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Manipulation of Conjugated Linoleic Acid in Milk and Meat through Dietary Management in Ruminant Animals: A Review

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ABSTRACT

Importance of conjugated linoleic acid (CLA) in the diets of humans as a vital health promoter has gained significance in recent times. CLA is a collective term used for a mixture of positional and geometrical (cis or trans) isomers of linoleic acid with conjugated double bonds. The major isomers are c9t11 and t10c12 besides many others. Most prevalent and biologically active CLA isomer is c9t11. Amongst, several beneficial health effects inhibition of cancer, coronary heart diseases and mutagenesis are most important aspects, besides reduction of body fat in humans. Even though, ruminant milk and meat and their products are richest sources of CLA, further enrichment of these products is required to obtain the health benefits of CLA in human population. This can be achieved by increase of CLA contents of above livestock products by way of various dietary manipulations in the ruminant animal production system. CLA is principally synthesized in the rumen by biohydrogenation of linoleic acid and endogenous desaturation of trans vaccenic acid (TVA), which is also produced in the rumen. Feeding ruminant animals with diets either rich in linoleic acid or poor but effectively increase the TVA production is the practical way for elevation of CLA contents in milk and meat. Grazing on pastures rich in grasses enhanced the milk and meat CLA contents. Supplementing ruminant diets with oilseeds/oils viz. soybean, rapeseed, linseed, sunflower, safflower etc. or fish meal/oil and some methods of feed processing like- cracking, rolling, roasting, extrusion etc. increased milk and meat CLA contents. Literature relates that high level of concentrate feeding did not support elevation of CLA, whereas feeding good quality roughage was always beneficial. Moreover use of ionophore antibiotics in the animal diet produced marginal increase; their role is limited due to prohibition. The effects of various other dietary factors and processing methods for increasing CLA levels in the ruminant livestock products have been discussed. Future research studies on aspects of synchronized ruminal fermentation along with effective biohydrogenation and optimization of passage rates are suggested. The role of alternate hydrogen sink in rumen such as organic acids, component of Krebs cycle may also be explored for potential of increasing CLA levels.

(Key Words: CLA, milk, meat, ruminants, oilseed, roughages, pastures)

INTRODUCTION

Amongst 400 odd fatty acids (FA) found in cow milk fat, one was recognized to contain conjugated double bonds by UV absorption technique (Booth et al. 1935). Its structure was later, recognized mainly as cis 9, trans 11-C18:2 (c9t11-C18:2) monocarboxylic acid. It was christened as *'Rumenic Acid'*, probably due to its origin from the rumen. It is formed as an intermediate compound during the microbial hydrogenation of linoleic acid to stearic acid and also produced endogenously in other body tissues from trans vaccenic acid (TVA), which also originate in the rumen (Griinari and Bouman 1999). The term conjugated linoleic acid (CLA) is collectively used for a mixture of positional and geometrical (cis or trans) isomers of linoleic acid having conjugated double bonds. The major isomers include c9t11, t9t11, t10t12, and c10t12 while the minor isomers include c9c11, c10c12, c10t12, and c10t12 (Lin et al. 1995). The most biologically active and abundant isomers of CLA

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are c9t11 and t10c12. However, former alone accounts for over 80% of total in milk fat (Bouman and Griinari 2001; Chin et al. 1992).

Different isomers of CLA and especially c9t11 possess beneficial effects on human health like suppression of carcinogenesis (Cantwell et al. 1999; Ha et al. 1990), mutagenesis (Ha et al. 1987) and atherosclerosis (McGuire and McGuire 2000). The other important physiological effects include its ability to reduce catabolic effects of immune stimulation (Cook et al. 1998), increased IgG and IgM production (*in vitro*) by spleen lymphocytes in rats (Yamasaki et al. 2000), immune stimulation and modulation (Cook et al. 1993) and prevention of distress (Ohgushi et al. 2001) in poultry, increased lean muscle tissue in humans (Beuker et al. 1999). It has also been shown to reduce the body fat (Pariza et al. 1996) and growth-promoting action (Chin et al. 1994) in rats and increased feed conversion efficiency in growing and finishing pigs (Thiel-Cooper et al. 2001). Emerging evidence indicates that c9t11 and t10c12 isomers of CLA produce different effects (Wang et al. 2005a) and later could be associated with decrease in level of HDL-cholesterol in humans (Martin and Valeille 2002).

Although CLA is found in foods both of animal and vegetable origin, but its content is much higher in animal products. Milk, meat and their products from ruminant animals are richer sources than their non-ruminant counter parts (3.32 vs 1.39 mg CLA/g fat) and are recognized as major dietary sources for human beings. Goat meat with 63.5 mg/g fat CLA contents was ten times higher than of pork and chicken (Takenoyama et al. 2001). Among various sources, level of CLA vary from as low as 0.2 mg/g in vegetable oils to 17 and 30 mg/g fat in beef and milk respectively, and c9t11 isomer contributes more than 90% of total CLA content in milk fat (O'Shea et al. 1998).

Owing to the considerable differences in CLA contents of common foods its dietary intake in humans varies to large extent. According to estimates the common people are not fully benefited by the advantages of CLA since they are not consuming appropriate quantity through the diets (Ip et al. 1994). German males and females had average CLA intake with 430 and 350 mg/d respectively. In USA and Finland, the daily intake of CLA for men and women together ranged from 52-137 and 40-310 mg, respectively (McGuire et al. 1999). In Indian context, no such estimates are available, but looking into the beneficial effects and consumption pattern of milk and meat by human population, the increase of CLA levels may be important. This could be achieved by adjusting the animal diet that can increase CLA levels. This article is aimed to review the influences of various dietary factors on the CLA content in milk and meat of ruminant animals and to suggest suitable research strategies.

CLA biosynthesis in ruminants

Membrane associated enzymes of some rumen bacteria carry out the process involving biohydogenation (BH) of polyunsaturated fatty acids (PUFA; Hughes et al. 1982). Typically, enzymes produced by fiber degrading rumen bacterium, *Butyrivibrio fibrisolvens* catalyze these reactions. Moreover, recently *Megashphaera elsdenii* (*YJ-4*) has been found responsible for the synthesis of t10c12-CLA in rumen (Kim et al. 2005). Principally c9t11 isomer of CLA is synthesized in rumen as BH intermediate of linoleic acid ($C18{:}2\Delta^{c9,c12}$ or C18:2) to stearic acid (C18:0), however BH also take place in the hind gut to a small extent. Henceforth term CLA will be used for c9t11 isomer or otherwise mentioned. Process of CLA biosynthesis in ruminant animals is depicted in Figure 1.

