantial Δ^9-desaturase activity include mammary gland, adipose tissues, and intestinal epithelium (Bauman *et al*., 2003).

There are four major fatty acid pairs in milk fat that represent a product/substrate ratio for Δ^9-desaturase, which are *c*-9, *t*-11 CLA/TVA, oleic/stearic, palmitoleic/palmitic, and myristoleic/myristic. The ratios of these four pairs of FA are regarded as the proxy for the activity of Δ^9-desaturase (Choi *et al*., 2000). It catalyzes the Δ^9-*cis* desaturation of fatty acyl-CoA substrates and synthesizes monounsaturated FA (Ntambi *et al*., 1999) and its activity is regulated by both the *c*-9, *t*-11 and *t*-10, *c*-12 isomers of CLA (Choi *et al*., 2000; Ntambi *et al*., 1999). Since endogenous synthesis of CLA has been shown to be the major source of CLA, contributing from a minimum of 64% (Griinari *et al*., 2000) or 80% (Lock and Garnsworthy, 2002) to a maximum of 91% (Kay *et al*., 2004) or even 100% (Kay *et al*., 2002) of the total CLA, activity of Δ^9-desaturase is important to explain some of the variation in milk fat CLA content among individual cows. It has been shown that individual cows may vary over 3-fold in the activity of Δ^9-desaturase (Kelsey *et al*., 2003; Lock and Garnworthy, 2002; White *et al*., 2001), which has been primarily implicated in the variation in milk fat CLA content among individual cows. However, Fievez *et al*. (2003) have shown that changes in CLA depended mainly on TVA supply and to a lesser extent on the activity of Δ^9-desaturase. No difference in the activity of Δ^9-desaturase for cows fed pasture or pasture supplemented with sunflower oil (Kay *et al*., 2004) supports this proposition. This may be the reason as to why the variation in both the CLA content and the activity of Δ^9-desaturase appeared to be higher when cows were fed diets such as FFES (Peterson *et al*., 2002), or a combination of LA and LNA (Lock and Garnsworthy, 2002) that would supply more TVA from the rumen. It is possible that the activity of Δ^9-desaturase is expressed only when the substrate is available in sufficient amounts. Moreover, the enzyme follows saturation kinetics (Choi *et al*., 2000; Ntambi *et al*., 1999). It could explain why there was no increase in milk fat CLA content when cows grazing on pasture did not produce increased concentration of CLA when supplemented with feed sources rich in LA, such as extruded soybeans (Khanal *et al*., 2002; Khanal *et al*., 2003b) or sunflower oil (Kay *et al*., 2004).

Kelly *et al*. (1998a) have observed a larger variation in milk fat CLA content for cows fed sunflower oil than either peanut or linseed oil. Similarly, variations in CLA content among individual cows were higher with diets such as all pasture (Kelly *et al*., 1998b), TMR supplemented with free oil (Kelly *et al*., 1998a), or a diet with higher forage:concentrate ratio (Jiang *et al*., 1996), all of which are conducive to higher CLA concentrations. Cows in confinement fed TMR had smaller variations in milk fat CLA content compared to cows grazing pastures (White *et al*., 2001). Similarly, variation in milk fat CLA content for the same group of individual cows was higher while grazing on pasture than when receiving either a TMR diet or a diet of conserved forage (*ad libitum* access) supplemented with grain (Khanal, 2004). Peterson *et al*. (2002) also found that the same group of cows had larger variation in milk fat CLA content when FFES was included in the diet compared to a TMR diet with no FFES. As mentioned above, it is possible that the variation in the activity of Δ^9-desaturase was expressed only under dietary conditions suitable for higher TVA contents that would lead to greater variation in milk fat CLA contents among the individual cows. A consistency in the individual hierarchy in desaturase ratios over time has been shown when cows are fed the same diet or when they are switched between diets (Peterson *et al*., 2002), suggesting that CLA content depended more on TVA supply than on Δ^9-desaturase activity. Breed, parity, and days in lactation had little relationship to the individual variation for milk fat CLA content or desaturase index in Holstein or Jersey cows (Kelsey *et al*., 2003). Individual variation among animals in CLA content of beef has not been reported nor has it been reported for other species of animals.

In a 2-yr study on the status of CLA content in 4 commercial dairies of Utah and Idaho, USA, Khanal (2004) found that dairy herds that graze cows during summer and supplement them with grains produce 60% or more CLA and TVA on an year round basis than the herd that fed 10% of DM as fresh cut grass during summer. A great majority of cows (~90%) in these commercial dairies had milk fat CLA content between 0.3 and 0.9%. It was also found that individual cow variation was greater during summer than in winter and in dairies that graze cows during summer than dairies that either did not graze or had only 1/3 of its cows grazed. It has been observed that concentration of CLA in milk fat was lowest during February-March and highest during August-September both in the USA (Khanal, 2004) and Canada (Riel, 1963).

