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Conjugated Linoleic Acid: A Milk Fatty Acid with Unique Health Benefit Properties

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1. Introduction

Human diet comprises of milk and milk products in both developed and developing parts of globe. Milk fat is the major energy source in Indian diet but due to the fear of hypercholesterolemia, saturated fats have lead to avoidance of dietary fats especially of animal origin. However, milk contains a number of components with beneficial properties, one such compound associated with the fat phase is Conjugated Linoleic Acid (CLA) which has potential health benefits towards human beings.

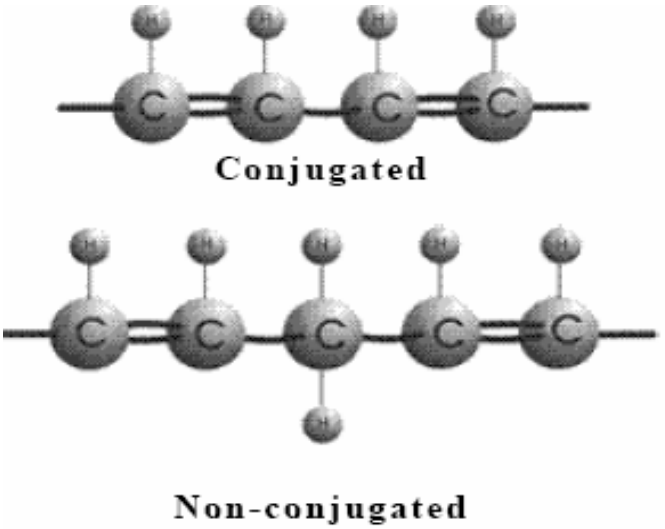
Conjugated Linoleic Acid (CLA) refers to a mixture of positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12, $C_{18:2}$) with two conjugated double bonds at various carbon positions in the fatty acid chain. It is formed as an intermediate during the biohydrogenation of linoleic acid by linoleic acid isomerase from the rumen bacteria *Butyrivibrio fibrisolvens* (Kritchevsky, 2000) or from the endogenous conversion of *trans*-11, $C_{18:1}$ (Transvaccenic Acid) another intermediate of linoleic or linolenic acid biohydrogenation by Δ^9-desaturase in the mammary gland (Corl *et al*., 2001).

Milk fat is the richest natural dietary source of CLA. Milk contains an average 4.5mg CLA/g of fat (Kelly *et* al., 1998). Recent studies have shown that the CLA content of milk fat can be markedly enhanced by dietary manipulation especially those involving dietary addition of plant oils which are high in unsaturated fatty acids (Griinari and Bauman, 1999). Dietary increase of linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) is one of the feeding strategies for increasing the CLA concentration in milk which is the main precursor of CLA. The main sources of linoleic acid for feeding animals are cereals, oil seeds, oils etc (Kelly *et al*., 1998)

There is an increasing research interest towards the CLA and its potential health benefits such as anticarcinogenic, antiatherogenic, antidiabetic and immunomodulatory effects (Belury, 2002; Tyagi and kathirvelan, 2006). The potential anti-cancer effect of CLA is well documented, with the majority of experimental work conducted *in vitro* or in animal models (Ip et al., 1994). It has been demonstrated that CLA has the ability to affect mammary cancer, stomach cancer, skin cancer and prostate cancer. Most of the anticarcinogens are of plant origin but CLA is unique, it is present in food from animal sources and its anti-cancer efficacy is expressed at concentrations close to human consumption level. The unique structural and functional properties of CLA appear to modulate cellular process involved in carcinogenesis.

1.1 Chemical structure of CLA

CLA is a term given to a group of positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12, $C_{18:2}$ LA) in which double bonds are conjugated, instead of being in the typical methylene interrupted configuration. Each double bond can be *cis or trans* configuration giving rise to possible CLA isomers (Kelly *et al.*, 1998). Although conjugation of double bonds occurs as part of free radical mediated oxidation of linoleic acid. CLA is a true isomer of LA, in that it does not possess additional oxygen (Vandenberg *et al*., 1995).

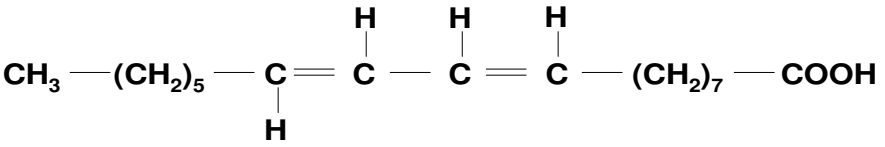


1.2 Isomers of CLA

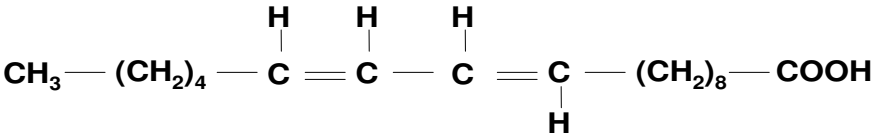
CLA therefore, includes 28 positional and geometrical isomers of which only *cis*-9, *trans*-11 and *trans*-10, *cis*-12 have thus far been proven to have biological activities (Park *et al*., 2003). Of the two physically important isomers *cis* -9, *trans* -11 (rumenic acid) is the most prevalent comprising 80-90% of the total CLA in food products from ruminants where as *trans*-10, *cis*-12 is present in small amounts at 3-5% of total CLA (Parodi, 2003). Analysis of cheese using silver ion- high performance chromatography and G-C electron ionization mass spectrometry showed that isomers of CLA present (in fat percent) total CLA were *cis*-9, *trans*-11 (78-84%); *trans*-7, *cis*- 9 plus *trans* -8, *cis* -10 (8-13%); *trans*-11,*cis*-13 (1-2%); *cis*-12,*trans*-14 (<1%) and the total *trans*/*trans* isomers (5-9%) (Parodi, 2003)

1.3 Sources of CLA

CLA occurs in many foods, however, the principal dietary sources are dairy products and other foods derived from ruminants. The CLA content of some common foods is shown in (Table 1).



cis-9, trans-11 CLA



trans-10, cis-12 CLA

Foodstuff	Total CLA (mg/g of fat)	Foodstuff	Total CLA (mg/g of fat)
Dairy products		*Meats*	
Homogenized milk	4.5	Ground beef	4.3
Condensed milk	7.0	Lamb	5.6
Butter milk	6.1	Pork	0.6
Mozzarella cheese	4.9	Chicken	0.9
Plain yogurt	4.8	Salmon	0.3
Ice-cream	3.6	Ground turkey	2.5

Table 1. CLA content of common foods (Chin *et al*., 1992)

2. Biosynthesis of CLA

CLA found in milk and meat of ruminants originates from two sources (Griinari and Bauman, 1999). CLA is formed during ruminal biohydrogenation of linoleic acid and the second source is CLA synthesized by animal tissues from *trans*-11 $C_{18:1}$, another intermediate in the biohydrogenation of unsaturated fatty acid. Hence, the uniqueness of CLA in ruminant edible products relates to incomplete biohydrogenation of dietary unsaturated fatty acids in the rumen.

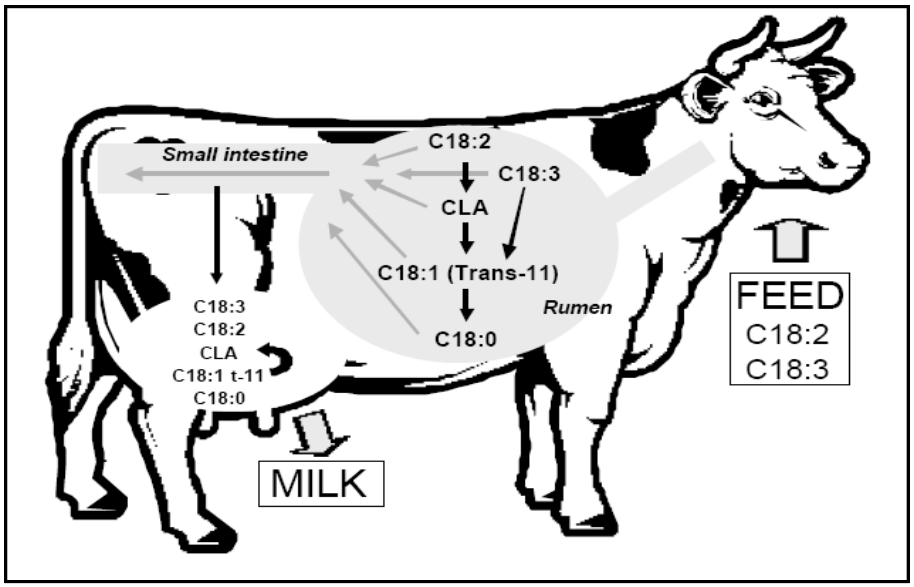
2.1 CLA synthesis in the rumen

Dietary lipids undergo two important transformations in the rumen (Devillard *et al*., 2004). First, lipolysis - hydrolysis of ester linkages catalyzed by microbial lipase and second, biohydrogenation of unsaturated fatty acid.

The first reaction in linoleic acid (*cis-9*, *cis-12*) biohydrogenation is isomerization of the *cis*-12 double bond. Linoleate isomerase is the enzyme responsible for forming conjugated double bonds from the *cis*-9, *cis*-12 double bond structure of linoleic as well as α and γ linolenic acids. The enzyme is bound to the bacterial cell membrane and demonstrates an absolute

substrate requirement for a *cis*-9, *cis*-12 diene system and a free carboxyl group. The second reaction is a reduction in which *cis*-9, *trans*-11 CLA is converted to *trans*-11 $C_{18:1}$(*trans*-vaccenic acid). Isomerization of the *cis*-12 double bond was followed by rapid conversion of *cis*-9, *trans*-11 CLA to *trans*-11 octadecenoic acid. Hydrogenation of the *trans*-11 monoene occurred less rapidly, and it increased in concentration (Qiu, 2004). Therefore, *trans*-11 $C_{18:1}$ reduction seems to be rate-limiting in the biohydrogenation sequence of unsaturated C_{18} fatty acids. As a consequence, this penultimate biohydrogenation intermediate accumulates in the rumen and is, therefore, more available for absorption.

Similar to biohydrogenation of linoleic acid, biohydrogenation of linolenic acid begins with an isomerization followed by a sequence of reductions and terminates with the formation of stearic acid. Rumen biohydrogenation of α-linolenic acid produces *cis*-9, *trans*-11, *cis*-15 conjugated octadecatrienoic acid as the predominant initial isomerization product, and this is followed by reduction of the *cis*-double bonds. As a consequence, *trans*-11 octadecenoic acid is a common intermediate in the biohydrogenation of both α-linolenic acid and linoleic acid (Fig.1)



2.2 Endogenous synthesis

Griinari and Bauman (1999) proposed that a major proportion of CLA in tissue and milk lipids synthesized endogenously from *trans* vaccenic acid (TVA) by Δ^9 – desaturase, in mammary gland (Fig.1). Griinari *et al* (2000) examined the potential for endogenous synthesis of CLA by infusing TVA abomasally and measuring the changes in milk fat CLA. By day three it resulted in a 31% increase in milk fat CLA indicating that an active pathway for endogenous synthesis existed in the mammary gland.