During the synthesis of CLA in the BH pathway, specific protonation of C^{13} atom in D-configuration of linoleic acid is carried out by an isomerase enzyme. It involves interaction between active site of enzyme and π-electrons of double bonds at C^9 position followed by transfer of proton (H^+) owing to additional bonding between an electronegative region of enzyme and -COOH group of substrate to produce c9t11 isomer (Kepler et al. 1971). Further, strong linear correlation between contents of CLA and TVA ($C18{:}1\Delta^{11}$ or t11-C18:1) suggest that, first two reactions i.e., up to the formation of TVA, are not rate limiting (Kepler et al. 1966) nevertheless, conversion of TVA to stearate is a rate limiting step in the complete BH of linoleic acid (Jiang et al. 1996). Accumulation of higher TVA in rumen could be an indicator of a decrease of BH process (Scollan et al. 1997) and reduced BH as evidenced by higher TVA could lead to accumulation of CLA (from linoleic acid) due to negative feedback and might yield higher CLA in the rumen (Enser et al. 1999).

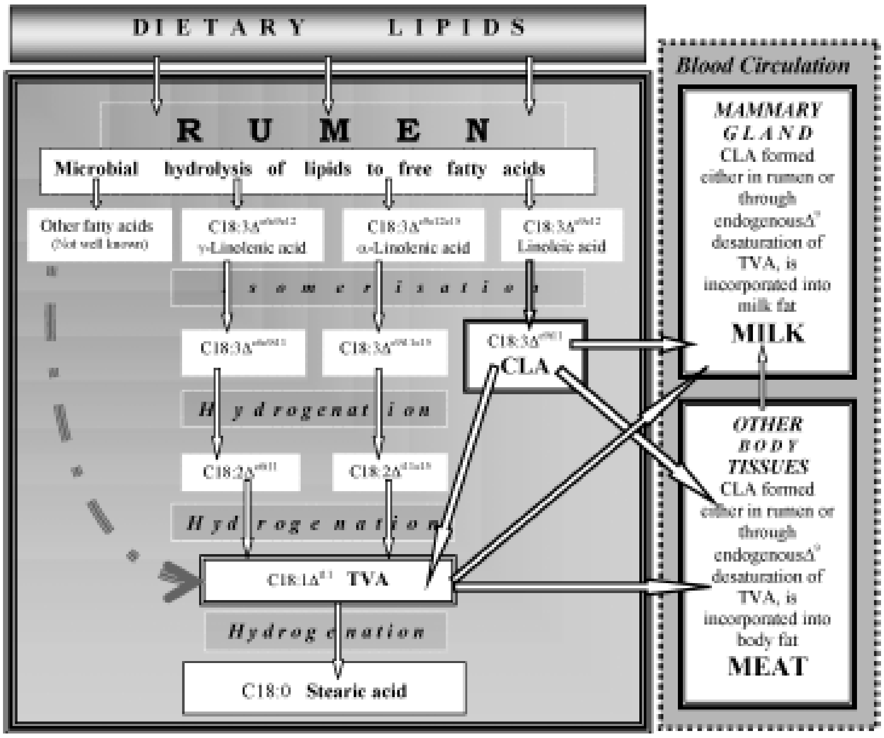

Figure 1: Schematic representation of biosynthesis and origin of CLA in milk and meat in ruminants

It is suggested that relatively small proportion of CLA escapes the rumen and after absorption made available for uptake by mammary gland or adipose tissues and thereby an increase of dietary supply of linoleic acid, being elementary precursor through oil supplement enhances the amount of CLA in milk and meat. Moreover, some studies indicate the presence of alternate mechanisms also. Feedstuffs which are not good sources of linoleic acid like pasture grasses (Palmquist 1988) and linseed (Table 1) upon consumption increase the CLA contents and conversion of linoleic acid by rumen microorganisms to CLA probably does not appear to be major source (Griinary and Bauman 1999). Choi and Song (2005) observed that addition of C18:3 FA produced higher level of CLA than radiolabeled C18:2 under *in vitro* system. On the other hand, γ-linolenic acid ($C18:3\Delta^{c6,c9,c12}$) which occur frequently in fungi, algae and certain oil seeds but present in small quantities in lipids from animal and higher plants (Kemp and Lander 1983) does not seem to be of much significance.

A mechanism involving the tissue conversion of TVA into CLA seems to be of major importance (Corl et al. 1998). The BH of linolenic acid ($C18:3\Delta^{c9,c12,c15}$) does not include CLA as an intermediate whereas TVA is a common intermediate for BH pathways of C18:2 and C18:3 FA. Both CLA and TVA after synthesis in rumen are absorbed in lower gastrointestinal tract and transported through blood circulation to different body sites, where these are incorporated into tissues. The TVA can be transformed to CLA by desaturase enzyme present in intestine, adipose tissue and mammary gland (Fig.1). Elevated CLA content in cow milk on post ruminal infusion of TVA provide good evidence in favour of tissue desaturation. According to few estimates, the milk fat CLA can arise to a tune of

Table 1: C18 Fatty Acid (FA) contents of some feedstuffs and vegetable oils

Item	FA content (g/100g total FA)			Reference
	C18:1	C18:2	C18:3	
Alfalfa	6.5	18.4	39.0	Palmquist 1988
Rye grass	2.2	14.6	68.2	-do-
Pasture grass	3.4	13.2	61.3	-do-
Barley silage	18.4	28.1	6.4	-do-
Vetch grass pasture	3.5	14.0	63.3	Valvo et al. 2005
Vetch grass hay	8.3	16.8	48.8	-do-
Barley grain	12.5	48.2	8.9	Gibb et al. 2005
Corn grain	22.8	58.1	1.6	-do-
Corn oil	30.5	52.0	1.0	Anonymous 1995
Cottonseed oil	19.0	52.0	Traces	-do-
Groundnut oil	37.0	41.0	0.3	-do-
Linseed oil	28.0	18.0	60.0	-do-
Rapeseed oil	56.0	26.0	10.0	-do-
Rice bran oil	42.3	37.0	1.3	-do-
Safflower seed oil	13.8	75.3	Traces	-do-
Hempseed oil	12.0	55.0	25.0	-do-
Jack fruit oil	6.4	40.2	9.4	-do-
Karanj seed oil	47.9	23.6	-	-do-
Poppy seed oil	11.0	72.0	5.0	-do-
Beef Tallow	40.0	4.0	2.5	-do-

