PJN
ISSN 1680-5194

# PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 3 (2): 72-81, 2004


# Biosynthesis of Conjugated Linoleic Acid (CLA): A Review

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

**Abstract:** Conjugated linoleic acid is a mixture of positional and geometric isomers of linoleic acid with two conjugated unsaturated double bonds at various carbon positions in the fatty acid chain. An increasing interest on CLA is attributed to its potential health benefits such as anticarcinogenic, antiatherogenic, antidiabetic and antiadipogenic effects. More than a dozen isomers of CLA are present in ruminant fat. Of the two isomers known for their physiological importance, *c*-9, *t*-11 is the most prevalent one comprising 80 to 90% of total CLA in food products from ruminants, whereas *t*-10, *c*-12 is present in small amounts at 3-5% of total CLA. Sequences in the biohydrogenation of dietary unsaturated fatty acids leading to the biosynthesis of CLA in the rumen from linoleic acid and in the mammary gland from *t*-11 $C_{18:1}$ by $\Delta^{-9}$ desaturase have been reviewed. Understanding the mechanisms involved in the biosynthesis of CLA will allow us to design feeding strategies for enhancing the concentration and output of CLA in milk and meat so we can derive the potential health benefits associate with it.

**Key words:** Rumen biohydrogenation, fatty acids, linoleic acid, linolenic acid, CLA, TVA

## Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (*c*-9, *c*-12 $C_{18:2}$, LA) with two conjugated unsaturated double bonds at various carbon positions in the fatty acid (FA) chain. Each double bond can be *cis* or *trans*, but those with one *trans* double bond are bioactive (Jensen, 2002). It is formed as an intermediate during the biohydrogenation of LA to stearic acid in the rumen by *Butyrivibrio fibrisolvens* (Kepler *et al*., 1966) and other rumen bacteria (Kritchevsky, 2000) or from the endogenous conversion of *t*-11 $C_{18:1}$ (transvaccenic acid, TVA), another intermediate of rumen biohydrogenation of LA or linolenic acid (*c*-9, *c*-12, *c*-15 $C_{18:3}$, LNA) by the $\Delta^{9}$-desaturase enzyme in the mammary gland (Corl *et al*., 2001; Griinari and Bauman, 1999). An increasing interest on CLA is attributed to its potential health benefits such as anticarcinogenic, antiatherogenic, antidiabetic and antiadipogenic effects (Banni *et al*., 2003; Belury, 2003; Kritchevsky, 2003; Pariza, 1999). Its role on vitamin A metabolism (Carta *et al*., 2002), bone modeling (Watkins *et al*., 2003) and immune response (Cook *et al*., 2003) has also been reported. Of the two physiologically important isomers (Fig. 1), *c*-9, *t*-11 is the most prevalent one comprising 80 to 90% of total CLA in food products from ruminants, whereas *t*-10, *c*-12 is present in small amounts at 3-5% of total CLA (Parodi, 2003).

Ruminant feedstuffs typically contain relatively small amounts of lipids. Forages and concentrate feeds both contain esterified FA. While forages contain mostly phospholipids and glycolipids, plant seeds used as concentrate feeds mostly contain triglycerides. While LNA is the predominant FA in forages, it is LA that predominates concentrate feeds. Some seeds and oils such as peanut, rapeseed, palm oil, etc. contain *cis*-$C_{18:1}$ as the major FA and those of marine origin are high in FA of 20 or 22 carbons. When dietary FA reaches the rumen, it is extensively modified by rumen bacteria with the help of a full complement of lipases hydrolyzing triglycerides, phospholipids, and glycolipids. Enhancing the CLA content in milk and meat from ruminants depends largely on the understanding of sequential processes involved in the biohydrogenation of dietary unsaturated FA in the rumen and how such processes could be modified to enhance CLA and TVA output from the rumen by manipulating the animal diet. This is first of the two reviews on CLA synthesis and its content and enhancement in milk and meat through manipulation of animal diet. The objective of this review is to provide an overview on the history of CLA, biohydrogenation of unsaturated FA leading to its synthesis in the rumen, and its endogenous synthesis in ruminants and non-ruminants, wherever possible.

**History of milk fat CLA:** It was probably Booth *et al*. (1935) who for the first time established the presence of conjugated FA in milk fat. They reported that when cows were turned out to pasture after winter, the FA of milk fat showed greatly increased absorption in the ultraviolet region at 230 nm. Moore (1939) later concluded that absorption at 230 nm was the result of two conjugated double bonds. Hilditch and Jasperson (1941, 1945) suggested that conjugated unsaturation occurred with polyunsaturated FA of 18 carbon chains. Bartlett and Chapman (1961) found a constant relationship between *trans*-$C_{18:1}$ and conjugated unsaturation in a large number of butter samples as determined by differential infrared spectroscopy, which prompted them to suggest a sequence of reactions that would help explain the

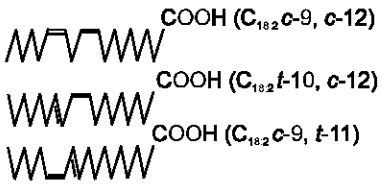


Fig. 1: Linoleic acid (*c*-9, *c*-12 $C_{18:2}$), *c*-9, *t*-11 CLA, and *t*-10, *c*-12 CLA

biohydrogenation of LA in the rumen. Riel (1963) showed a 2-fold increase in milk fat conjugated dienes during summer when cows were grazing on pasture compared with winter when cows were fed total mixed rations (TMR). Parodi (1977) subsequently determined that the conjugated double bonds were *c*-9 and *t*-11 of $C_{18:2}$ (Fig. 1). Conjugation at other positions was found later.

**Biosynthesis of CLA:** As pointed out above, Parodi (1977) first established *c*-9, *t*-11 octadecadienoic acid as the major CLA component in bovine milk fat. However, Kepler *et al.* (1966) had identified it a decade earlier as the first intermediate of linoleic acid biohydrogenation in the rumen by *Butyrivibrio fibrisolvens*. It was regarded as the only source of CLA in the milk and meat of ruminants until recently, because there was a relatively constant ratio of *trans*-$C_{18:1}$ to *c*-9, *t*-11 CLA (Wolff, 1995) that prompted Enser *et al.* (1999) to conclude that CLA synthesis occurred in the rumen only. However, CLA is now accepted to have two different origins in the rumen and endogenously in the tissues.