Griinari *et al.* (2000) reported that the contribution of endogenous synthesis to the over all CLA content in milk fat was 84%, making it the primary source. Lock and Garnsworthy (2002) estimated the endogenous synthesis of CLA to be more than 80% of total collected

duodenal samples and estimated the rumen synthesis of CLA to be 4-7%. Endogenous *cis*-9, *trans*-11 CLA would originate from desaturation of *trans*-11 $C_{18:1}$ by delta-9 desaturase (Fig. 1). The enzymatic reaction introduces a cis double bond between carbons 9 and 10 of fatty acids.

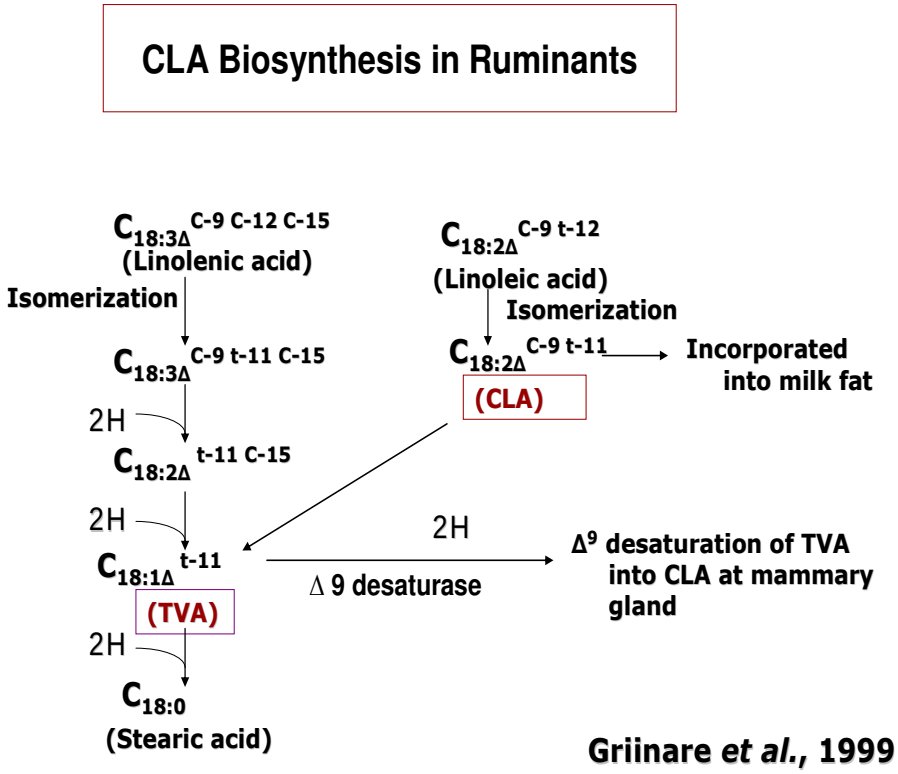


Fig. 1. CLA Biosynthesis in Ruminants

3. Role of rumen bacteria in CLA synthesis

Ruminants rely on microbial digestion of forages and supplementary feed materials in the rumen to provide nutrients that would otherwise be unavailable to the host animal. Ruminant diets typically contain polyunsaturated fatty acids, which are toxic to many rumen microorganisms (Harfoot and Hazlewood, 1997). However, to protect themselves against these toxic effects, rumen microorganisms have mechanisms to hydrolyse and biohydrogenate dietary lipids (Kemp and Lander, 1984).

Rumen bacteria play a major role in biohydrogenation. They convert C_{18} unsaturated fatty acids to stearic acid, via a number of intermediates. Biohydrogenation intermediates have received a lot of attention recently because of their potential effects on human health. CLA and *trans* vaccenic acid are one of the intermediates of fatty acid biohydrogenation in the rumen.

3.1 Biohydrogenation of unsaturated fatty acid by rumen bacteria

Rumen fluid contains bacteria, fungi and protozoa. Small amount of contribution is made by fungi and protozoa in biohydrogenation. Among which the major source of biohydrogenation is bacteria when compared to fungi and protozoa (Nam and Garnsworthy, 2007). Bacteria involved in biohydrogenation process can be divided into two groups i.e., A and B

Group A bacteria: They hydrogenate linoleic and linolenic acid to *trans*-11 octadecadienoic acid, and are not able to or incapable of hydrogenating octadecadienoic acid. Examples are *Butyrovibrio*, *Micrococcus*, *Ruminococcus* and *Lactobacillus.*

Group B bacteria: They are capable of hydrogenating a wide range of octadecadienoic acids, including *cis*-9 (oleic) and *trans*-11 (transvaccenic) acids as well as linoleic acid to stearic acid. Examples are *Fusocillus S*p and gram negative rods.

More than 250 bacterial strains from 14 genera were examined and strains belonging to the genera *Enterococcus*, *Micrococcus*, *Propionobacterium* and *Lactobacillus* were found to produce considerable amounts of CLA from linoleic acid.

Butyrivibrio fibrisolvens

CLA formation in rumen has mainly been associated with bacterial activity. The ruminal bacterium *Butyrivibrio fibrisolvens* has been used as a model for *cis*-9, *trans*-11 CLA production (Grinarii *et al.,* 2000). *Butyrivibrio fibrisolvens* a gram negative curved rod, strict anaerobic bacteria of moderate butyric acid producers with potent linoleic acid isomerase activity by using roll tube technique with ATCC media from rumen of cross bred cattle (Fig 2; Fig 3),has been isolated in our lab and research work is under publication.

Fig. 2. Translucent colony of *Butyrivibrio Sp* in roll tube.

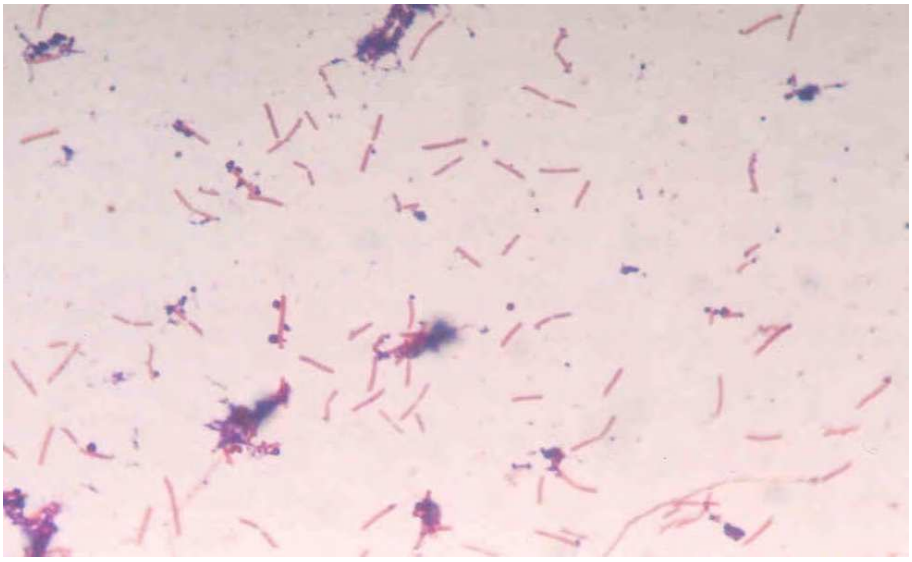


Fig. 3. Gram – ve, curved/straight rods of *Butyrivibrio Sp.*

3.2 Bacteria other than rumen origin involved in CLA synthesis

In addition to rumen microflora, microbial CLA production has also been reported in *Propionibacteria freudenreichii* used as dairy starter cultures (Jiang *et al.*, 1998). Ogawa *et al.*, (2005) reported the production of CLA from free linoleic acid by *Lactobacillus acidophilus*. Kishino *et al.*, (2002) found that *Lactobacillus plantarum* formed high levels of CLA from free linoleic acid upon extended incubation. *Bifidobacteria* also produce CLA, mainly the *cis*-9, *trans*-11 isomer (Coakley *et al.*, 2003).

4. Factors affecting CLA content in milk

A cow's diet, breed, age, non-nutritive feed additives, such as ionophores (Table 2) can affect the CLA content in milk fat. Among these factors, the diet is known to strongly influence the CLA content of milk and includes feedstuffs such as pasture, conserved forages, plant seed oils, cereal grains, marine oils and feeds and animal fat.

4.1 Lipolysis and biohydrogenation in rumen

Any change in the process of lipolysis or biohydrogenation will influence the supply of their intermediate and end products, including CLA, to the small intestine and ultimately their contents in the milk and meat. Replacement of forages with grain in the diet reduced the rates of lipolysis and biohydrogenation (Gerson *et al.*, 1985). Increased proportion of nitrogen in the diet resulted in increased rates of lipolysis and biohydrogenation by rumen contents *in vitro* (Gerson *et al.*, 1983). Lipolysis and hydrogenation reactions were more rapid with feed particles ranging from 1-2 mm size than from 0.1 to 0.4 mm size and this was due, at least in part, to microbial population density (Gerson *et al.*, 1988). Biohydrogenation of fatty acids averaged 47% in the rumen of cows fed diets containing calcium salts of palm oil

and 71% with diets containing fat from animal or vegetable sources (Wu and Palmquist, 1991). Factors affecting ruminal fermentation and microbial population are undoubtedly the keys to control the regulation of biohydrogenation and CLA synthesis.

4.2 Diet, feed to the animal

CLA are highly correlated with either linoleic or alpha linolenic acid intake. It is well known that linoleic and linolenic acid are both indirect precursors of the CLA isomer *cis*-9, *trans*-11 (Aii *et al.,* 1999) but the highest concentration of this CLA isomer is generally obtained with linolenic acid rich diets (Dhiman *et al.*, 2000). Dhiman *et al.*, (1999) reported that cows grazing pasture had 500% higher CLA content in milk fat compared to cows fed a diet containing 50% conserved forage (hay and silages) and 50% grain. Tyagi *et al* (2007) reported that three fold (187 %) increase in total CLA content in milk of cow and buffaloes by feeding berseem fodder than the concenrtae feeding. About 48 to 56% of the total fatty acids in fresh forages consist of $C_{18:3}$ (Bauchart *et al.*, 1984) and form substrate for ruminal biohydrogenation.