43% via endogenous conversion of TVA (Griinary and Bauman 1999). Interestingly, Adolf et al. (2000) obtained 30% conversion efficiency via desaturation of deuterium labeled TVA at Δ^9 positions. A higher level of 91% of ruminant milk fat CLA was traced to be of endogenous origin via desaturation of TVA (Kay et al. 2004). Thus formation of CLA in ruminant animal is a consequence of partial BH of dietary FA (C18:2 and C18:3) and endogenous Δ^9 desaturation of TVA in mammary gland and other parts like subcutaneous or intarmuscular adipose tissues (Raes et al. 2004).

Influence of various dietary factors on CLA content in milk and meat

Various dietary conditions affect the rumen environment and consequently supply of precursors for CLA synthesis. Concentration and duration of feeding lipid substrates and energy sources could affect microbial FA metabolism in the rumen and could be major determinants at ruminal CLA production (Bessa et al. 2000). Fundamentally, linoleic acid is a better CLA precursor than linolenic acid but others not fully recognized precursors may also be contributing through many other reactions viz. desaturation, chain elongation and many other modifications. Recently, Kay et al. (2004) have suggested that endogenous synthesis is responsible for more than 91% of the c9t11-CLA secreted in milk fat of cows fed fresh pasture. Therefore, different tissues differ in CLA distribution, probably because of differences in activities of desaturase enzyme apart from various dietary factors (Mir et al. 2000). Like milk fat, CLA in meat also depends upon similar dietary factors, but sizable literature is available.

Effect of roughage based diets

The pasture grasses, especially lush green with young leaves are rich sources of linolenic acid i.e., C18:3$\Delta^{c9,c12,c15}$ (Young et al. 2000), mostly as galactosyl-glyceride esters. Usually, these are also rich in readily fermentable sugars and fiber fractions, which go on decreasing as plant matures. Legume forages are rather not good source of FA like C18:2 and C18:3. The content of readily fermentable carbohydrates and fiber portion is also reduced considerably in silages, haylages and hay or straws. The young lush green grasses have characteristically higher digestibility and passage rates than silages (Mambrini and Peyraud 1994) or dry roughages. Probably, these conditions result in the more incomplete BH, and therefore responsible for the increased flow of TVA and CLA with concurrent reduction in stearic acid production in rumen. Several workers have observed an array of effects on different dietary treatments.

Kelly et al. (1998a) reported an increase of over 200% in milk fat CLA when cows were exclusively grazed on grass rich (>75%) lush green pasture as compared to cows fed a total mixed ration (TMR) containing corn silage-24, legume silage-18.8, legume hay-4.2, rolled corn-25 and whole cottonseed-12.5 parts. The increase in CLA may be attributed to increased availability of TVA coupled with its desaturation in tissues specially the mammary gland, since C18:3 FA did not directly yield CLA. Further, the decreased DMI would have affected some rumen bacteria due to reduced energy supply to complete the BH process (Jahereis et al. 1997; Stantone et al. 1997). Dhiman et al. (1999a) observed a 4.5 fold increase in milk fat CLA content when cows were allowed 100% grazing on pastures rich in grasses. There was 4.5 and over 8 folds increase in stearate and oleate contents respectively, and 12 and 25 folds decrease in C18:2 and C18:3 FA contents respectively in milk fat on pasture grazing.

Elgersma et al. (2003) demonstrated that both milk fat CLA and TVA progressively decreases as dairy cows were shifted from pasture grazing to silage based diets during the commencement of winter (Fig. 2). Esterified FA present in grasses become free on silage making and are highly susceptible for easy hydrogenation in the rumen. Overall, CLA (mg/g milk fat) contents in milk of

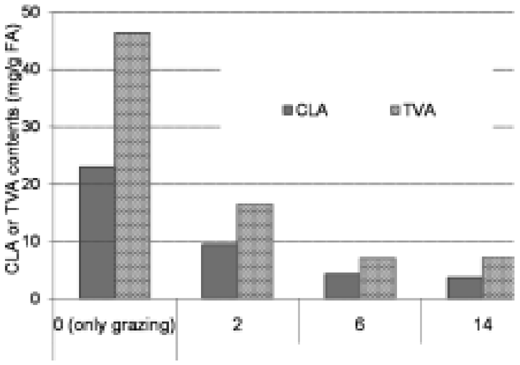

Figure 2: Effect of change of dairy cows from pasture grazing to silage feeding on milk fat CLA and TVA (data adapted from Elgersma et al. 2003)

individual cow were in range of 14-36 on grazing while it was 4.0-5.8 at the end of 14th day on silage based diet. Jehris et al. *(*1997a) also observed lower level of milk fat CLA and TVA from cows fed silage based diets as compared to pasture fed conditions. Rego et al. (2004) reported higher concentration of milk fat TVA and CLA on pasture grazing as compared to cows fed a TMR consisting of corn silage (60%) and concentrate (40%) on DM basis. Further, moderate (i.e. 1.0 kg/d) low fat concentrate supplementation of grazing dairy cows increases performance without compromising the FA profile of milk fat.

Bargo et al. (2006) evaluated two pasture allowances (25 and 40 kg DM/cow/d), which were supplemented with or without 1 kg concentrate for every 4 kg milk production. Concentrate supplementation has reduced DMI by 2.0 or 4.4 kg/d at low or higher pasture, respectively, and also the contents (g FA/100g fat) of TVA, c8t11 and t10c12 CLA in milk fat (Table 2). A reduction in TVA content of milk fat was found on concentrate supplementation and c9t11-CLA by 13%. The ratio of two CLA isomers was not affected by concentrate supplementation. It was suggested that the reduction in milk fat is due to a reduction in contribution of pasture. An Indian study indicated that a total roughage based diet containing berseem (*Trifolium alexandrnum)* and wheat straw (87:13) was best for enhancement of contents of CLA as well as ideal ratio of ω-3 and ω-6 FA in milk of buffalo (Anonymous 2005).