Some of the variation observed between herds may have been due to breed. It has been shown that given the same diet Holsteins produce higher CLA in milk fat than do Jerseys or Normandes (White *et al.*, 2001; Lawless *et al.*, 1999). Kelsey *et al.* (2003), however, have observed minor variation in milk fat CLA content between Holstein and Jerseys fed the same diet, and that the breed contributed only about 1% of the total variation. They also observed that parity contributed about 10% of the variation. It was similar to the earlier finding where cows and water buffaloes with 7 or higher lactation produced an average of 15% more CLA in milk fat compared with cows and water buffaloes of either 1 to 3 or 4 to 6 lactations (Lal and Narayanan, 1984). Similarly, Stanton *et al.* (1997) reported that increased lactation number had a positive effect on milk fat CLA content. When Ca salts of CLA were supplemented to cows during transition and early lactation, there was no significant difference in milk fat CLA content compared with palm fatty acid distillate supplemented control (Bernal-Santos *et al.*, 2003). Information on the effect of breed, parity, or stage of lactation on milk fat CLA content in other species of animals is limited.

Post harvest related factors: The effect on CLA content of post-harvest related factors, such as processing conditions, storage, cooking, aging, etc., or converting one product to another, such as milk into cheese or yogurt is controversial. It could be argued that the CLA content of all these processed products depends largely on the CLA content of the original milk or meat. Therefore, the CLA content of the original product needs to be taken into account whenever processing effects are investigated. However, it is likely that the starter cultures used for making other dairy products from milk would contain enzymes that can isomerize LA into CLA, and thereby increase their CLA content. Several species of bacteria such as *Lactobacillus acidophilus*, *L. Casei*, *L. delbruckii,* and *Propionibacterium frudenbruckii* that are routinely used for making cheese, yogurt, or other fermented milk products have been shown to convert free LA into CLA (Alonso *et al.*, 2003; Ogawa *et al.*, 2001; Lin, 2000; Jiang *et al.*, 1998). Lin (2000) studied three cultures of *Lactobaciallus* sp., two of *Lactococcus* sp. and one of *Streptococcus* sp. for the effects of sucrose, fructose, lactose, and NaCl added to skim milk and found that *L. acidophilus* produced the highest CLA content. Small differences in CLA levels at various processing stages were observed when raw milk was processed into cheddar cheese aged to 6 months (Lin *et al.*, 1999). In contrast, there was no increase in CLA content of mozzarella (Dhiman *et al.*, 1999b) or cheddar cheese (Allred, 2004; Khanal *et al.*, 2002) compared with original milk nor any effect of aging of cheese was observed on CLA content (Allred, 2004). Similarly, Baer *et al.* (2001) found no difference in CLA contents of raw milk, cream or butter. The effect of pasteurization, almost a mandatory process now, if any, is of academic value only. The CLA content of cooked or uncooked extra lean ground beef, ground beef, or rib roast was similar (Ma *et al.*, 1999). Cooking, however, increased the CLA content of sirloin tip roast (Ma *et al.*, 1999), reasons for which were not clear.

Overall, the changes in CLA content during processing are small. Parodi (2003) opined that such changes claimed to be due to processing variables were often less than the expected measurement error. Moreover, such minor changes may not be of great significance considering the large variations caused by the diet and individual physiologic factors regulating the synthesis of CLA and TVA.

Conclusion: Of more than a dozen isomers of CLA detected in foods of ruminant origin, *c*-9, *t*-11 and *t*-10, *c*-12 are the ones with known physiological importance. While *c*-9, *t*-11 comprises 80 to 90% of total CLA, *t*-10, *c*-12 comprises 3 to 5% of the total. Another isomer of quantitative importance is *t*-7, *c*-9, which comprises 3 to 16% of the total CLA. Although *c*-9, *c*-11 isomer has been found to be more potent than *c*-9, *t*-11 or *t*-10, *c*-12 isomers against breast cancer cells recently, information about its content in milk, meat, or egg lipids is limited. The CLA content in milk, meat, or egg varies greatly from a low 0.1% or less to a high 2% or more of the milk, tissue, or egg yolk lipids, with milk lipids from ruminants having highest concentrations. Although food products from ruminants are the richest source of CLA for humans, it is possible to enhance the CLA content of foods from non-ruminants by supplementing CLA and probably TVA in the diet. Whether such an effort is of practical significance needs to be investigated. A host of factors appear to affect the CLA content in milk, meat, and other food products from various species of animals, which could be broadly classified into diet, animal, and post-harvest related factors. Of all these factors, diet is the primary one that could be easily manipulated for enhancing the concentration of CLA in food products, both from ruminants and non-ruminants. While animal-to-animal variation is also of great significance, post-harvest related factors appear to be of minor importance.

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