Dietary Factor	**Effect on CLA Content of Milk Fat**
Lipid Substrate	
Unsaturated vs saturated fat	Increased by addition of unsaturated fat
Type of plant oil	Greatest with oils high in $C_{18:2}$
Level of plant oil	Dose dependent increase
Calcium salts of plant oils	Increased as with free oils
High oil plant feeds	
High oil corn	Minimal effect
Soybeans	Heat processing will increase
Rapeseed vs soybean	Similar effect
Modifiers of Biohydrogenation	
Forage: concentrate ratio	Increased with high ratio
Fish oils	Greater increase than with plant oils
Monensin - ionophore	Variable effect
Dietary buffers	Little effect
Combination	
Pasture vs conserved forages	Higher on pasture

Source: Bauman *et al.*, (2001)

Table 2. Different substrate (dietary factors) that affect CLA content in milk fat

4.3 Rumen pH

Rumen pH has an important role in maintaining a viable rumen environment suitable for *Butyrivibrio fibrisolvens* involved in the biohydrogenation of linoleic and linolenic acid. Ruminal pH of 6.0 or above has a positive effect on TVA and CLA content in rumen cultures (Troegeler-Meynadir *et al.,* 2003). Qiu *et al.* (2004) observed that reduced ruminal pH reduces total and cellulolytic bacterial number and thus reduce biohydrogenation which in turn

increase CLA. Martin and Jenkins (2002) from their continuous culture data suggest that culture pH seems to have most influence on the production of *trans*-$C_{18:1}$ and CLA isomers by mixed rumen bacteria. When mixed ruminal bacteria were maintained in continuous culture on mixed soluble carbohydrate at a dilution rate of 0.05/hr, concentration of *trans*-$C_{18:1}$ were significantly reduced at a culture pH of 5.5. Because *trans*-$C_{18:1}$ monoenes serve as a precursor of CLA, to maximize CLA synthesis in rumen diet need to be formulated to maintain ruminal pH above 6.0.

5. Feeding strategies to enhance the CLA content in milk

Milk fat is the richest natural dietary source of CLA. Milk contains an average 4.5 mg CLA/g of fat (range 3-6 mg/g). The level of CLA in milk reflects the quantity, which is available for intestinal absorption (Loor and Herbein 1997). Therefore, there is a need to manipulate the feed in such a way to have higher CLA output in the reticulo-rumen for its increased absorption from the intestinal tract and eventually its secretion in the milk.

Dietary factors that affect CLA content have been grouped into four categories related to the potential mechanisms through which they act (Bauman *et al.*, 2001).

1. The first category includes dietary factors that provide PUFA substrates for rumen production of CLA and *trans*-11 18:1. This typically corresponds to increasing the dietary supply of plant and/or fish oils.
2. The second group consists of dietary factors that affect rumen bacteria involved in biohydrogenation, either directly or via changes in rumen environment. For example, modifying the forage: concentrate ratio of the diet, inclusion of ionophores and fish oil typically alters the biohydrogenation of PUFA.
3. The third category includes dietary factors that involve a combination of lipid substrates and modification of rumen biohydrogenation. For example, several investigations have demonstrated that feeding fresh grass to dairy cows doubles the CLA content of milk fat (Lock and Garnsworthy, 2002) and this cannot be fully explained in terms of PUFA supply to the rumen. Other factors or components of grass must promote the production of CLA in the dairy cows.
4. The fourth category is dietary supplements of CLA or *trans*-11 18:1 fatty acids. These must be protected from rumen biohydrogenation, typically with calcium soaps or formaldehyde.

5.1 Vegetable oil – An effective feeding strategy

Manipulation of animal diet primarily involves supplying linoleic acid or linolenic acid as substrates for rumen biohydrogenation. The vegetable/plant oil rich in these fatty acids seems to be an effective strategy for milk CLA manipulation in ruminants.

Feeding plant seed oils, such as sunflower, soybean, peanut, canola, and linseed also increases CLA content in milk (Dhiman *et al.*, 1999). There are a number of other research reports suggesting that feeding processed soybeans, canola, or flax seeds to dairy cows was more effective in increasing milk CLA content than feeding unprocessed seeds (Stanton *et al.*, 1997; Chilliard *et al.*, 2003).

Kathirvelan (2007) reported that buffaloes fed with three different concentrate mixtures (Table 3) with different fatty acid composition (Fig 4) had different level of CLA (Table 4 & Fig 5) and concluded that milk CLA increased 185 percent in buffaloes fed on mustard oil

(2%) plus mustard cake containing rations (19.50 mg/g fat) as compared to GNC fed buffaloes (6.84mg/g fat). Tyagi *et al. (*2006), reported higher level of total CLA (7.12 mg/ g fat) on feeding 30 percent mustard oil cake in concentrate mixture to lactating buffaloes than those fed on GNC based concentrate mixture (6.74 mg/g fat). In another experiment, the same author reported (Table 5) that feeding berseem fodder to goats resulted in high milk CLA content (13.3 mg/g fat) than concentrate plus berseem fed goats (8.5 mg/g fat).

Ingredients	**concentrate mixture (GNC)**	**concentrate mixture (Mustard cake)**	**concentrate mixture (Mustard cake+2% mustard oil)**
Groundnut cake (Expeller)	27	-	-
Mustard cake (Expeller)	-	39	39
Mustard oil	-	-	02
Maize	50	41	41
Wheat bran	20	17	17
Mineral mixture	02	02	02
Salt	01	01	01

Table 3. Ingredients in concentrate mixture (parts)

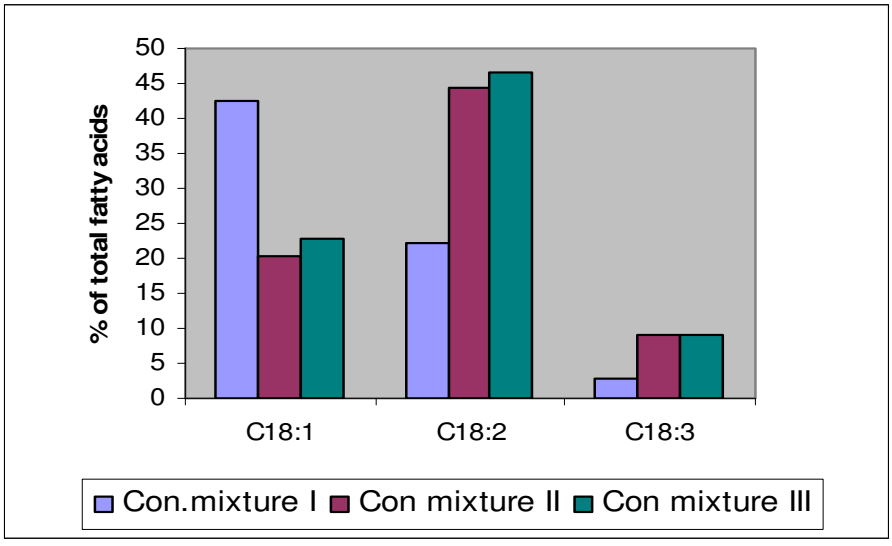


Fig. 4. The levels of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ in three different concentrate mixtures

Fatty acid	concentrate mixture (GNC)	concentrate mixture (Mustard cake)	concentrate mixture (Mustard cake+2% mustard oil)
C4:0	30.99 ± 0.50	30.95 ± 0.32	30.72 ± 0.26
C6:0	15.63 ± 0.45	16.72 ± 0.45	14.84 ± 0.28
C8:0	8.27± 0.08	9.88 ± 0.64	9.21 ± 0.34
C10:0	16.17± 0.37	18.01± 0.74	17.38 ± 0.52
C12:0	23.13± 0.59	24.21 ± 0.35	22.75 ± 0.50
C14:0	105.20± 0.47	106.82 ± 0.84	105.23 ± 0.41
C14:1	5.36± 0.10	4.87 ± 0.24	5.63 ± 0.32
C16:0	265.12 ± 0.79	265.98± 1.55	271.42 ± 1.75
C16:1	13.40 ± 0.36	13.44 ± 0.34	14.77 ± 0.71
C18:0	144.43^{a} ± 2.19	139.33^{b} ± 0.82	133.06^{c} ± 1.06
C18:1 9-t	1.73 ± 0.17	1.96 ± 6.03	1.94 ± 0.12
C18:1 9-c	238.41 ± 1.68	238.27 ± 0.89	239.98 ± 1.16
C18:1 11-c (VA)	9.55^{a}± 0.45	13.01^{b} ± 0.06	16.98^{c} ± 0.04
C18:2 9-c,12-c	11.80± 0.25	11.38 ± 0.28	12.17 ± 0.34
C20:0	11.94 ± 0.43	9.27 ± 0.59	10.1 2 ± 0.20
C18:3 6-9-12-c	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.04
C18:3 9-12-15-c	3.23 ± 0.16	9.37 ± 0.17	9.09 ± 0.03
Total C18:3	3.23± 0.16	9.37 ± 0.17	9.17 ± 0.01
C18:2 9-c,11-t	6.17^{a}± 0.25	10.51^{b}± 0.23	16.94^{c}± 0.30
C18:2 10-t,12-c	0.67^{a} ± 0.08	1.61^{b} ± 0.16	2.56^{c}± 0.05
Total CLA	6.84^{a} ± 0.33	12.12^{b} ± 0.04	19.50^{c} ± 0.32
Total Omega 3 FA	4.12^{a} ± 0.16	10.36^{b}± 0.25	10.50^{b}± 0.06
Total Omega 6 FA	13.02 ± 0.34	12.64± 0.38	12.47± 0.36
Omega 6 : Omega3	3.18^{a} ± 0.15	1.22^{b}± 0.02	1.19^{b} ± 0.03

Values are Mean ± SE for n=5
Values with different superscript across a row differ significantly (P<0.05)

Table 4. Fatty acid composition (mg/g fat) in milk

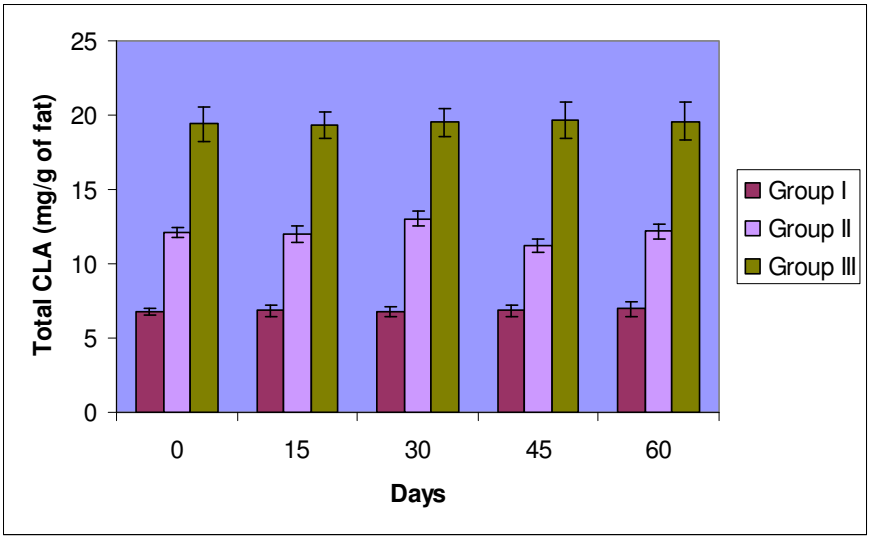


Fig. 5. Total CLA in milk (mg/g of fat) at fort night intervals (Zero day or first sampling after 30 days of adaptation period