Table 2: Effect of two levels (25 vs 40 kg) of pasture with or without concentrate supplementation on contents of TVA and CLA isomers (g FA/100g fat) in milk of cows

Fatty acid	Low pasture		High pasture	
	Without concentrate	With concentrate	Without concentrate	With concentrate
TVA	3.37	2.72	3.58	2.85
c9t11-CLA	1.35	1.11	1.36	1.24
t10c12-CLA	0.17	0.07	0.16	0.08

(adapted from Bargo et al. 2006)

All grass fed conditions in beef steers increased CLA incorporation into meat over silage or concentrate fed conditions (Dufey 1999). Shantha et al. (1997) in an experiment involving 21 steers, fed either pasture or pasture plus cracked corn for 150 days, observed that all pasture group had 1.5 fold higher CLA content (7.4 vs 5.1 mg/g fat) in semi membranous muscle than grain supplemented diet. The c9t11 isomer represented 95-98% of total CLA in all pasture groups. Tsuneishi et al. (1999a) reported a positive correlation ($P<0.05$) between roughage intake from chopped rice straw and CLA. In steers fed on concentrate and chopped rice straw ad libitum for 11 months; from 15-26 months of age, showed CLA contents of 0.44, 0.34 and 0.25% in subcutaneous, intermuscular and perinephric fats respectively. A difference was observed in mean CLA concentrations amongst the adipose tissues. Coefficient of correlation between percentage of CLA in subcutaneous and intermuscular fat was 0.897 ($P<0.01$), in subcutaneous and perinephric fat 0.680, and in intermuscular and perinephric fat 0.643. Correlations between the proportion of roughage and total feed consumed and CLA concentrations in the subcutaneous fat, intermuscular fat and perinephric fat were 0.761, 0.658 and 0.393, respectively. Roughage intake was significantly correlated with CLA concentrations in the subcutaneous fat. Relatively higher percentage of CLA was observed in the subcutaneous fat of beef cattle which had higher intake of fibrous feed.

Valvo et al. (2005) observed significant increase in milk fat CLA and TVA in ewes that were allowed grazing before about 30 days of lambing as compared to concentrate feeding. The CLA and TVA contents in meat (*Longissimus dorsi)* of lambs, determined after 38 days of sole milk feeding from respective ewes also indicated similar trend (Fig. 3). Knight et al. (2004) also reported similar results

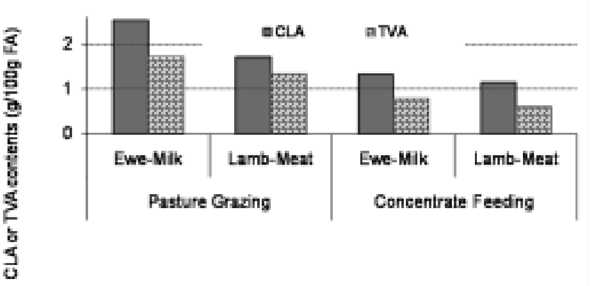

Figure 3: Effect of grazing and concentrate feeding on CLA and TVA contents in milk and meat of ewes and lambs

(data adapted from Valvo et al. 2005

in CLA contents in meat (*Longissimus dorsi)* samples of lambs raised on ewe's milk containing high or low levels of CLA. Poulson et al. (2004) in a study involving beef cattle demonstrated that raising cattle on forage and pasture without grain supplementation enhances beef CLA content. Additionally, finishing cattle on pasture increased the vitamin E content of beef by 300% compared to beef from animals finished on a traditional high-grain diet.

Effect of oilseed supplementation

On supplementation of oil rich feed ingredients to the diets, the increased concentration of CLA in milk fat is primarily due to increased supply of precursor to the rumen. Such effects may be observed in animals fed oilseeds and/or other grains rich in either linoleic acid or linolenic acid. Oilseeds rich in former produced higher CLA contents. A smaller increase of dietary lipids from cereals failed to yield any significant change in milk fat CLA content. Dhiman et al. (1999a) did not find the beneficial impact of dietary inclusion of high oil corn (7.5% EE) at 32% level in TMR over normal corn (3.8% EE) on milk fat CLA content. However, higher dietary lipid level in the form of oilseeds enhanced the CLA content (Takamistu et al. 1999). Lawless et al. (1998) observed different ranges of CLA (mg/g milk fat) i.e., 6.8-25.7 in control (3 kg/d unmolassed beet pulp and pasture *ad libitum*), 10.6-33.5 in rapeseed (1.65 kg/d full fat rapeseed, 1.2 kg/d unmolassed beet pulp, 150 g/d molasses and pasture *ad libitum*) and 8.8-30.5 in soybean (3.1 kg/d full fat soybean and pasture *ad libitum*) supplemented diets in cattle.

Aii et al. (1999) reported a 3.5 fold increase in CLA content on linseed supplementation and Stantone et al. (1997) reported 165% increased milk fat CLA content on 1.65kg/d linseed supplementation over control. At 3.63 kg/d linseed supplementation, further increase of 172% was recorded. There was substantial increase in FA like C14:0, C16:0, C18:0 and C18:1 from diet to milk fat with reduction in C18:2 and C18:3. Collomb et al. (2004) have observed 4.07, 5.47, 7.46, 15.46, 4.85 and 7.37 mg/g fat of c9t11-CLA in the milk samples of dairy cows on without supplementation or ground oilseeds supplementation with rapeseed-1, sunflower-1 and 1.4, linseed-1 and 1.4 kg respectively. Zhang et al. (2006) have reported significant increase of TVA and CLA contents in milk and cheese on supplementation of flax and sunflower seeds (300 and 260 g/kg concentrate) to a diet having 78:22 forage to concentrate ratio (Table 3).