**Synthesis in the rumen:** Reiser (1951) noticed that the body fats of ruminants possessed less LNA than horses on the same high LNA diet and suspected something to have happened in the rumen. He incubated LNA in rumen fluid and demonstrated the formation of *trans* FA in the rumen (Reiser, 1951). Utilizing rumen contents from fistulated sheep grazing pasture, Shorland *et al.* (1955) confirmed the existence of *trans* FA as a result of rumen biohydrogenation. Shorland *et al.* (1957) also showed that conjugated dienoic acids accumulated when LA was incubated with rumen contents, but not when LNA was incubated with it. Subsequent work (Ward *et al.*, 1964; Wilde and Dawson, 1966) further confirmed the formation of an array of $C_{18}$ FA with varying degrees of unsaturation and positional isomerization, including conjugated dienes.

In a classic experiment, Kepler and Tove (1967) showed the production of *c*-9, *t*-11 CLA from LA by *B. fibrisolvens*. When the same bacteria was incubated using LNA as the substrate, *c*-9, *t*-11, *c*-15 $C_{18:3}$ was produced, which later was found to be hydrogenated to TVA (Harfoot and Hazlewood, 1988). No *c*-9, *t*-11 CLA was formed from LNA. The biohydrogenation of LA and LNA occurs in a similar manner. The first reaction in LA biohydrogenation is the isomerization where the double bond at carbon-12 position is transferred to carbon-11 position forming *c*-9, *t*-11 CLA. It is followed by the rapid hydrogenation of *cis*-9 bond leaving TVA. Both these steps are carried out by group A bacteria, while the last step of biohydrogenation of oleic to stearic acid is carried out by group B bacteria (Harfoot and Hazlewood, 1988). The enzyme responsible for the conjugation of *cis*-9, *cis*-12 double bonds was identified as linoleic acid isomerase (EC 5.3.1.5). It is a particulate enzyme bound to the bacterial cell membrane (Griinari and Bauman, 1999) and demonstrates an absolute substrate requirement for a *cis*-9, *cis*-12 diene system and a free carboxyl group (Kepler *et al.*, 1970), found in both LA and LNA. Similarly, the LNA is first isomerized at *cis*-12 position to form *c*-9, *t*-11, *c*-15 $C_{18:3}$, which is then reduced at both the *cis* bonds to produce TVA. The final step is similar to that of LA. A common intermediate during the biohydrogenation of LA and both $\alpha$ and $\gamma$ LNA was found to be TVA (Harfoot and Hazlewood, 1988). Its reduction appears to be rate limiting in the complete biohydrogenation of unsaturated $C_{18}$ fatty acids resulting in the accumulation of TVA in the rumen (Keeny, 1970). This is the predominant pathway of rumen biohydrogenation of LA and LNA and is given in Fig. 2. Subsequently, a product-precursor relationship between TVA and CLA was observed both in vitro and in vivo (sheep) with increasing concentrations of LA in the diet (Noble *et al.*, 1974). Noble *et al.* (1974) also observed that biohydrogenation of LA derived from triglyceride followed a different pathway from that presented as free acid.

With a low fiber diet, however, a change in *trans*-octadecenoic acid profile of milk occurred and *trans*-10 octadecenoic acid became the predominant *trans*-octadecenoic acid in milk fat (Griinari *et al.*, 1998). This led Griinari and Bauman (1999) to propose another pathway for the ruminal synthesis of *t*-10, *c*-12 CLA (Fig. 3) involving bacterial *c*-9, *t*-10 isomerase with the formation of a *t*-10, *c*-12 double bond as the first step in the process. The *c*-12, *t*-11 isomerase from *B. fibrisolvens* can hydrogenate *t*-10, *c*-12 octadecadienoic acid (Kepler *et al.*, 1966), thus producing *t*-10 octadecenoic acid. It has been shown that more than 50% of the LA was converted to *t*-10, *c*-12 isomer of CLA and only 10% was converted to *t*-10 octadecenoic acid by anaerobic *Propionibacterium* isolated from mouse cecum (Verhulst *et al.*, 1987). Another rumen bacteria *Megasphaera elsdenii* YJ-4 have also been shown to produce *t*-10, *c*-12 isomer of CLA (Kim *et al.*, 2000). The *t*-10, *c*-12 isomer was formed from LA but not from either of the LNA as was the case with *c*-9, *t*-11 isomer of CLA. It is not clear whether *t*-10 octadecenoic acid is desaturated at *cis*-12 position to produce *t*-10, *c*-12 isomer in the rumen or in some other tissues endogenously. Recently, Mosley *et al.* (2002) have shown *in vitro* that oleic acid also forms several *trans*-