Attribute	Treatment	
	Group I	Group II
Fat (%)	3.7 ± 0.15	3.8± 0.08
SNF (%)	6.6± 0.19	7.0 ± 0.16
Total solids (%)	10.3 ± 0.43	10.8 ± 0.26
CP (%)	3.5 ± 0.12	3.5 ± 0.19
	Fatty acids (mg per g)	
C4-14	203.0a ±7.21	227.8 [b] ±5.23
C15:0	18.7±0.65	21.4±0.53
C16:0	253.54[a] ±7.21	255.4 [b] ±6.14
C18:0	134.0 [a] ±4.25	148.9 [b] ±4.28
C18:1 11-trans (TVA)	50.8 [a] ±1.52	35.4 [b] ±0.85
C18:1 9-c	200.1 [a] ±5.24	231.2 [b] ±4.56
C18:2 9-c, 11-t	18.5 [a] ±1.12	10.5 [b] ±0.98
C18:2 10-t, 12-c	0.5±0.08	0.4±0.04
Total CLA	18.9 [a] ±1.30	10.94 [b] ±1.15
C18:3	13.3 [a] ±0.80	8.5 [b] ±1.30

Values with different superscripts across a row differ significantly (P<0.01)

Table 5. Milk composition of goats fed with berseem and berseem plus concentrate

Besides, the feeding of fish oil has been shown to enhance the CLA contents of milk fat. In some studies, fish oil/ fish meal was more effective at enhancing the CLA content of milk than adding similar amounts of soybean oil or combinations of fish oil and soybean oil through extruded soybeans or soybean meal (Ramaswamy *et al.*, 2001) since fish or marine oils are usually rich in long chain PUFA. The inclusion of marine feeds, such as fish meal or sea algae, into dairy cow diets has also been shown to enhance the CLA content of milk (Abu-Ghazaleh *et al.*, 2002)

5.2 Fatty acid composition of different vegetable oils

Dietary effect on CLA content is related probably more to the fatty acid composition of the diet/ feed used than any other factor. Poly unsaturated fatty acid composition of different oils (Table 6) and fatty composition of feeds and fodders used at NDRI cattle yard are reported in Table 7 (Kathirvelan, 2007)

Plant oil	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
Sunflower oil	22.8	67.0	0.45
Soybean oil	19.5	53.2	9.1
Corn oil	27.3	57.5	1.0
Cotton seed oil	16.5	57.4	0.33
Mustard oil	24.12	41.16	9.54
Ground nut oil	46.77	35.67	0.58
Linseed oil	17.7	15.4	57.2

Table 6. Oleic, Linoleic and Linolenic fatty acids concentration in different vegetable oils

Fatty acid	Maize Fodder	Wheat straw	Wheat bran	Maize grain	Groundnut cake	Mustard cake/oil
$C_{14:0}$	1.82	1.26	0.89	0.42	0.53	0.45
$C_{16:0}$	11.24	10.91	20.12	15.14	14.72	8.33
$C_{16:1}$	0.00	0.00	0.00	0.11	0.00	0.24
$C_{18:0}$	5.41	0.00	4.26	3.25	7.28	1.45
$C_{18:1}$	6.62	7.15	16.35	28.42	46.77	24.12
$C_{18:2}$	12.69	10.15	48.90	47.07	25.67	41.16
$C_{18:3}$	45.26	0.00	4.06	1.02	0.96	9.54
$C_{20:0}$	0.00	0.00	0.00	0.00	0.00	0.36
$C_{22:1}$	0.00	0.00	0.00	0.00	0.00	17.82

Table 7. Fatty acids composition of feeds and fodders (% of total fatty acids)

6. Effect of milk process on CLA content in dairy products

Processing of milk into a number of dairy products under normal conditions has no influence on the CLA contents (Shantha *et al.*, 1995). Use of different starter culture, processing conditions and aging periods had negligible effect on the total CLA concentration in dairy products (Shantha *et al.*, 1995). Kathirvelan (2007) reported that when milk was converted into ghee by creamery method, similar level of CLA was found in ghee

(19.54 mg/g fat) as in milk (19.50mg/g fat). Tyagi *et al* (2007) reported an increased level of CLA in ghee prepared by the indigenous method of ghee preparation due to lactic culture fermentation but no such increase was found in cheese and paneer (Table 8). However, Aneja and Murthi (1990) reported substantial increase in CLA content in ghee during its clarifying process at 120^{o}C.

Milk	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	Total C18:3	t10, c12 CLA	C18:2 c-9t11 CLA	Total CLA	Total Ω-3	Total Ω-6	Ω-3: Ω-6
							Cheese							
Group I	18.1±1.9	20.2±1.7	102.7±4.2	281.6±5.6	167.2±6.3	296.5±11.5	13.9±0.7	3.9±0.3	0.8±0.03	7.0±0.3	7.7±0.4	4.5±0.2	16. ±0.7	1:3.6
Group II	15.9±0.6	22.6±0.7	112.3±2.2	292.2±6.2	160.6±8.0	289.7.±6.7	13.1±0.4	9.1±0.4	1.7±0.01	11.7±0.7	13.4±0.7	9.9±0.4	16.0±0.5	1:1.6
Group III	14.0±0.8	20.8±0.9	110.1±3.1	317.9±6.1	124.9±4.9	293.7±10.8	13.8±0.6	13.5±0.8	2.5±0.02	14.4±1.1	17.0±1.2	14.2±1.0	16.0±0.6	1:1.1
LSD	3.3*	3.4	7.5*	14.5*	16.8*	8.9	2.5	4.2*	0.14*	2.2*	2.5*	1.8*	1.9	
							Paneer							
Group I	13.8±0.9	19.5±1..2	101.8±5.2	287.0±7.2	169.5±2.8	293.8±13.8	13.4±0.4	7.4±1.9	0.9±0.02	7.1±0.2	8.0±0.4	8.2±1.9	16.2±0.3	1:1.19
Group II	15.7±0.4	21.8±0.8	111.3±1.0	297.7±7.2	160.7±2.0	284.5±11.6	11.1±1.3	7.1±1.0	1.6±0.01	11.1±1.0	12.0±1.0	7.6±1.1	13.0±1.9	1:1.7
Group III	13.7±0.6	20.2±0.9	110.8±4.2	323.1±5.9	134. ±6.8	284.3±14.2	12.9±1.0	12.2±0.8	2.3±0.03	14.1±1.0	16.3±1.3	13.1±0.7	14.3±1.1	1:1.1
LSD	1.1*	1.6*	5.6*	9.6*	6.7*	9.7	2.6	2.2*	0.13*	1.1*	1.4*	1.6*	1.5*	
							Ghee							
Group I	14.0±1.2	20.2±1.8	103.9±6.6	285.5±6.2	167.8±4.2	296.2±22.1	13.3±0.6	6.4±0.9	0.8±0.02	8.3±1.2	9.1±1.3	7.3±1.0	16.1±0.5	1:2.2
Group II	14.1±0.3	19.7±0.5	104.9±0.8	297.3±3.3	151.8±10.9	293.5±15.0	14.2±0.9	10.4±2.8	1.8±0.02	11.5±2.1	13.4±2.6	11.6±3.1	16.5±0.6	1:1.4
Group III	14.4±0.7	21.0±0.8	110.7±1.9	308.1±10.3	132.3±12.5	292.8±10.5	13.3±0.3	11.8±1.6	2.3±0.04	16.0±2.5	18.3±3.2	13.1±2.0	15.2±0.7	1:1.1
LSD	1.1	1.4	5.8*	8.4*	6.1*	8.8	1.8	0.5*	0.14*	1.8*	2.1*	0.6*	0.7*	
Group I	13.8±0.9	19.6±1.1	102.3±5.5	288.2±6.3	170.9±3.6	302.4±11.1	13.4±0.3	6.0±0.1	0.8±0.02	7.5±0.2	8.2±0.1	6.6±0.2	16.3±0.2	1:2.5
Group II	13.9±0.6	20.5±0.6	107.1±3.7	290.2±2.3	161.7±2.2	301.5±14.5	13.3±0.1	9.1±0.5	1.2±0.04	11.5±0.2	12.7±0.6	10.2±0.6	16.0±0.2	1:1.5
Group III	13.6±0.6	19.7±1.0	106.2±3.5	308.7±5.1	125.4±4.7	301.1±10.5	14.2±0.8	14.1±0.5	2.3±0.02	16.5±2.0	18.8±2.1	15.7±0.4	16.0±0.9	1:1.0
LSD	1.2	1.4	5.5	9.6*	14.5*	8.6	1.7	2.8*	0.13*	3.1*	3.9*	2.9*	0.9	

*P<0.01 The nutritional requirements of Group-I buffaloes were fulfilled through concentrate mixture, In Group- II concentrate mixture + Berseem and in Group –III, Berseem only.

Table 8. Level of CLA (mg/g) in buffalo milk and the dairy products prepared through different processing treatments

7. Potential health benefits of CLA

The biological properties of dietary CLA are currently attracting considerable interest because of its diverse physiological outcomes in animal studies. Beyond its nutritional value, dietary CLA is effective in suppressing tumor development during initiation, promotion and progression phases of carcinogenesis (Belury, 2002). Not only CLA is a powerful anticarcinogen, but it also has antiatherogenic, immunomodulating, growth promoting, lean body mass enhancing and antidiabetic properties. Hence CLA is considered being a functional food.

Growing evidences suggest that CLA has numerous health benefits towards human being. Milk and meat from ruminants are richest natural source of CLA, which has been shown to have anticancer properties (Parodi, 1994). CLA reduced plasma lipoproteins and early atherosclerosis in animals (Lee *et al.,* 1994). CLA was able to normalize impaired glucose tolerance in diabetic rats (Houseknecht *et al.,* 1998). CLA has been shown to have immunomodulatory properties by enhancing mitogen induced lymphocyte blastogenesis, lymphocyte cytotoxic activity and macrophage killing ability (Wong *et al.,* 1997)

7.1 Anticarcinogenic property of CLA

The recent interest in CLA began with the isolation from hamburger meat as an anticarcinogenic factor. Partially purified extracts from fried ground beef was shown to contain mutagenic modulator activity inhibiting the initiation of mouse epidermal carcinogenesis by 7, 12-dimethylbenz[a]anthracene, a pro-carcinogen (Ha *et al*. 1987). CLA was repeatedly shown to have anticarcinogenic effects in animal models for stomach neoplasia (Ha *et al*. 1990), mammary tumors (Ip *et al*., 1997), and skin papillomas (Belury *et al*. 1997). As low as 0.05% level of CLA is enough to significantly decrease the induced mammary tumors in rodents (Ip *et al*. 1997). CLA is effective in reducing the size and metastasis of transplanted human breast cancer cells and prostate cancer cells in severely compromised immunodeficient mice. CLA-enriched butterfat was reported to alter mammary gland morphogenesis and also to reduce the risk of cancer in rats (Ip *et al*., 1997).