Table 3: Effect of oilseed supplementation to high forage diets on TVA and CLA contents in milk and cheese (g/kg of total FA) of lactating ewes

Products	TVA/CLA	High Forage (HF)	HF + Flax	HF + Sunflower
Milk	TVA	9^b	15^a	15^a
	CLA-c9t11	10^c	15^b	23^a
	CLA-t10c12	1^b	2^a	2^a
Cheese	TVA	9^b	15^a	15^a
	CLA-c9t11	9^c	15^b	22^a
	CLA-t10c12	1^b	1^b	2^a

Figures bearing different superscripts differ significantly ($P<0.05$) across a row.(adapted from Zhang et al. 2006)

Abu-Ghazaleh et al. (2001) investigated the effect of replacing soybean meal with fish meal on feed intake, milk yield and milk composition in 12 multiparous Holstein cows at 48±8 days in milking with 4 x 4 Latin square design of 21-day periods. Fishmeal substituted for soybean meal on an isonitrogenous basis at 0, 25, 50 and 100% of supplemental protein. Total mixed diets were (DM basis) 25% corn silage, 25% lucerne hay and 50% concentrate mix. Intake of DM and milk yield was similar for all diets. Milk protein percentages (3.23, 3.24, 3.31 and 3.35%) increased with 100% fishmeal supplementation and tended to be higher with 50% fishmeal supplementation compared with 100% soybean meal diet. Milk fat percentages (3.18, 2.99, 3.04 and 2.87) and yield were lower with the 100% fishmeal than with the 100% soybean meal diet. Concentrations (g/100g of FA) of CLA (0.39, 0.44, 0.46 and 0.72) and TVA (1.09, 1.19, 1.28 and 1.54) were higher with the 100% fishmeal diet than with 100% soybean meal diet.

Effect of processing of oilseeds on CLA has been investigated. Perhaps, roasting of oilseeds caused brittleness thereby enhancing the efficiency of oil release, de-esterification and early escape of precursors from the rumen. On the other hand extrusion causes partial gelatinisation and also increases the substrate in the form of readily available oil. These conditions improve the rumen passage leading to more incomplete BH of dietary oils. Moreover, a shift in rumen environment due to dry or moist heat treatment of full fat oilseeds may also be responsible for enhanced CLA content in milk fat. Over 200% increase (8.1 vs 3.8 g/d) in cow milk fat CLA yield was noticed when roasted full fat soybean was supplemented with raw cracked full fat soybean at 18% level in TMR of dairy cattle (Dhiman et al. 2000). Similar to roasting, extrusion of whole soybean and cottonseed has also shown to influence the CLA content in milk fat. The supplementation of soybean meal in cow diet produced 0.34 g/d CLA whereas full fat extruded cottonseed and soybean supplementation at similar level resulted in 0.72 and 0.96 (g/d) CLA content in milk fat (Dhiman et al. 1999b).

Like the case of milk fat CLA, small increase in supply of precursors from increased dietary lipids did not increase its contents in meat. When silage portion was increased from 12 to 20% level in isocaloric TMR containing either 74% high oil corn (7.04% EE) or 82% normal corn (4.86% EE), the CLA content in *longissimus dorsi* muscle was increased ($P<0.06$) from 3.87 to 4.87 mg/g fat in finishing steers. A level of silage at 12% in either of the diets did not bring any significant change. However, a small decrease (3.81 from 3.92 mg/100g) in CLA content was observed when they offered TMR containing 83% high oil corn (7.04% EE) and 12% silage as compared to similar levels of normal corn (4.86% EE). Feeding silage at 20% level from 80% high oil corn (isocaloric TMR to normal corn) resulted in a significant ($P<0.06$) increase in CLA content (McGuiree et al. 1998).

Stasiniewicz et al. (2000) have carried out an experiment on bulls to investigate the effect of feeding rapeseed oil cake or linseed on performance and meat quality. Bulls were fed ad libitum on a basic complete feed and barley straw (control group) or with similar amounts of supplemental fat as linseed, rapeseed oil cake, or rapeseed oil. The fat samples in the *M. longissimus dorsi* from linseed fed bulls showed highest content of linolenic acid and CLA. The level of cholesterol in the same sample of animals fed the experimental complete feed with vegetable oils was significantly lower than in the control group. On supplementing cracked hempseed at 0, 9 and 14% levels in the diets of feedlot cattle, there was an increase in CLA content in fats from both brisket and costalis diaphragmatic meat samples, but former contained higher amounts (Gibb et al. 2005).

Effect of vegetable oil supplementation

Oil supplementation is more efficient than oilseed feeding. It may be due to partial deviation in bacterial activity on account of lipid coating over bacterial surface (Devendra and Lewis 1974) coupled with rapid escape of CLA and TVA from the rumen and partial suppression of cellulolytic bacterial activity. Usually oils provide fair quantities of C18:2, C18:3 and other PUFA (table 1). The oils supplying higher quantities of C18:2 FA yield more CLA. On supplementation at 5.3% (DM basis) levels of peanut (oleic acid rich), sunflower (linoleic acid rich) and linseed (linolenic acid rich) oils yielded 13.3, 24.4 and 16.7 mg CLA/g milk fat respectively in three groups of lactating cows (Kelly et al. 1998b). Supplementing canola oil at 1 kg/d in the diet had increased the duodenal flows and milk concentrations of TVA and CLA. The feed intake, ruminal fermentation characteristics, ruminal and total tract digestibilities of nutrients were not significantly affected (Chelikani et al. 2004).

Up to a certain limit the increased level of supplementation of some vegetable oil has further increased milk fat CLA. A level of 4.8 g/d CLA increased to 18.3 g/d when soybean oil was increased from 0.5-4.0% (DM basis) in cow diets (Dhiman et al. 2000). On supplementation of 3.0% canola oil (DM basis), Loor and Herbein (1997b) also reported similar results. Mir et al. (1999) observed increase in milk fat CLA content when goats were supplemented with 0, 2, 4 and 6% rapeseed oil (DM of grain intake). The CLA content increased ($P<0.01$) from 10.35 to 19.42 and 32.05 mg/g milk fat, when goats fed 0, 2 and 4% rapeseed oil respectively with linear and quadratic increase ($P<0.01$) in C18:1 and quadratic decrease in medium and short chain FA. Atkinson et al. (2006) reported that increase of level of safflower oil (77% C18:2) supplementation from 3-9% in the diet of sheep has relatively decreased the ruminal lipolysis and increased the amount (g/d) of TVA and CLA flow through duodenum but absorption or disappearance of these metabolites in small intestine remained similar. Szumacher et al. (2001) have studied the influence of the addition of rapeseed oil, linseed oil and hydrogenated rape seed oil at 4, 8 or 10% of fat (DM basis) to the basal diet consisting of 60% concentrate and 40% meadow hay (control group) in milking ewes. Rapeseed oil and hydrogenated rapeseed oil had no effect on the CLA content in milk, whereas the addition of linseed oil significantly increased ($P<0.05$) the CLA level in milk.