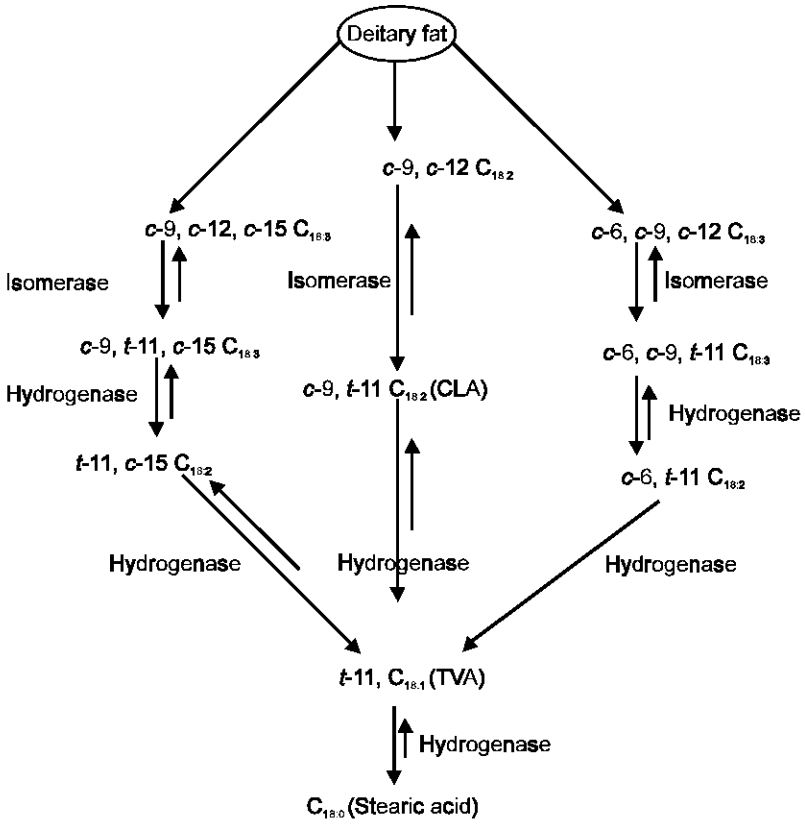


Fig. 2: Predominant pathways of biohydrogenation of dietary linoleic and linolenic acids in the rumen (Adapted from Harfoot and Hazlewood, 1988). Note that *c*-9, *t*-11 CLA is formed only in the biohydrogenation of *c*-9, *c*-12 $C_{18:2}$, whereas TVA is formed from both linoleic and linolenic acids

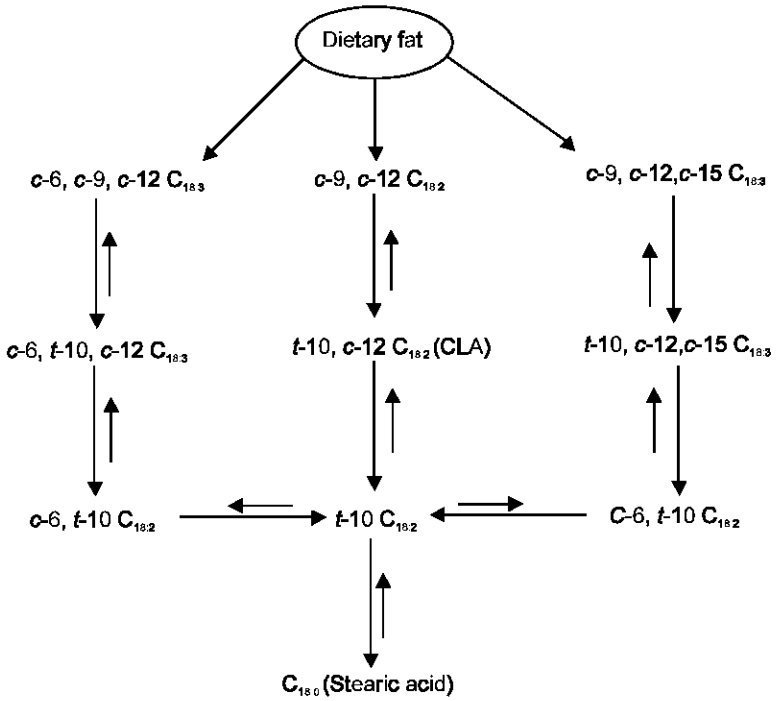


Fig. 3: Pathways of biohydrogenation of dietary linoleic and linolenic acids in the rumen involving *c*-9, *t*-10 isomerase (Adapted from Griinari and Bauman, 1999). Note that *t*-10, *c*-12 CLA is formed from linoleic acid only

$C_{18:1}$ including TVA during its biohydrogenation to stearic acid, which may have implications for the endogenous synthesis of CLA. Moreover, oils and seeds of peanuts, rapeseed, palm, canola, high-oleic sunflower etc. contain higher proportion of oleic acid that may find practical applications for enhancing the milk fat CLA and TVA from dairy cows and possibly other ruminants. From South-Asian point of view, rapeseed, peanut and palm seeds and oils, which are readily available and less expensive, have greater implications for enhancing milk fat CLA. Pathways for the synthesis of other isomers of CLA have not been elucidated in detail.

Rumen pH has an important role in maintaining a viable rumen environment suitable for *B. fibrisolvens* involved in the biohydrogenation of LA and LNA. It has been shown that ruminal pH at 6.0 or above has a positive effect on TVA and CLA contents in rumen cultures (Troegeler-Meynadir *et al.*, 2003; Martin and Jenkins, 2002). It is of higher importance in high yielding dairy and beef animal diets where large amounts of grain are included in the diet and thus decrease the rumen pH below 6.0. Other than its positive effects on *B. fibrisolvens*, how rumen pH affects overall biohydrogenation of unsaturated FA of 18, 20 or 22 carbons in the rumen in relation to CLA and TVA has not been investigated in detail.

It has been shown that CLA could be increased by supplementing TMR diets with feed sources such as fish oil or marine algae (*Schizochytrium sp.*), which are rich in 20 or 22-carbon FA (Franklin *et al.* 1999; Donovan *et al.*, 2000) that do not yield either CLA or TVA during biohydrogenation in the rumen. The mechanism by which supplementation of fish oil or marine algae increases concentration of milk fat CLA and TVA is not clear. It has been proposed that the longer chain poly-unsaturated FA from fish oil inhibit the complete biohydrogenation of $C_{18:2}$ in the rumen by inhibiting the growth of bacteria responsible for hydrogenating TVA or through the inhibition of their hydrogenases (Griinari and Bauman, 1999) leading to an increased escape of TVA from the rumen. Further research is needed on the pathway for rumen biohydrogenation of longer chain poly-unsaturated FA from fish oils and other FA sources of marine origin to clearly define the mechanisms involved in enhancing CLA and TVA contents in food products from ruminants.

Water buffalo is the species of choice for milk production in South Asia. It is also used for producing meat in many parts of the sub-continent. However, literature on the CLA content of milk and meat from this important species of animal is very limited not only in this part of the world, but also elsewhere where it is domesticated for various purposes. These animals are raised more on subsistence type of farming in most of South and South East Asia, where rice/wheat straw may be the only forage available with little supplementation of grains and/or oil cakes. The process of rumen biohydrogenation of dietary lipids under such conditions may be entirely different from that observed among the high yielding dairy cows of the west, where cows are kept largely in confinement and are fed a TMR diet containing 50% conserved forage and 50% concentrate. This is an area where further research is warranted.