Kathirvelan (2007) reported that feeding CLA enriched ghee (composition of rat diet given in Table 9) lowered around 37 percent gross tumors incidence (Table 10) in 7, 12 dimethyl benz (a) anthrazene induced mammary gland carcinogenesis in female Wistar rat than the control (soybean oil based diet) group. The mean tumour weight was high in soybean oil fed group (3.30 g) (Fig 6 & Fig 7) than the high CLA ghee fed group (1.29 g) (Fig 8). The common type of tumour (benign) found in the three groups were fibroma, adenoma and fibroadenoma but adenocarcinoma (malignant) type (Fig 9) was found only in soybean oil based diet group. Thus, CLA feeding not only inhibited the benign type tumour but malignant tumour as well.

7.2 Cancer modulation mechanism of CLA

There are various intriguing possibilities regarding its anti-carcinogenic action. However, the concrete anti-carcinogenic mechanism still is unclear.

7.3 CLA acts as an antioxidant

This is the first theory proposed in support of its anticarcinogenic action. CLA through dietary fat gets incorporated into neutral lipids and phospholipids, preferably in the former.

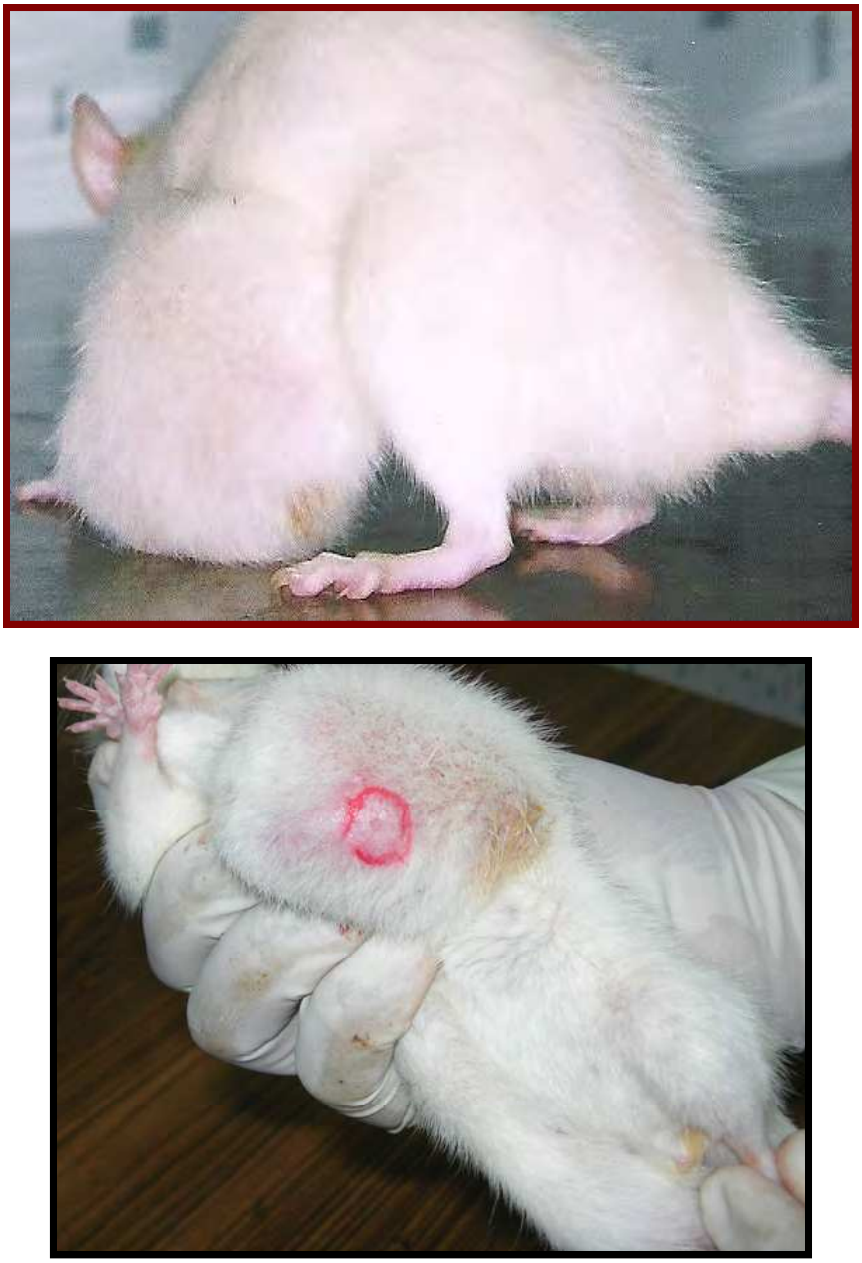
They act as free radical scavengers thereby providing an *in situ* 'defence mechanism' against membrane attack by dangerous oxygen free radicals (Ha *et al*., 1990).

Component	Soybean oil based diet	Low CLA (12.12 mg/g fat) ghee based diet	High CLA (19.54mg/g fat) ghee based diet
Bengal gram	540.0	540.0	540.0
Wheat	130.0	130.0	130.0
GNC	60.0	60.0	60.0
Soybean oil	200.0	-	-
Low CLA ghee	-	200	-
High CLA ghee	-	-	200
Skimmed milk powder	44.4	44.4	44.4
Mineral mixture	21.6	21.6	21.6
Vitamin mixture	2.0	2.0	2.0
Choline chloride	2.0	2.0	2.0

Table 9. Composition of rat diets (g/kg of diet)

Groups	**Percent tumour incidence***	**Percent Individual tumour incidence**				**Mean tumour weight (g)**
		Fibroma	**Adenoma**	**Fibro adenoma**	**Adenoma sarcoma**	
Soybean oil based diet	83.33 (25/30)	36.00 (9/25)	24.00 (6/25)	28.00 (7/25)	12.00 (3/25)	3.30 ± 1.39
Low CLA (12.12 mg/g fat) ghee based diet	63.33 (19/30)	42.11 (8/19)	21.05 (4/19)	36.84 (7/19)	0.00	2.42 ± 0.88
High CLA (19.54mg/g fat) ghee based diet	46.70 (14/30)	57.14 (8/14)	14.29 (2/14)	28.57 (4/14)	0.00	1.29 ± 0.27

Table 10. Effect of feeding CLA enriched ghee on mammary gland tumour incidence in 7, 12 DMBA administered rats



Rats were killed 32 weeks after DMBA administration
* Includes both palpable and histo pathological examination

Fig. 6. Rat showing the mammary tumour with the diameter approximately 3.4 cm (Soybean oil based diet)

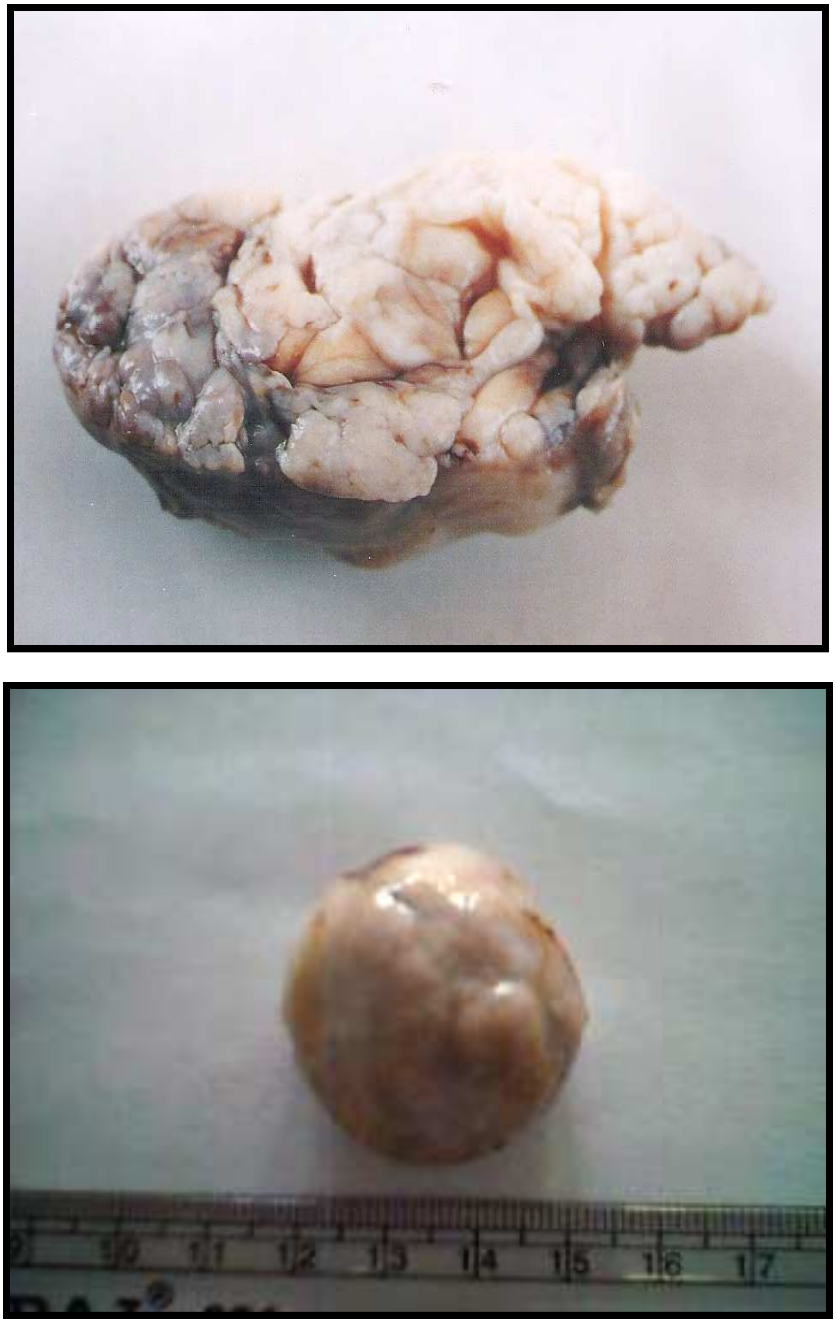


Fig. 7. Excised tumour showing 3.0 cm diameter (Soybean oil based diet)