As compared to low (2.2%), high (4.4%) level of linseed oil supplementation (DMI basis) decreased the average daily yield (16.6 vs 12.5 g) of CLA in milk of dairy cows. However, supplementation of soybean oil at 0.5, 1.0, 2.0 and 4.0% DM level an expected increase was observed (7.1, 8.5, 13.8 and 18.1 g CLA/d) in milk of cows. It may be inferred that higher level of linseed oil (increasing level of C18:3 FA) probably had negative effect on bacterial activity, which might have reduced the CLA content and supplementing soybean oil is relatively beneficial over linseed oil at higher levels (Dhiman et al. 2000).

Supplementation of lipids from different sources also affects the CLA contents in meat. In a study by Enser et al. (1999), steers were supplemented with megalac, a mixture of saturated FA, linseed oil (high C18:3), fish oil (high C20:5 and C22:6) and a mixture of linseed oil and fish oil for 120 days to 60% grass silage and 40% concentrate (barley and sugar beet based) diet. The levels of CLA (g/kg of total FA) in longimissus muscle were 3.2, 8.0, 5.7 and 7.3 in respective groups. Dhiman et al. (1999c) also observed similar results in steers fed 2 or 4% soybean oil. Thus, dietary availability of both C18:2 and C18:3 FA has increased the muscle CLA content.

Mir et al. (2000) suggested that supplementation of lamb feedlot diets with a source of linoleic acid was a successful method of increasing CLA content of tissues. They compared the relative increase in the CLA content of lamb tissues by dietary CLA supplementation (0.33 g/d for 21 days prior to weaning) to milk replacer of pre-ruminant lambs or by feeding linoleic acid rich oil (safflower oil, 6% DM-SAFF) to weaned ruminating lambs with that of lambs receiving unsupplemented milk-replacer and pelleted feed. Dietary supplementation with safflower oil increased fat content of subcutaneous adipose tissue only, but CLA content of all the tissues was increased ($P<0.05$) by more than 200%. Dietary safflower oil increased C16:2 in all tissues and C16:0 in the diaphragm, and decreased ($P<0.05$) C18:1 and C18:3 content in all tissues. Supplementation of the diet with pre-formed CLA prior to weaning decreased fat content of the adipose tissue with decreases occurring in C18:0 relative to animals receiving the unsupplemented diet, however, tissue CLA content was not affected by provision of dietary CLA to pre-ruminant lambs.

Szumacher et al. (2001) have studied the influence of the addition of rapeseed oil, linseed oil and hydrogenated rapeseed oil to the diet for milking ewes and growing lambs on the concentration of CLA, in milk and meat respectively. Supplementing diets for fattened lambs with 6% rapeseed oil, linseed oil or hydrogenated rapeseed oil had no significant effect over non-supplementation on the CLA content in meat. Hristov et al. (2005), fed finishing cattle with high grain (78.6%) diet, supplemented with either high (76.5%) linoleic or oleic acid rich oils at 5% level. Subcutaneous fat contained significantly higher CLA (% of total FA) at 0.37 and 0.29 on linoleic or oleic acid rich oil supplementation than kidney fat with 0.23 and 0.17 on similar diet composition. However, CLA contents of different muscles viz. longissimus, semitendinosus and semimembranous were similar and were in a range of 0.21 to 0.25% of total FA on both type of supplementation.

Supplementation of rumen-protected vegetable oils did not promote the CLA synthesis in animals. Triglycerides, amides, esters or calcium salts are required to be hydrolyzed to respective fatty acids with free –COOH group before any BH can occur in body. The degree of protection may have some impact on CLA or TVA flow from rumen of supplemented animals. Canola oil has comparable C18 FA composition to oleamide with 61.8% C18:2 and 21.7% C18:3 FA. In dairy cows when 53% concentrate based TMR was supplemented with increasing levels from 0 to 3% of rumen-protected canola oil product *canolamide* with concurrent reduction in canola oil, the CLA content did not change. However, the CLA content was higher in oil-supplemented groups than control i.e., without oil (DeLuca and Jenkins 2000). Milk fat CLA did not change (0.28 vs 0.23 g/100gm) when *canolamide* was added at a level of 3.0 % to TMR. A level of 1.5 % each of *canolamide* and canola oil increased the CLA to 0.44 g/100g milk fat in dairy cows (Loor and Herbein 1997b). *In vitro* (*Rusitech*) study by Buccuioni et al. (2006) indicated that supplementation of diet with toasted soybean provided better CLA contents in rumen liquor than soap of olive oil.

Effect of fish and animal origin lipid supplementation

Generally, fish oils and ruminant animal body fats are rich in long chain PUFA and saturated FA respectively. Marine fish oils are richer source of C20:5n-3 or C22:6n-3 FA. Fish oil provides necessary precursors for the CLA synthesis and it increases the milk fat CLA content. Fundamentally, both fish oil and tallow interfere with fiber digestion and BH process. The C18:3 and (or) long chain PUFA may coat rumen bacteria. The higher levels of fish oil may have damaging effect as high concentration of unsaturated fatty acids in fish oil are effective inhibitors of fiber degrading bacteria in rumen (Jenkins and Jenny 1989). Fish oil inhibited the reduction of TVA in the rumen and elevated the supply of TVA which was mainly responsible for enhanced milk fat CLA content (Shingfield et al. 2003).

Offer et al. (1999) observed about ten-fold increase in milk fat CLA content when 250 g fish oil was supplemented to total mixed diets in cattle. On supplementing fish oil there was an increase in the C16:1, C18:1, TVA and CLA with reciprocal changes in C18:0, C18:3n-3 and total saturated fatty acids in milk fat (Donovan et al. 2000). They reported 0.60, 1.58, 2.23 and 1.5 g CLA/100 g milk fat on 0, 1, 2 and 3% dietary supplementation of menhaden fish oil (DM basis) in cattle. Fish oil supplementation up to 2% level increased the milk fat CLA and further increase lowered it.

Tallow did not seem to be good for CLA increase. Combining tallow with increasing levels of fish oil to TMR in cows improved CLA concentration in milk fat (Jones et al. 2000). Other marine products that are similar in fatty acid composition to fish oil, too promote the CLA content. Franklin et al. (1999) demonstrated six times increase in milk fat CLA of cows supplemented with marine algae (*Schzochytrium* sps).