**Endogenous synthesis:** While the origin of CLA from LA by *B. fibrisolvens* in the rumen was accepted, it was not sufficient to account for all the CLA present in milk or meat. Furthermore, Banni *et al.* (1996) found that lactating sheep grazing pastures with no supplemental LA produced a high level of *c*-9, *t*-11 CLA in milk fat. The puzzle as to why the milk fat showed greatly increased absorption in the ultraviolet region when cows were turned out to pasture, even though the pastures are high in LNA and not LA, continued to intrigue the scientists. The mystery got murkier when supplementation of fish oil, which is high in polyunsaturated FA of 20 or more carbons and does not produce *c*-9, *t*-11 CLA or TVA as the intermediate during biohydrogenation, also increased *c*-9, *t*-11 CLA content in the milk of cows (Chouinard *et al.*, 1998). Given these findings, Griinari and Bauman (1999) came to the conclusion that ruminal synthesis of CLA was only marginal and could not account for the amount of CLA present in milk and meat from ruminants. Overall these findings suggested that the CLA formed during the biohydrogenation of LA in the rumen was not the only source and that another source needed to be explored.

Based on the observations by Holman and Mahfouz (1981) and Pollard *et al.* (1980), who described desaturation of *trans* monoenes to *cis*, *trans* $C_{18:2}$, it was proposed that CLA could be synthesized endogenously from TVA by $\Delta^9$-desaturase (Parodi, 1994). Earlier, Bartlett and Chapman (1961) have also shown a close linear relationship between *trans*-$C_{18:1}$ and conjugated dienes in butter samples (n = 300). Griinari and Bauman (1999) proposed that a major proportion of CLA in tissue and milk lipids originated from endogenous synthesis by $\Delta^9$-desaturase, activity of which is higher in the mammary gland. Griinari *et al.* (2000) subsequently examined the potential for endogenous synthesis of CLA by infusing TVA abomasally and measuring the changes in milk fat CLA. By day 3, it resulted in a 31% increase in milk fat CLA, indicating that an active pathway for endogenous synthesis existed in the mammary gland. They also infused sterculic oil, which contains sterculic acid and malvalic acid that are potent inhibitors of $\Delta^9$-desaturase (Bauman *et al.*, 2003), abomasally to lactating cows and showed that it not only decreased CLA concentration in milk fat by 45%, but also decreased other $\Delta^9$-desaturase products in milk fat such as *cis*-$C_{14:1}$, *cis*-$C_{16:1}$, and *cis*-$C_{18:1}$. Conversion of TVA to *c*-9, *t*-11 CLA by $\Delta^9$-desaturase is given in Figure 4 and the relationship between *c*-9, *t*-11 CLA and TVA is

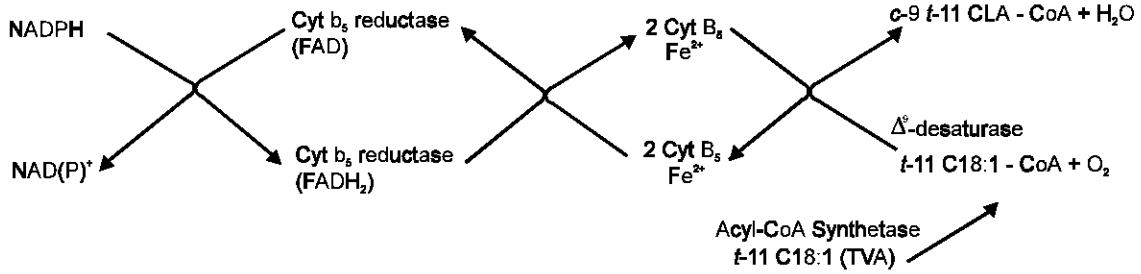


Fig. 4: The $\Delta^9$-desaturase enzyme showing the conversion of TVA to *c*-9 *t*-11 CLA (Adapted from Ntambi, 1999)

given in Fig. 5. It appears that the relationship of *c*-9, *t*-11 CLA with TVA is better under dietary conditions that favor the higher concentrations of *c*-9, *t*-11 CLA and TVA in milk (Khanal, 2004) or meat (Poulson, 2001). It is not surprising given the fact that a minimum of 64% (Griinari *et al.*, 2000) to a maximum of 100% (Kay *et al.*, 2002) of *c*-9, *t*-11 CLA have been estimated to be synthesized de novo.

Griinari *et al.* (2000) also showed that the contribution of endogenous synthesis to the overall CLA content in milk fat was 64%, making it the primary source. Later, Lock and Garnsworthy (2002) estimated rumen output of CLA in nonlactating cows and then extrapolated the results to lactating cows on the basis of feed intake. They estimated the endogenous synthesis of CLA to be >80% of the total. Piperova *et al.* (2002) collected duodenal samples and estimated rumen synthesis of CLA to be 4 to 7% and the rest derived through endogenous synthesis. Kay *et al.* (2002) even estimated 100% of CLA to be derived from endogenous synthesis. It is not surprising, considering the fact that the amount of CLA detected in the blood serum of cows is none or very small (Khanal *et al.*, 2002; Loor *et al.*, 2002a). It is possible that a higher proportion of CLA is synthesized endogenously in cows fed all pasture diets compared with cows fed grains, oil seeds, or oils, because LNA, which is high in fresh pastures, does not produce CLA as the intermediate product during its biohydrogenation in the rumen. However, all these studies used an indirect approach to estimate the CLA synthesized endogenously and it may be over or underestimated under different feeding regimens. A direct approach, which may be difficult, is probably needed to measure the actual uptake of CLA and its incorporation into milk fat by the mammary gland to estimate the mammary synthesis of CLA more accurately. Estimation of TVA entering and leaving the mammary gland and its amount in milk fat will provide further insight into the mammary synthesis of CLA.