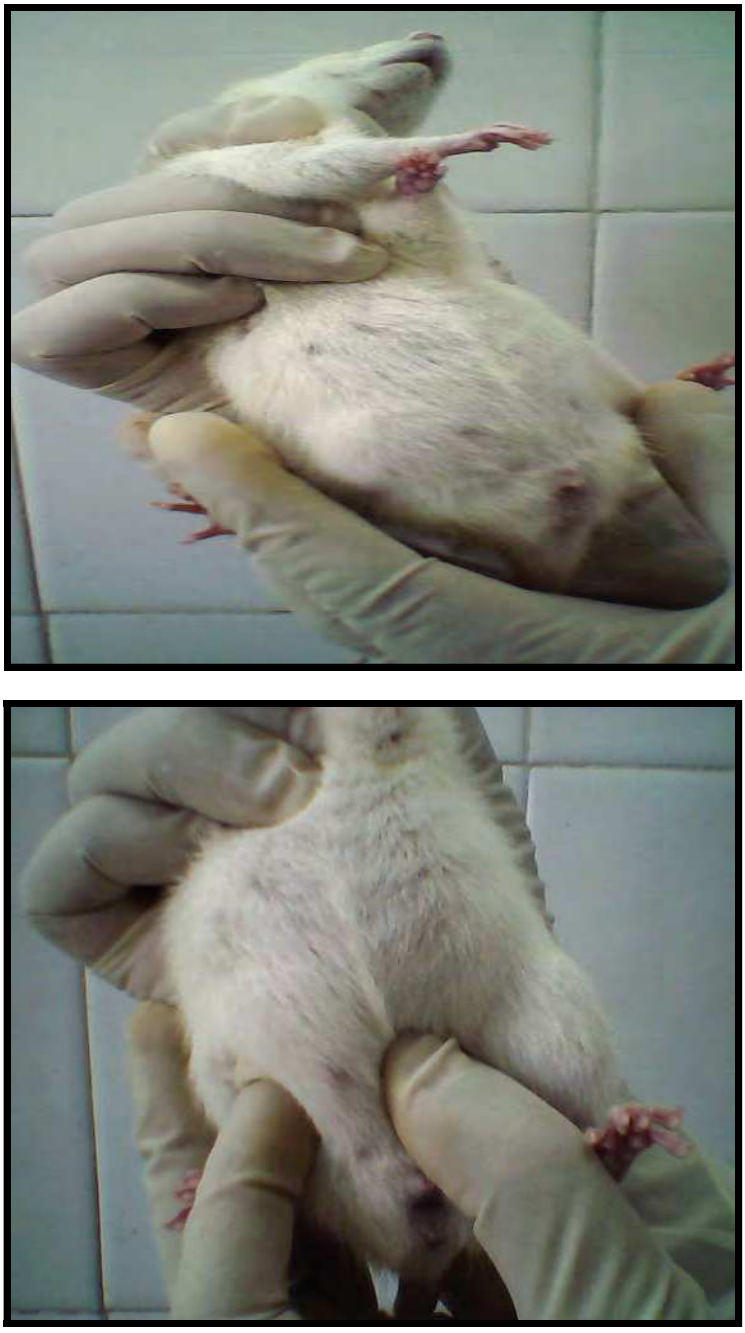
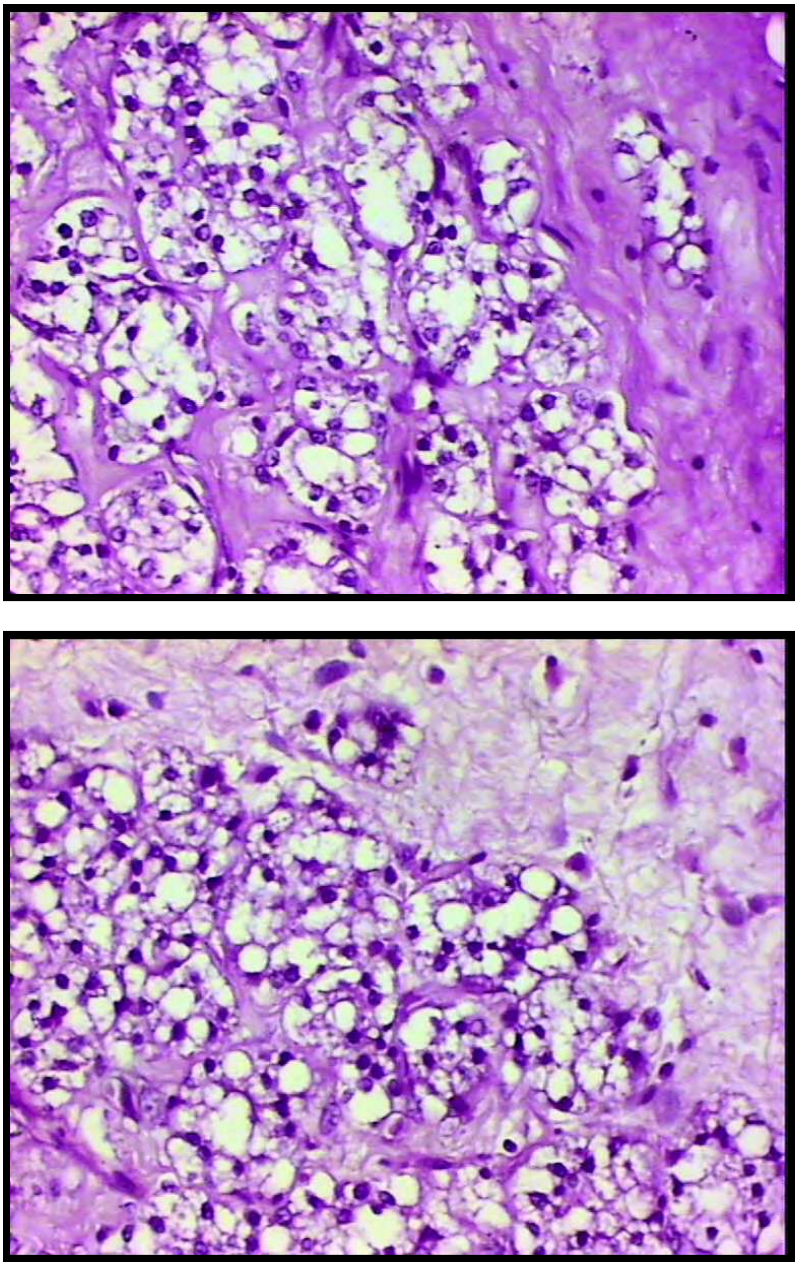


Fig. 8. Rats showing tumour with diameter of 1.1 cm (CLA ghee based diet)



(Soybean oil based diet) H&E x 400

Fig. 9. Adenocarcinoma showing stromal invasions with tumor cells

Compared with other antioxidants, CLA has been shown to be more potent antioxidant. It is more potent than alpha-tocopherol and as effective as butylated hydroxytoluene (BHT) in inhibiting iron-thiocyanate induced peroxide formation. In addition, CLA was shown to be as effective as vitamin E and butylated hydroxyanisole in inhibiting the formation of thio barbituric acid reactive substances (TBARS), as a bio marker often used to assess oxidation in biological systems.

Liver is the major organ in which most of the chemicals, drugs and carcinogens undergo metabolism (Krishnaswamy and Raghuramulu, 1999). Several environmental carcinogens have been reported to elicit hepatic oxidative stress and alter the activities of detoxifying and antioxidant enzymes during extra hepatic tumorigenesis (Aruna and Sivaramakrishnan, 1996). Antioxidant and detoxification enzymes can block carcinogenesis by acting as inhibitors of environmental carcinogens or mutagens (Cunningham and Lokesh, 1983).

Catalase and superoxide dismutase are the primary antioxidant enzymes involved in direct elimination of toxic free radicals which may result in amelioration of oxidative damage. Amelioration of oxidative damage by scavenging of free radicals formed during oxidative stress may protect cell against carcinogenicity and toxicity (Ketterer *et al.,* 1990). Glutothione-S-transferase is a biotransformation enzyme involved in the detoxification of xenobiotics, carcinogens, free radicals and peroxides by conjugating these toxic substrates with GSH (Glutathione reduced) thus ultimately protecting cells and organs against induced toxicity. Hence, enhancement of these enzymes by a natural or synthetic component may result in the amelioration or inhibition of extra hepatic tumorigenesis and CLA acts as chemopreventive agent by counteracting the carcinogen induced oxidative stress and elevating the levels of detoxifying enzymes (Ip *et al*., 1999). Ip *et al*. (1999) have clearly established a relationship between dietary CLA, oxidation and mammary carcinogenesis. Dietary CLA 0.25% or higher reduced the formation of TBARS in non initiated mammary tissue *in vivo* in parallel with CLA inhibition of mammary carcinogenesis. Kathirvelan (2007) observed that feeding CLA enriched ghee resulted in increased antioxidative as well as detoxifying enzymes in liver and mammary gland than the soybean oil based diet fed rats (Table 11).

Liver enzymes	**Cancer groups**		
	Soybean oil based diet	**Low CLA ghee based diet**	**High CLA ghee based diet**
Catalase (U/mg protein)	$479.91^{a} \pm 11.9$	$609.60^{b} \pm 7.62$	$730.67^{c} \pm 11.36$
SOD (U/mg protein)	$9.63^{a} \pm 0.79$	$15.86^{b} \pm 0.84$	$20.60^{c} \pm 1.41$
GST (U/mg protein)	$0.79^{a} \pm 2.50$	$1.19^{b} \pm 3.77$	$1.42^{c} \pm 4.51$
Mammary gland			
Catalase (U/mg protein)	$22.26^{a} \pm 1.71$	$35.66^{b} \pm 1.41$	$47.52^{c} \pm 1.43$

Values are Mean ±SE for n=12, One CAT Unit (U) is equivalent to 1µmol of H_2O_2 consumed /minute/ mg of protein at 25°C, One SOD unit is equivalent to the amount of enzymes that inhibit the reaction 50% , One GST unit is expressed as mmoles of cDNB conjugated /minute/mg protein.
Values with different superscript across a row differ significantly at ($P<0.05$)

Table 11. Effect of feeding CLA enriched ghee on liver and mammary gland enzymes activities in cancer induced groups

7.4 Inhibit arachidonic acid ($C_{20:4}$) derived eicosaonoid metabolism in the target organ (Belury et al., 1997)

Many types of cancer cells have an enzyme called cycloxygenase (Cox-2) and use it as a biological fuel to propagate rapidly. CLA inhibits the enzyme thereby modulation the prostaglandin biosynthesis from linoleic acid in normal course (Urquhart *et al*, 2002). The prostaglandins (PGE_2) secreted by cancer cells under the activity of Cox-2 acts as a tumor promoter. Unusual PUFAs ($C_{18:3.}$ $C_{20:3}$) derived from CLA leads to the formation of arachidonic acid which behaves differently than that of its usual nature when derived from linoleic acid in normal course (Iwakiri *et al*, 2002). This cerueval behavior leads to eicosaonoid inhibition viz. inhibition of prostaglandins and leukotrienes which further leads to reduction of diacylglycerol (Tumor promoting factor) upto 50% (Kritchersky et al, 2000). The cancer preventive activity of CLA is unlikely to be indicated by interference with the metabolic cascade involved in converting linoleic acid to eicosaonoid.