The inclusion of fish meal, replacing the soybean meal at 0, 25, 50 and 100% in the TMR fed for 21 days have resulted in decrease of milk fat (3.18, 2.99, 3.04 and 2.87%) and increase of milk protein (3.23, 3.24, 3.31 and 3.35%), CLA (0.39, 0.44, 0.46 and 0.72 g/100g FA) and TVA (1.09, 1.19, 1.28 and 1.54 g/100g FA) in an observation by Abu-Ghazaleh et al. (2001).

Effect of concentrate feeding

Certain level of concentrate in diet reduces the cellulolytic bacterial activity in the rumen. This condition may be responsible for the incomplete BH leading to higher accumulation of TVA (and may be CLA) in the rumen with simultaneous proportionate reduction in stearate level. By Δ^9 desaturation activity some of the absorbed TVA is converted into CLA (Jiang and Jiang 1998). The decreased proportion of fiber coupled with increased concentration of starch in diet, resulted in decrease of final BH step and an increased formation of TVA as main product (Jiang et al. 1996). Further, a higher starch content in the diet with one or two meals as compared to continuous feeding i.e., steady state condition, lowers the rumen pH. This condition causes a greater reduction of the fibrolytic microbial activity, and at extreme conditions BH process may stop completely. However, mild acidic conditions may produce increased levels of TVA. In case of corn based concentrate feeding, further higher milk fat content of CLA and TVA may also be attributed to increased supply of precursors like C18:2 and C18:3 FA in addition to normal action of starch from diet. On very high concentrate (starch) based diet, a substantial supply of CLA precursors would fail to increase CLA unless more forage is offered.

In many developed countries, ruminant production is principally dependent upon high concentrate feeding. Usually, such diets do not support fibrolytic bacterial population, as they require particulate material for their attachment in rumen, and also lowering of pH. To maintain a fairly constant rumen pH, sodium bicarbonate and magnesium oxide have been used as buffers. Though no direct work has been reported in this regard, use of these buffers may play a role in changing the CLA content since TVA can be converted to CLA in the tissues of ruminant animals. Piperova et al. (1997) used 0.5% magnesium oxide and 1.5% sodium bicarbonate as buffers on medium (40%) and high (75%) concentrate diets in cattle. There was no marked effect of buffer in medium concentrate diet, while, there was 0.19-unit increase in rumen pH and TVA contents were reduced from 5.7 to 2.9 g/100g milk fat. It was proposed that more complete BH might have been responsible for such depression. Kalscheur et al. (1997) have also noticed similar results in their study using sodium bicarbonate. Qiu et al. (2004) observed a decrease (24.5 vs 17.9 mg/g FA) in the milk fat CLA content on addition of 0.8% sodium bicarbonate as buffer to the diets of cows on high concentrate (63.8%) with 2% fish oil. However, the values were still higher than similar diet with 2% soybean oil (10.1 mg/g FA).

Effect of ionophore antibiotic supplementation

Ionophores antibiotic substances alter the rumen fermentation by selectively influencing hydrogen producing bacteria. Monensin was first introduced as growth promoter. It improved feed efficiency with reduced DMI and prevented acidosis. These compounds have been used for the reduction of methane production in the ruminants. However, European Union Commission proposed to ban its use in animal production from year 2006 (McCartney 2002) and other countries may follow similar ban.

Dhiman et al. (1999a) reported a positive effect of monensin addition to diet on milk CLA. However, Chouinard et al. (1998) did not observe effect of monensin on milk fat CLA in cows. In *in vitro* study, Fellner et al. (1997) reported that monensin increased the total CLA isomers and it inhibited the growth of *Butyrivibrio fibrisolvens,* major bacterium involved in ruminal BH. Dhiman et al. (1999) in a study in lactating cows noticed some beneficial effects on ionophore supplementation. The cows in different groups were without supplementation (control) or supplemented daily with 3% fish meal, 250 mg monensin or fish meal with monensin. The relevant contents of CLA in milk (mg/g of FA) were 5.3, 8.6, 6.8 and 8.9 respectively.

To study the mechanism of BH of C18-unsaturated FA of safflower oil and CLA production by mixed ruminal bacteria on ionophore or fish oil supplementation Wang et al. (2005a) conducted an *in vitro* study. Commercially manufactured concentrate (1%, w/v) with safflower oil (0.2%, w/v) was added to mixed solution (600 ml) of strained rumen fluid and McDougalls artificial saliva (control). Monensin (10 ppm, w/v, MO), mixed fish oil (0.02%, w/v, absorbed to 0.2 g alfalfa hay, FO) or similar amounts of monensin and fish oil (MO+FO) to MO and FO was also added into the control solution. All the culture solutions prepared were incubated in the culture jar anaerobically at 39^0C up to 12 h. Supplementation of MO or MO+FO increased ($p<0.001$) the proportions of C18:2. The MO alone reduced ($p<0.022$) the proportion of CLA compared to FO in all incubation times. The FO supplementation increased the proportion of CLA. An additive effect of MO to FO in the production of CLA was observed at 6 h incubation. *In vitro* supplementation of monensin reduced hydrogenation of C18 unsaturated FA, while fish oil supplementation increased the production of CLA.

In another in vitro study, Wang et al. (2005b) observed that addition of 10 ppm monensin did not affect the total VFA concentration, but propionate was increased during overall incubation intervals. VFA concentration and proportion was not affected by fish oil supplementation. Content of total C18 FA increased as incubation increased in case of control and fish oil supplementation, while it was reduced on monensin with fish oil supplementation. Monensin reduced proportion of c9t11, but increased t10c12 isomer of CLA when compared to control or fish oil supplementation. Monensin appears to incresase t10c11 synthesis and blocks the last step of BH of unsaturated FA while fish oil contributes to increase of c9t11-CLA and TVA production in the ruminal culture.