No similar investigations have been reported to establish the importance of endogenous synthesis of CLA in the body fat of ruminants. It could be speculated that the contribution from endogenous synthesis is similar to that reported for milk fat because TVA and $\Delta^9$-desaturase are the two primary prerequisites. The $\Delta^9$-desaturase enzyme is active in ruminant adipose tissues (Porter, 2003; St. John *et al.*, 1991), their mRNA is well expressed (Martin *et al.*, 1999; Cameron *et al.*, 1994), and increased concentrations of tissue fat CLA have been reported with increased concentrations of TVA (Madron *et al.*, 2002). A high correlation ($r^2$ = 0.84) was observed between tissue fat concentrations of CLA and *trans*-$C_{18:1}$ (Poulson, 2001), suggesting that a substantial amount of CLA might be synthesized endogenously. Detailed analysis of beef fat demonstrated the similar range of isomers and the predominance of *c*-9, *t*-11 CLA as observed for milk fat (Fritsche *et al.*, 2000). Based on ratios of TVA to *c*-9, *t*-11 CLA in duodenal flow and adipose tissues, Gillis *et al.* (2003) estimated that over 86% of *c*-9, *t*-11 CLA in beef fat is originated from desaturation of TVA. Moreover, the increase in forage level from 12 to 36% in beef steer diets increased the duodenal flow of TVA linearly without altering the flow of *c*-9, *t*-11 CLA (Sackman *et al.*, 2003). It suggested that endogenous desaturation of TVA to *c*-9, *t*-11 CLA increases with the increased proportion of forage in the beef cow diets.

In other ruminants, information on the proportion of rumen and endogenous origin of CLA is limited. However, the fact that increased CLA content in lamb meat (Bolte *et al.*, 2002) and goat milk fat (Chilliard *et al.*, 2003) was associated with high TVA contents indicates that post ruminal synthesis could be the predominant one. LeDoux *et al.* (2002) have shown that TVA is the predominant *trans*-$C_{18:1}$ in goat milk and has similar proportions of *trans* isomers of $C_{18:1}$ to that present in bovine milk fat. A very high correlation ($r^2$ = 0.99) of CLA with TVA in goat milk fat (Chilliard *et al.*, 2003) suggests that mammary synthesis predominates over rumen synthesis of CLA. Noble *et al.* (1974) have shown a product-precursor relationship of CLA with TVA in the sheep rumen, suggesting that TVA accumulated in the rumen could be used for post-ruminal synthesis of CLA, which might be more important quantitatively than its ruminal synthesis. There was a concomitant increase in TVA in plasma triglycerides of sheep (Noble *et al.*, 1974), which could potentially be utilized by the mammary $\Delta^9$-desaturase for increased synthesis of CLA in milk fat.

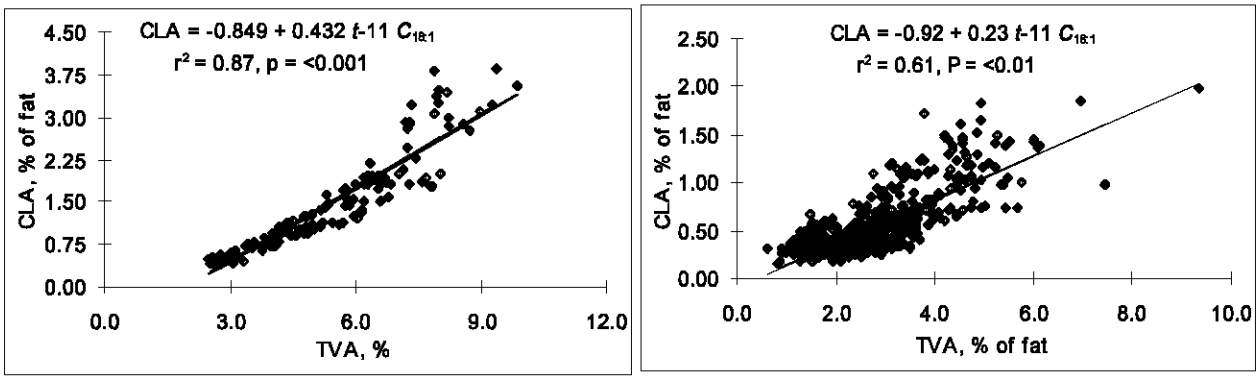


Fig. 5: Relationship between *c*-9 *t*-11 CLA and TVA in milk fat. Milk samples were obtained either from cows grazing on pasture (left panel) or from commercial dairies that either grazed cows on pasture with supplemental grains or did not graze cows at all (right panel) (Source: Khanal, 2004)

Information about the synthesis of CLA in water buffalo seems to be very limited.

**Source of other CLA isomers:** Several other *trans*-$C_{18:1}$ are found in rumen digesta, and milk and tissue lipids (Ward *et al*., 1964; Wilde and Dawson, 1966; Piperova *et al*, 2002). However, the pathways presented in Fig. 2 and 3 do not account for all of them. It has been proposed that double bond migration results in the production of these *trans*-$C_{18:1}$ FA during enzymatic biohydrogenation by anaerobic rumen bacteria (Shorland *et al*., 1957). It has also been proposed that ruminal bacteria possess several specific *cis*, *trans* isomerases that help produce a wide range of *trans*-$C_{18:1}$ FA in milk, meat or rumen. Results finding several isomers of CLA in milk fat (Piperova *et al*., 2002) support this theory. The origin of these several isomers of CLA is undoubtedly the rumen and their further reduction in the rumen would produce a range of *trans*-$C_{18:1}$ FA mentioned above. However, theory of double bond migration leading to a wide range of *trans*-$C_{18:1}$ FA is not studied in detail.

Unlike *c*-9, *t*-11 CLA, no detailed studies have been conducted to study the endogenous synthesis of other isomers of CLA, including *t*-10, *c*-12, probably because many of them contribute 0.05% or less in milk or meat fat and their biological significance has not been established. Piperova *et al*. (2002) detected several of them in the duodenal fluid and the quantities were greater in rumen fluid than in milk fat. Information is limited as to the effect of diet and other factors on the milk or meat fat contents of these minor isomers of CLA, except probably on *t*-10, *c*-12 and *t*-7, *c*-9 isomers. Increased production of *t*-10 $C_{18:1}$ and *t*-10, *c*-12 CLA in the rumen have been associated with an increased content of these intermediates in the milk fat of lactating cows, and is implicated in reduced milk fat content (Baumgard *et al*., 2001). The *t*-7, *c*-9 CLA is quantitatively the second most important CLA present in ruminant fat and constitutes 3 to 16% of total CLA in milk fat (Yurawecz *et al*., 1998). Corl *et al*. (2002) suggested its synthesis to be almost exclusively of endogenous origin in the mammary gland by $\Delta^9$-desaturase. Piperova *et al*. (2002) also concluded that almost all *t*-7, *c*-9 CLA present in milk fat was produced postruminally. The *t*-7, *c*-9 CLA is also the second most prevalent isomer of CLA in beef fat, representing 8 to 15% of total CLA (Fritsche *et al*., 2000). Its endogenous synthesis in the body fat is not investigated in any greater detail, but could be expected to be similar to that of *c*-9, *t*-11 CLA since both of them are associated with the activity of the same enzyme.