7.5 Reduce the formation of carcinogen – DNA adducts

CLA leads to a reduced formation of a carcinogen and DNA adducts (Moon *et al*, 2003) in a similar manner to substrate legend binding. This may be due to CLA check at the initiation point of carcinogenesis further preventing the binding of electrophile intermediates of DNA thereby causing permanent DNA lesion. This DNA containing cell when allowed to proliferate under promotion stage may lead to neoplastic cell.

7.6 Stimulate the lymphocyte proliferation

The lymphocytes had enhanced proliferation when stimulated with CLA, *in vivo* and *in vitro* (Miller *et al*, 1994) when a blend of isomers (cis-9, *trans*-11 and *trans*-10 *cis*-12) in the ratio 80:20 are given through the diet, the lymphocyte proliferation is observed (Majumdar *et al,* 2002). This in turn enhances cellular immunity by modulating phenotype and effect or functions of CD^{8+} lymphocytes (cells involved in both adaptive and innate immunity) involving T-cell receptors. The CD^{8+} lymphocytes are essential for the development of cell mediate protection against neoplastic cells. CLA enhanced the cytotoxic potential of peripheral blood lymphocytes by inducing the proliferation of cytotoxic CD^{8+} cells. Further, CLA was found to increase CD^4 lymphocyte population and NK cell function and number (Ochoa *et al*, 2004). CD^4 cells get differentiated into cytokines TH_1 cells and TH_2 cells (Thymus responsive cells). The TH_1 cells produce interleukin 1, 2 and γ-interferon, activate macrophages and further delayed hypersensitivity reaction whereas TH_2 cells produce interleukin -4, 5, 10 induces esinophiles decrease in number (CLA has anti allergic effect) and is more specialized in inducing B cells for immunoglobin production (CLA has immune stimulation effect) interleukin 1, 2 so derived by induction of TH_1 responsive cells further stimulate growth of γ-interferon in T-cells and NK cells. The γ-interferon inhibits PGE_2 synthesis which acts as a tumor promoter factor. Gamma interferon kill tumour either by usual mechanism of secretion of tumour necrosis factor (TNF) or by activated macrophages mediated selective cellular toxicity. NK cells in addition to direct lysis of tumour cells can also participate in antibody dependant cellular cytotoxicity.

7.7 Modulate the activity of phase-1 enzymes of Mixed Function Oxidase system (Benjamin et al, 2003)

CLA has been found to modulate the activity of Cytochrome-P450 (Cyt.P) and reduce the induction of ornithine decarboxylase (ODC) and GTPase activating protein kinase C (PKC).

This facilitates the oxidation and reduction of Cyt.P and hydroxylation as a result of which activated toxicant-Cyt.P complex and subsequently hydroxylated toxicant is available in the end of phase-1 reaction. The enzymes ODC+PKC play key roles in the activation of carcinogens and therefore are tumor production indicators.

7.8 Modulate gene expression (Carta et al, 2002)

The potential molecular mechanism by which CLA shows its anti-carcinogenic activity says that CLA acts as a legend for peroxisomes proliferation receptor (PPAR) which is a steroid hormone receptor (nuclear receptor) . This binding after the transcription process through sequential steps. The regulatory gene is lost and so the target genes are altered. This activates the endonuclease action; influx of Ca^{2+} takes place in the cytoplasm which leads to DNA fragmentation. The cell growth and differentiation is arrested and thus induce apoptosis.

7.9 CLA and atherosclerosis

While considerable research has focused on a potential anticarcinogenic effect of CLA, there are few studies indicating that CLA may also reduce the risk of cardiovascular diseases in animal models. Unlike linoleic acid, there is a paucity of information regarding the effect of dietary conjugated linoleic acid on plasma lipoproteins and aortic atherosclerosis.

Lee *et al.*(1994) first tested whether CLA might affect the initiation and progression of atherosclerosis lesions in rabbits through its effect on lipid peroxidation. They reported that rabbits fed an atherogenic diet and supplemented with 0.5g CLA per day for 22 weeks had significantly less plasma triglyceride, plasma LDL-cholesterol (LDL-C) and LDL-C/HDL-C ratio than control animals. CLA feeding also resulted in fewer aortic fatty lesions. Subsequently, (Nicolosi *et al*., 1997) reported that hamsters fed CLA collectively had significantly reduced levels of plasma total cholesterol, non-high density lipoprotein cholesterol, (combined very-low and low-density lipoprotein) and triglycerides with no effect on high density lipoprotein cholesterol, as compared to controls. Kathirvelan (2007) reported that low and high CLA feeding reduced total cholesterol by 52.17 and 26.06 percent, triglycerides by 23.00 and 11.00 percent, however, HDL-c increased by 17.82 and 33.26 percent than soybean oil fed rats (Table 12). He concluded that, antiatherogenic property of CLA was proved by decreasing the total cholesterol, triglycerides, LDL-cholesterol (Fig 10) and atherogenic index (Fig. 11) and increasing the HDL-cholesterol blood plasma. In addition to this, the level of cholesterol and triglyceride deposition in liver and aorta were lower in CLA fed groups (Table 13).

In the literature, dietary CLA has been reported to have controversial results on atherosclerosis in mice. Noone *et al*., (2002) reported that CLA actually did not reduce the incidence of atherosclerosis, but increased the incidence of fatty acid streaks in mice compared to control-fed mice. Study conducted by Munday *et al.* (1999) contradicts the finding of studies conducted in rabbits and hamsters (Lee *et al*., 1994: Nicolosi *et al*., 1997). It is possible that dietary CLA has different influences on the fatty acid and cholesterol metabolism in different animal species. More research is necessary to elucidate the effects of dietary CLA on lipid metabolism and atherogenesis in animal models and eventually human beings.

Since it is difficult to study the effect of CLA on atherosclerosis in humans, an indirect approach by measuring various potential heart disease markers is required (Belury and Kempa-steczko, 2000). Lipid atherogenic risk markers were more favorably influenced by *cis*-9, *trans*-11 isomer than a mixture of CLA or fish oil (Valeille *et al*., 2004) when healthy human subjects were used in a double-blind placebo controlled intervention trial. Noone *et*

al. (2002) demonstrated that CLA isomers improved very low-density lipoprotein cholesterol and plasma triacylglycerol metabolism suggesting that some of the cardio-protective effects of CLA shown in animal studies were relevant to humans as well.

Parameters	Base line levels	Groups		
		Soybean oil based diet	Low CLA ghee based diet	High CLA ghee based diet
Total Cholesterol (mg/dL)	58.09 ± 1.24	81.33^{a} ± 2.91	74.40 ^{b} ± 1.68	69.93^{c} ± 1.44
HDL-C (mg/dL)	34.72 ± 0.78	35.51^{a} ± 0.91	41.84^{b} ± 0.54	47.32^{c} ± 0.96
Triacylglycerol (mg/dL)	40.87 ± 0.77	73.23^{a} ± 1.08	65.01^{b}± 1.16	56.21^{c} ± 0.42
LDL-C (mg/dL)	14.26 ± 1.72	26.79^{a} ± 3.53	19.57^{b} ± 1.86	14.38^{c} ± 1.62
Atherogenic Index (AI)	0.410 ± 0.06	0.971^{a} ± 0.12	0.570^{b} ± 0.05	0.244^{c}± 0.04

Values are Mean±SE for n=8
Values in rows with different superscript differ significantly (P<0.05)

Table 12. Plasma Lipids profile of Wistar rats at baseline and after 16 weeks of CLA enriched ghee feeding.

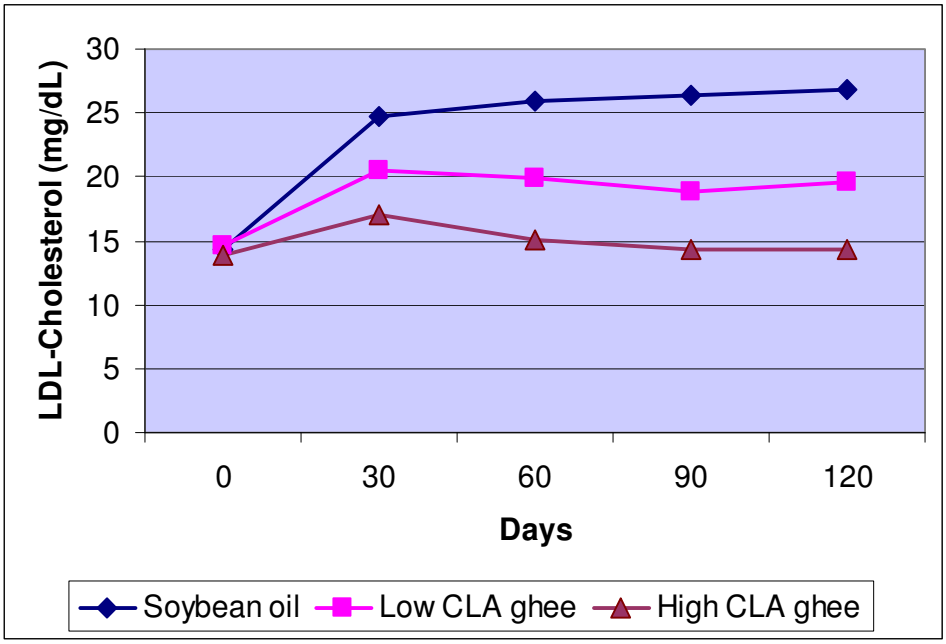


Fig. 10. Plasma LDL (mg/dL) levels in rats fed on CLA enriched ghee diet

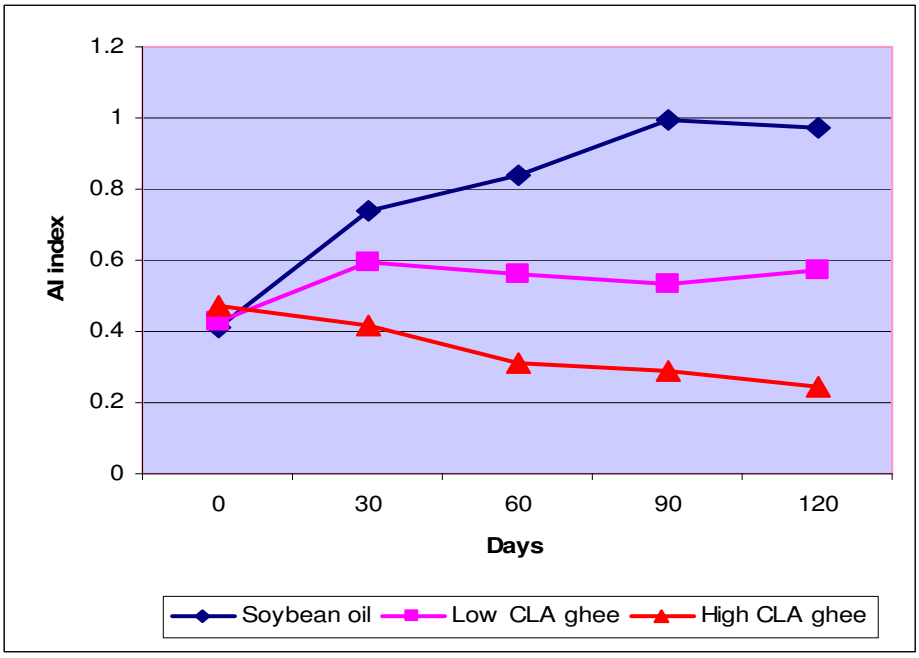


Fig. 11. Atherogenic index in rats fed on CLA enriched ghee diet

Groups	Liver		Aorta	
	Cholesterol	Triglycerides	Cholesterol	Triglycerides
Soybean oil based diet	$1.05^{a} \pm 0.029$	$10.08^{a} \pm 0.145$	$9.46^{a} \pm 0.029$	$7.06^{a} \pm 0.073$
Low CLA ghee based diet	$0.84^{b} \pm 0.013$	$7.12^{b} \pm 0.218$	$6.95^{b} \pm 0.015$	$5.09^{b} \pm 0.123$
High CLA ghee based diet	$0.53^{c} \pm 0.008$	$4.50^{c} \pm 0.143$	$3.64^{c} \pm 0.009$	$2.49^{c} \pm 0.109$