Effect of post ruminal infusion of CLA mixture

Direct abomasal infusion of preformed CLA mixture enhanced its availability at mammary gland and adipose tissues. Due to increased supply of different FA, especially TVA, mammary *de novo* synthesis of C:4 to C:14 FA was reduced (Loor and Herbein 1997a). Chauinard et al. (1998) reported a linear decrease in milk yield and milk fat percentage with out any effect on DMI, when different levels (ranging from 0 to 150 g/d) of CLA-60 mixture containing 14.5% c9t11 isomer and 45% of other CLA isomers was infused. There was 4-fold increase in c9t11 isomer in milk fat at highest level of infusion. Kraft et al. (1999) reported a 7-fold increase in CLA on 200 g/d CLA mixture infusion in duodenum for five days period. They also observed about 40% reduction in milk fat content. Gervais et al. (2005), on contrary under commercial conditions observed that supplementation of ruminaly inert CLA, containing 7.9% c9t11 and 8.6% t10c12 isomers at 8-32 g/d to dairy cows did not impact the level of CLA in the milk, but decreased the overall fat content and total CLA yield (g/d).

Effect of nutritional status of animal

The dietary restriction alters the rumen fermentation and nutrient partitioning in the lactating animals. Lower feed intake associated with condition such as higher intake of PUFA and fiber, promoted the CLA synthesis in rumen (Jahreis et al. 1997b; Stantone et al. 1997). The higher level of feeding increased the insulin activity that in turn promote lipogenesis and after absorption FA are partitioned for lactation and adipose tissues. This condition perhaps, lowers the availability of CLA and TVA for milk synthesis. When animals receive the diets required for lactation alone they do not store FA in adipose tissue and all available CLA and/or TVA are directed towards milk. Where as, the animals receiving a deficient diet will mobilize CLA and TVA from body reserves and this condition might increase CLA content (Jiang et al. 1996). However, on longer exposure to such conditions the milk fat CLA content is ought to remain in close association with availability of dietary CLA precursors.

Stantone et al. (1997) offered 16, 20 and 24 kg grass (DM/head) to lactating cows. The 16 and 20kg DMI groups received approximately 67 and 80% of DM requirement respectively as compared to last group. The CLA and TVA contents were 3.94 and 3.91; 6.84 and 5.92; 5.71 and 5.52 mg/g milk fat for respective groups. The CLA content for 20 kg group at 12-week period of feeding were significantly ($P<0.01$) higher. Kelly et al. (1998a) reported increased level of milk fat CLA and TVA when cows received, 19% less DM on pasture than cows maintained on 100 or 50% TMR. On average animals lost approximately 40 kg body weight during experimental period, which might have caused mobilization of CLA and TVA from body reserves into milk fat. Jiang and Jiang (1998) also reported higher production of CLA with strong positive correlation ($P<0.01$) in animals on restricted diets.

Tsuneishi et al. (2001) studied the influence of anatomical location and nutritional status on CLA concentration in goats. Fat tissues located at body surfaces contain higher CLA than *perinepheric* fat tissue. As compared to dietary restriction full fed condition showed improvement in tissues from both locations. They observed two groups of growing goats in 90 days long experiment. The first group that received maintenance ration had growth rate of 15 g/d and second full fed group had growth rate of 210 g/d. The full fed animals had significantly higher contents of total unsaturated fatty acids, TVA and CLA in meat. Further, *subcutaneous* fat tissue from full fed animals had higher contents of total unsaturated FA and CLA, while TVA concentration was higher in same tissue from first group of animals. This study indicates that the level of feeding over maintenance increases the availability of CLA precursors and D^9 desaturase activities play an important role on *ad libitum* feeding since adipose tissue in growing ruminant animals has the greatest desaturase activity. It also seems that this activity is more pronounced in tissues located on the body surfaces as *perinepheric* fat contain less CLA than *subcutaneous* fat in both maintenance and full fed groups.

EPILOGUE

Grass rich lush green pasture, feeding high concentrate diet with optimum fiber level and oilseeds are advantageous to enhance the CLA content in ruminant milk and meat. Although no estimates are available, the prevailing conditions of northeastern hill region where ruminants receive ample supply of lush green grasses during the rainy season would also produce richer CLA contents in their milk and meat. Dry and moist heat treatments of oilseed also elevate CLA to the tune of 200 to 300%. Up to a certain level of vegetable oil supplementation encouraging results were obtained. Fish oil addition produced 3 to 4 fold increase. The post rumen preformed CLA infusion also proved beneficial. However, feeding animal based saturated and rumen protected fat supplemented diets failed to exhibit useful impact on CLA content in milk and meat. The CLA out put vary considerably on different diets because of variation in supply of precursors, rumen environment and endogenous desaturation. Different dietary regimens affected the rumen microbial activity for more incomplete process of BH. Therefore, a balance has to be achieved for optimal growth of biohydrogenating bacteria and availability of CLA precursor like linoleic or linolenic acid with faster passage rate so that these products can evade complete hydrogenation in rumen and are available for the host animal for synthesis into milk and meat.

Future Research Approach

So far, various feeding strategies have been demonstrated for maximizing the levels of CLA in milk and meat of ruminant animals. In order to ascertain accurate estimates of synthesis and potential availability of the CLA in milk or other tissues, not only the adequate dietary supply of CLA precursors in shape of lipids is essential but also it has to be synchronized with optimal rate of lipolysis and subsequent optimum intensity of BH in the rumen. Following approaches may be tried for future research, so that optimum health benefits of CLA can be achieved in human beings on consumption.

- Elucidation of levels of CLA in the milk and meat of ruminant animals under the prevailing feeding practices in northeastern region of India.
- Certain substances that influence the rumen passage like common salt etc. may be used with supplementation of vegetable oils/oilseeds rich in either linoleic or linolenic acids.
- Other feed processing methods that are capable of modifying rumen environment needs to be worked out.
- Various secondary plant metabolites viz. tannins and saponins/sarsaponin, especially later have recently been demonstrated to alter the rumen metabolism and inhibit the methane production. Effect of these compounds on CLA may also be worked out.
- The conversion efficiency of TVA into CLA needs to be validated in different tissues with regard to trace minerals and vitamins as probable desaturase enzyme co-factors.
- Like ionophore antibiotics that are banned for use in animal production, bacteriocins (nisin), derived from strains of probiotic bacterial sps. like *Lactococcus lactis* could have promising potential for CLA enhancement.
- Other compounds that act as alternate hydrogen sink viz. organic acids especially components of Krebs cycle like fumarate and malate can also be tried for enhancement of CLA production potential in ruminant animals and their products.

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