**CLA synthesis in non-ruminants:** Santora *et al*. (2000) have shown that TVA is desaturated to CLA when pure TVA was fed to mice and that conversion of TVA to CLA occurred presumably in adipose tissue even though the site of fat synthesis in non-ruminants is the liver, not adipose tissues. Banni *et al*. (2001) demonstrated that feeding rats with increasing amounts of TVA resulted in a progressive increase in tissue CLA concentrations. Corl *et al*. (2003) showed that conversion of dietary TVA to CLA resulted in a dose-dependent increase in the accumulation of CLA in the mammary fat pad of rats. There was a high correlation between tissue concentrations of TVA and CLA in liver, plasma and mammary fat pad of rats fed varying concentrations of TVA and CLA (Corl *et al*., 2003), suggesting that endogenous synthesis of CLA occurred using TVA as the precursor. In lactating mice fed dietary supplementation of TVA, Loor *et al*. (2002b) showed an increased amount of CLA in blood, plasma, milk and tissue lipids. The CLA concentration in liver and carcass of the pups suckling these mothers was also higher. Greater concentrations of CLA in mammary tissue and milk fat of lactating mice fed TVA might have been related to increased activity of $\Delta^9$-desaturase in the

mammary tissue, but not in liver (Loor *et al*., 2002b), suggesting adipose tissue to be the major site for the bioconversion of CLA from TVA. Palmquist and Santora (1999) observed similar results in mice where TVA was desaturated to CLA mainly in adipose tissues but not in liver. Synthesis of CLA from TVA has been shown to occur in humans (Adolf *et al*., 2000) and several species of bacteria derived from the human intestine can synthesize CLA (Coakley *et al*., 2003; Alonso *et al*., 2003). However, the amount of CLA synthesized endogenously or from intestinal bacteria is not estimated in humans or other non-ruminants. Given the fact that humans or other non-ruminants do not have any appreciable amount of bacteria in their digestive system indicates that endogenous synthesis could be the only appreciable source. Synthesis of CLA in other monogastric herbivores is not reported. It needs to be verified whether the CLA content in poultry and pigs is merely the result of feeding ruminant products such as meat and bone meal, blood meal, etc., or whether they can actually synthesize CLA, because investigators have detected very little or no CLA in chickens (Raes *et al*., 2002; Yang *et al*., 2002; Chin *et al*., 1992) and swine (Chin *et al*., 1993).

**Conclusion:** The presence of more than a dozen isomers of CLA in the milk and body fat of ruminants is associated with the bacterial biohydrogenation of unsaturated FA in the rumen. Of all the isomers, *c*-9, *t*-11 is the major one comprising 80 to 90% of total CLA followed by *t*-7, *c*-9 comprising 3 to 16% of the total. The *t*-10, *c*-12 isomer, though important physiologically, is present in amounts of 3 to 5% of the total CLA. Although *c*-9, *t*-11 isomer originates as an intermediate during the biohydrogenation of LA in the rumen, its major source is the endogenous conversion of TVA by $\Delta^{-9}$ desaturase in the mammary gland and possibly adipose tissues. While the *t*-10, *c*-12 isomer of CLA is produced only ruminally, *t*-7, *c*-9 isomer is produced only endogenously by the same enzyme that converts TVA into *c*-9, *t*-11 CLA in the mammary gland. Several other isomers of CLA present in very small amounts in milk and tissue fat of ruminants probably have their origin in the rumen. Other than the dairy cows, information on the synthesis of CLA in the rumen and mammary gland/tissue fat in other species of ruminants is very limited.

## References


Adolf, R., S. Duval and E. Emeken, 2000. Biosynthesis of conjugated linoleic acid in humans. Lipids, 35: 131-135.

Alonso, L., E.P. Cuesta and S.E. Gilliand, 2003. Production of free conjugated linoleic acid by *Lactobacillus acidophilus* and *Lactobacillus casei* of human intestinal origin. J. Dairy Sci., 86: 1941-1946.

Banni, S., E. Angioni, E. Murru, G. Carta, M.P. Melis, D. Bauman, Y. Dong and C. Ip, 2001. Vaccenic acid feeding increases tissue levels of conjugated linoleic acid and suppresses development of premalignant lesions in rat mammary gland. Nutr. Cancer, 41: 91-97.

Banni, S., C. Carta, M.S. Contini, E. Angioni, M. Deiana, M.A. Dessi, M.P. Melis and F.P. Corongiu, 1996. Characterization of conjugated diene fatty acids in milk, dairy products and lamb tissues. J. Nutr. Bichem., 7: 150-155.

Banni, S., C.S.D. Heys and K.W.J. Wahle, 2003. Conjugated linoleic acid as anticancer nutrients: Studies in vivo and cellular mechanisms. In J. Sebedio, W.W. Christie and R. Adolf (ed) Advances in Conjugated Linoleic Acid Research, Vol. 2, pp: 267-281. AOCS Press.

Bartlett, J.C. and D.G. Chapman, 1961. Detection of hydrogenated fats in butter fat by measurement of *cis-trans* conjugated unsaturation. Agri. Food Chem., 9: 50-53.

Bauman, D.E., B.A. Corl and D.G. Peterson, 2003. The biology of conjugated linoleic acid in ruminants. In J. Sebedio, W.W. Christie and R. Adolf (ed) Advances in Conjugated Linoleic Acid Research, Vol. 2, pp: 146-173. AOCS Press, Champaign, IL.

Baumgard, L.H., B.A. Corl, D.A. Dwyer, A. Saebø and D.E. Bauman, 2001. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol., 278: R179-84.

Belury, M.A, 2003. Conjugated linoleic acids in type 2 diabetes mellitus: implications and potential mechanisms. In J. Sebedio, W.W. Christie and R. Adolf (ed) Advances in Conjugated Linoleic Acid Research, Vol. 2, pp 302-315. AOCS Press, Champaign, IL.