Values are Mean ± SE for n=8
Values in rows with different superscript differ significantly (P<0.01)

Table 13. Lipid profile (mg/g tissue) in liver and aorta tissue of rats fed on CLA ghee diet

7.10 CLA and lipid metabolism

A major effect of CLA in this respect is the reduction in lipid uptake by adipocytes (Pariza *et al.*, 2003) which leads to the reduction in body fat gain (Kim *et al.*, 2002). Azain *et al.* (2000) reported that the reduction in body fat mass in rats was the result of reduced adipose tissue size rather than cell number. Similarly Poulous *et al.* (2001) found a reduction in cell size, but not the cell number in rats which had less body fat in response to CLA supplementation.

Carta *et al.* (2002) concluded that a regular intake of CLA and or TVA as its precursor should work as an excellent preventive agent that would modulates lipid metabolism. Kathirvelan (2007) found that feeding CLA ghee (20 % in the diet) to male wistar rats doesn't increase the body weight as compared to the control group (Table 14)

week	Average weekly body weight (gram)		
	Soybean oil diet (Control)	Low CLA ghee diet	High CLA ghee diet
0	131.13 ± 4.70	125.00 ± 2.85	128.57 ± 3.45
2	169.29 ± 5.09	165.01 ± 4.03	163.14 ± 4.68
4	187.86 ± 6.04	185.08 ± 6.22	185.71 ± 7.02
6	203.57 ± 3.45	198.52 ± 5.59	195.29 ± 4.17
8	215.00 ±5.34	214.29 ± 7.58	212.86 ± 4.93
10	231.69 ± 8.55	228.33 ± 7.42	227.51 ± 6.95
12	243.33 ± 7.42	240.00 ± 6.35	239.17 ± 4.47
14	254.00 ± 5.88	257.50 ± 8.14	252.51 ± 6.32
16	263.33 ± 4.81	265.00 ± 6.67	262.12 ± 4.21
Over all mean	211.02 ±13.51	208.81±14.23	207.43 ±13.73

Values are Mean ±SE for n=8

Table 14. Average weekly body weight of rats fed on CLA enriched ghee diet

Studies in rats and mice have shown that feeding CLA at the level of 0.5% in total diet produced small reduction in body fat gain in growing animals (Pariza *et al.,* 1997; Park *et al.,* 1997). Whilst the actions of CLA in inhibiting body fat accumulation have received considerable attention because of increasing concern for marked increases in obesity in western societies, care should be taken in extrapolating these findings to man until more information is available. The specific mechanism by which dietary CLA reduces body fat content is likely to vary from one animal species to another. The results of animal studies are also not conclusive. The mechanism by which CLA alters lipid metabolism and body composition in animals is not fully elucidated. It may be tissue and species-specific. In rodents, CLA-induced alteration of lipid metabolism appears to involve increase in rates of lipolysis and fatty acid oxidation. Support for this mechanism comes from the observation of increased hormone sensitive lipase activity and enhanced carnitine palmitoyl transferase activity in several tissues of mice fed CLA (Pariza *et al.,* 2003).

Peroxisome proliferators activated receptor alpha (PPAR∝), one of the nuclear receptors related to the modulation of environmental and dietary stimuli (Schoonjan *et al.,* 1996), is likely to be involved in the regulation of the gene expression of fatty acid beta oxidation enzymes by dietary CLA. It has been demonstrated that CLA is a potent legend and activator of PPARα (Belury *et al.,* 1997) but Peterson *et al.* (2003) observed that a mixture of CLA isomers increased the gene expression of hepatic fatty acid beta oxidation enzymes through both PPARα dependent and independent mechanisms. The activity of fatty acid

synthatase (FAS), a key enzyme of fatty acid synthesis, was also significantly decreased by dietary CLA as compared to the control diet in the liver of rats.

7.11 CLA and diabetes

Feeding of CLA to rats prone to developing diabetes normalized glucose tolerance and improved hyperinsulinemia as effectively as currently used medications (Houseknecht *et al*., 1998). CLA was fed at 1.5% (by weight) of the diet for 2 week in diabetes induced rats. The study was short-term and needs to be replicated and extended before the results can be applied to human health. Nonetheless, if CLA can improve glucose homeostasis and inhibit body fat accretion as demonstrated in mice, rats, and pigs, then CLA may be beneficial to humans prone to diabetes (Ip *et al*., 2003)

7.12 CLA and immune system:

Cook *et al* (1993) showed that CLA not only enhances immune response, but also protects tissues from collateral damage. Sugano *et al* (1999) proposed that the immune enhancing effect of CLA was by modulating eicosanoid and immunoglobin production. CLA also diminished lipopolysaccharide induced inflammatory events in macrophages through reduced mRNA and protein expression of nitric oxide synthatase and cyclooxigenase-2 as well as subsequent production of nitric oxide and prostaglandin E2 (Cheng *et al*., 2004). Kathirvelan (2007) observed that feeding of CLA enriched ghee to mammary gland cancer induced female wistar rats reduced level of plasma nitric oxide than the non CLA fed rats (Table 15). Cook *et al* (1993) suggested that CLA prevents immune associated wasting by

Days	Cancer groups		
	Soybean oil based diet	Low CLA ghee based diet	High CLA ghee based diet
0	9.25^{a} ±0.31	8.59^{a} ±0.17	8.41^{a} ±0.18
30	18.52^{a} ±0.33	16.45^{b} ±0.41	15.45^{c} ±0.38
60	25.70^{a} ±0.50	19.44^{b} ±0.20	18.38^{c} ±0.23
90	30.28^{a} ±0.49	25.43^{b} ±0.22	23.43^{c} ±0.45
120	38.18^{a} ±0.33	33.16^{b} ±0.53	28.20^{c} ±0.28
150	40.92^{a} ±0.50	35.34^{b} ±0.31	32.54^{c} ±0.25
180	42.46^{a} ±0.46	35.83^{b} ±0.54	32.24^{c} ±0.37
210	43.26^{a} ±0.29	38.50^{b} ±0.28	33.20^{c} ±0.15
240	43.00^{a} ±0.41	37.56^{b} ±0.34	33.07^{c} ±0.47
Over all mean	32.40 ± 3.87	27.81 ± 3.39	24.99 ± 2.86

Values (μmol/ml) are Mean □ SE for n=12
Values in a row with different superscript differ significantly ($P<0.05$)

Table 15. Effect of feeding CLA enriched ghee diet on plasma nitric oxide (μmol/ml) level in mammary gland cancer induced rats

protecting nonlymphoid tissues from the adverse effects of cytokines, which are growth suppressants, because CLA influences the immune system by altering the effects of cytokine, interleukin, leukotriene and many immunoglobulin. Sebedio *et al*., (2000) hypothesized that CLA indirectly affects the function/production of tumor necrosis factor-α

(TNF-α) and interleukin-1 (IL-1). Yu *et al*. (2002) has shown that CLA exhibits anti-inflammatory effects by negatively regulating the expression of certain pro-inflammatory genes. By inducing the activity of peroxisome proliferators activated receptor gamma (PPAR-γ) via CLA, there is a decrease in the production of pro-inflammatory products such as nitric oxide and TNF-α (Yu *et al*., 2002).

7.13 CLA and bone metabolism

Watkins *et al* (1999) found a higher rate of bone formation in chicks fed butterfat, which was suggested to be due probably to increased CLA intake. Dietary CLA led to differences in CLA enrichment of various organs and tissues, bone marrow and periosteum containing the highest concentration of CLA and brain the lowest. Enrichment of chondrocytes with CLA affected collagen synthesis in a dose dependent fashion (Watkins *et al.,* 1999). Reduced production of arachidonic acid and PGE_2 in the chondrocytes was suggested to be the possible mechanism. Such changes in bone biomarkers and bone formation rates in rats were associated with increased *cis*-9, *trans*-11 CLA in bone tissue lipids. McDonald (2000) suggested that increased ash content in CLA fed animals (Park *et al*, 1997) is due to protection of bone loss from cytokines. Further investigation is needed as to how bone metabolism is affected by CLA and mechanism of action related with it.

8. Conclusion

Conjugated linoleic acid is unique; unlike the most naturally occurring anticarcinogenic substances found mainly in plants, it is present in food from animal sources. Milk fat is the richest natural source of CLA and could be increased by manipulation of the nutritional regimes of the animals. Diverse biological roles of CLA in mediating cancer, diabetes, lipid metabolism, atherosclerosis, immune function, bone modeling etc. observed in animal models are quite compelling. Although there have been a few attempts at verifying the positive effects of CLA in human health through case control studies, it is not yet possible to clearly state that CLA supports all the those benefits in humans as well. Limited available literature in humans, however also supports the findings observed in animals and tissue culture models even though the results are inconclusive and even conflicting at times. As a result, natural enrichment of food products through manipulation of animal diet may contribute to the overall goal of obtaining the positive health benefits associated with CLA in the immediate future.

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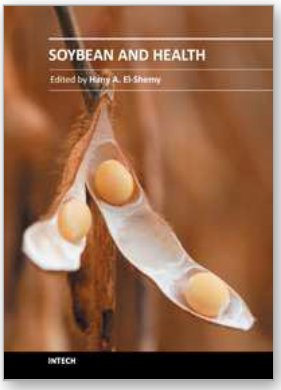

Soybean and Health

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