Bolte, M.R., B.W. Hess, W.J. Means, G.E. Moss and D.C. Rule, 2002. Feeding lambs high-oleate or high linoleate safflower seeds differentially influences carcass fatty acid composition. J. Anim. Sci., 80: 609-616.

Booth, R.G., S.K. Kon, W.J. Dann and T. Moore, 1935. A study of seasonal variation in butter fat. II. A seasonal spectroscopic variation in the fatty acid fraction. Biochem. J., 29: 133-137.

Cameron, P.J., M. Rogers, J. Oman, S.G. May, D.K. Lunk and S.B. Smith, 1994. Stearoyl coenzyme A desaturase enzyme activity and mRNA levels are not different in subcutaneous adipose tissue from Angus and American Wagyu steers. J. Anim. Sci., 72: 2624-2628.

Carta, G., E. Angioni, E. Murru, M.P. Melis, S. Spada and S. Banni, 2002. Modulation of lipid metabolism and vitamin A by conjugated linoleic acid. Prostaglandins Leukot. Essent. Fatty Acids., 67: 187-191.

Chilliard, Y., A. Ferlay, J. Rouel and G. Lamberet, 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci., 86: 1751-1770.

Chin, S.F., J.M. Strokson and M.W. Pariza, 1993. Conjugated dienoic derivatives of linoleic acid: A new class of anticarcinogens. Food Flavor and Safety pp: 262-271. American Chemical Society.

Chin, S.F., W. Liu, J.M. Storkson, Y.L. Ha and M.W. Pariza, 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Comp. Anal., 5: 185-197.

Chouinard, P.Y., L. Corneau, D.E. Bauman, W.R. Butler, Y. Chiliard and J.K. Drackley, 1998. Conjugated linoleic acid content of milk from cows fed different sources of dietary fat. J. Dairy Sci., 81 (suppl. 1): 223 (Abstr.).

Coakley, M.R., P. Ross, M. Nordgren, G. Fitzgerald, R. Devery and C. Stanton, 2003. Conjugated linoleic acid biosynthesis by human-derived *Bifidobacterium* species. J. Appl. Microbiol., 94: 138-145.

Cook, M.E., D. Butz, G. Li, M. Pariza, L. Whigham and M. Yang, 2003. Conjugated linoleic acid enhances immune responses but protects against the collateral damage of immune events. In J. Sebedio, W.W. Christie and R. Adolf (ed) Advances in Conjugated Linoleic Acid Research, Vol. 2, pp: 283-291. AOCS, Champaign, IL.

Corl, B.A., D.M. Barbano, D.E. Bauman and C. Ip, 2003. *cis*-9, *trans*-11 CLA derived endogenously from trans-11 18:1 reduces cancer risk in rats. J. Nutr., 133: 2893-2900.

Corl, B.A., L.H. Baumgard, D.A. Dwyer, J.M. Griinari, B.S. Phillips and D.E. Bauman, 2001. The role of delta(9)-desaturase in the production of cis-9, trans-11 CLA. J. Nutr. Biochem., 12: 622-630.

Corl, B.A., L.H. Baumgard, J.M. Griinari, P. Delmonte, K.M. Morehouse, M.P. Yurawecz and D.E. Bauman, 2002. Trans-7, cis-9 conjugated linoleic acid is endogenously synthesized by $\Delta^9$-desaturase in dairy cows. Lipids, 37: 681-688.

Donovan, D.C., D.J. Schingoethe, R.J. Baer, J. Ryali, A.R. Hippen and S.T. Franklin, 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci., 83: 2620-2628.

Enser, M., N. Scollan, N. Choi, E. Kurt, K. Hallett and J. Wood, 1999. Effect of dietary lipid on the content of CLA in beef cattle. Anim. Sci., 69: 143-146.

Franklin, S.T., K.R. Martin, R.J. Baer, D.J. Schingoethe and A.R. Hippen, 1999. Dietary marine algae (*Schizochytrium sp.*) increases concentrations of conjugated linoleic, docosahexaenoic and *trans* vaccenic acids in milk of dairy cows. J. Nutr., 129: 2048-2054.

Fritsche, J., S. Fritsche, M.B. Soloman, M.M. Mossoba, M.P. Yurawecz, K. Morehouse and Y. Kim, 2000. Quantitative determination of conjugated linoleic acid isomers in beef fat. Eur. J. Lipid Sci. Tec., 102: 667-672.

Gillis, M.H., S.K. Duckett, J.S. Sackman and D.H. Keisler, 2003. Effect of rumen-protected conjugated linoleic acid (CLA) or linoleic acid on leptin and CLA content of bovine adipose depots. J. Anim. Sci., 81 (Suppl. 2): 12 (Abstr.).

Griinari, J.M. and D.E. Bauman, 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza and G.J. Nelson (ed) Advances in Conjugated Linoleic Acid Research. Vol. I., pp: 180-200. AOCS Press, Champaign, IL.

Griinari, J.M., B.A. Corl, S.H. Lacy, Chouinard, P.Y., K.V.V. Nurmela and D.E. Bauman, 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by $\Delta^9$-desaturase. J. Nutr., 130: 2285-2291.

Griinari, J.M., D.A. Dwyer, M.A. McGuire, D.E. Bauman, D.L. Palmquist and K.V.V. Nurmela, 1998. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy. Sci., 81: 1251-1261.

Harfoot, C.G. and G.P. Hazlewood, 1988. Lipid metabolism in the rumen. In P.N. Hobson (ed) The Rumen Microbial Ecosystem, pp: 285-322, Elsevier, London.

Hilditch, T.P. and H. Jasperson, 1941. Milk fats from cows fed on fresh pasture and on ensiled greed fodder. I. Observations on the component fatty acids. J. Soc. Chem. Ind., 60: 305-310.

Hilditch, T.P. and H. Jasperson, 1945. The polyethenoid acids of the C18 series present in milk and grass fats. J. Soc. Chem. Ind., 64: 109-111.

Holman, R.T. and M.M. Mahfouz, 1981. Cis- and trans-octadecadienoic acid as precursors of polyunsaturated fatty acids. Prog. Lipid Res., 20: 151-156.

Jensen, R.G, 2002. The composition of bovine milk lipid: January 1995 to December 2000. J. Dairy Sci., 85: 295-350.

Kay, J.K., T.R. Mackle, M.J. Auldist, N.A. Thompson and D.E. Bauman, 2002. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in pasture-fed dairy cows. J. Dairy Sci., 85 (suppl. 1): 176 (Abstr.).

Keeny, M., 1970. Lipid metabolism in the rumen. In Phillipson, A.T. (ed.) Physiology of Digestion and Metabolism in the Ruminant. pp: 489-503. Oriel Press, Newcastle-upon-Tyne.

Kepler, C.R. and S.B. Tove, 1967. Biohydrogenation of unsaturated fatty acids. J. Biol. Chem., 242: 5686-5692.

Kepler, C.R., W.P. Tucker and S.B. Tove, 1966. Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. J. Biol. Chem., 241: 1350-1354.

Kepler, C.R., W.P. Tucker and S.B. Tove, 1970. Biohydrogenation of unsaturated fatty acids. IV. Substrate specificity and inhibition of linoleate $\Delta^{12}$-*cis* $\Delta^{11}$-*trans* isomerase from *Butyrivibrio fibrisolvens*. J. Biol. Chem., 245: 3612-3620.

Khanal, R.C., 2004. Dietary Influence on Conjugated Linoleic Acid Content of Milk and Consumer Acceptability of Milk and Cheese Naturally Enriched with Conjugated Linoleic Acid. Ph. D. Dissertation. Utah State University, Logan, Utah, USA.

Khanal, R.C., T.R. Dhiman, D.J. McMahon and R.L. Boman, 2002. Influence of diet on conjugated linoleic acid content of milk, cheese and blood serum. J. Dairy Sci., 85: (Suppl. 1): 142 (Abstr.).

Kim, Y.J., R.H. Liu, D.R. Bond and J.B. Russell, 2000. Effect of linoleic acid concentration on conjugated linoleic acid by Butyrivibrio fibrisolvens A38. Appl. Environ. Microbiol., 66: 5226-5230.

Kritchevsky, D., 2000. Antimutagenic and some other effects of conjugated linoleic acid. Br. J. Nutr., 83: 459-465.

Kritchevsky, D., 2003. Conjugated linoleic acid in experimental atherosclerosis. In J. Sebedio, W.W. Christie and R. Adolf (ed) Advances in Conjugated Linoleic Acid Research, Vol. 2, pp: 292-301. AOCS Press, Champaign, IL.

Kritchevsky, D., S.A. Tepper, S. Wright, P. Tso and S.K. Czarnecki, 2000. Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. J. Am. College Nutr., 19: 472S-477S.

LeDoux, M., A. Rouzeau, P. Bas and D. Sauvant, 2002. Occurrence of *trans*-$C_{18:1}$ fatty acid isomers in goat milk: effect of two dietary regimen. J. Dairy Sci., 85: 190-197.

Lock, A.L. and P.C. Garnsworthy, 2002. Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows'milk. Anim.Sci., 74: 163-176.

Loor, J.J., J.H. Herbein and C.E. Polan, 2002a. *trans*18:1 and 18:2 isolmers in blood plasma and milk fat of grazing cows fed a grain supplement containing solvent-extracted or mechanically extracted soybean meal. J. Dairy Sci., 85: 1197-1207.

Loor, J.J., X. Lin and J.H. Herbein, 2002b. Dietary trans-vaccenic acid (*trans*11-18:1) increases concentration of *cis*-9,*trans*-11 conjugated linoieic acid (rumenic acid) in tissues of lactating mice and suckling pups. Reprod. Nutr. Dev., 42: 85-99.

Madron, M.S., D.G. Peterson, D.A. Dwyer, B.A. Corl, L.H. Baumgard, D.H. Beerman and D.E. Bauman, 2002. Effect of extruded full-fat soybeans on conjugated linoleic acid content of intramuscular, intermuscular and subcutaneous fat in beef steers. J. Anim. Sci., 80: 1135-1143.

Martin, G.S., D.K. Lunt, K.G. Britain and S.B. Smith, 1999. Postnatal development of stearoyl coenzyme A desaturase gene expression in bovine subcutaneous adipose tissue. J. Anim. Sci., 77: 630-636.

Martin, S.A. and T.C. Jenkins, 2002. Factors affecting conjugated linoleic acid and *trans*-$C_{18:1}$ fatty acid production by mixed ruminal bacteria. J. Anim. Sci., 3347-3352.

Moore, T., 1939. Spectroscopic changes in fatty acids. VI. General. Biochem. J., 33: 1635-1638.

Mosley, E.R., G.L. Powell, M.B. Riley and T.C. Jenkins. 2002. Microbial biohydrogenation of eleic acid to trans isomers in vitro. J. Lipid Res., 43: 290-296.

Noble, R.C., J.H. Moore and C.G. Harfoot, 1974. Observations on the pattern of esterified and unesterified linoleic acid in the rumen. Br. J. Nutr., 31: 99-108.

Ntambi, J.M., 1999. The regulation of Stearoyl-CoA desaturase (SCD). Prog. Lipid Res., 34: 139-150.

Palmquist, D.L. and J.E. Santora, 1999. Endogenous synthesis of rumenic acid in rodents and humans. In M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza and G.J. Nelson (ed) Advances in Conjugated Linoleic Acid Research, Vol. I., pp: 201-208. AOCS Press, Champaign, IL.

Pariza, M.W., 1999. The biological activities of conjugated linoleic acid. In M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson (ed) Advances in Conjugated Linoleic Acid Research, Vol. I., pp: 12-20. AOCS Press, Champaign, IL.

Parodi, P., 2003. Conjugated linoleic acid in food. In J. Sebedio, W.W. Christie and R. Adolf (ed) Advances in Conjugated Linoleic Acid Research, Vol. 2, pp: 101-121. AOCS Press, Champaign, IL.

Parodi, P.W., 1994. Conjugated linoleic acid: an anticarcinogenic fatty acid present in milk fat. Aust. J. Dairy Tec., 40: 93-97.

Parodi, P.W., 1977. Conjugated octadecadienoic acids of milk fat. J. Dairy Sci., 60: 1550-1553.

Piperova, L.S., J. Sampugna, B.B. Teter, K.F. Kalscheur, M.P. Yurawecz, Y. Ku, K.M. Morehouse and R.A. Erdman, 2002. Duodenal and milk *trans* octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. J. Nutr., 132: 1235-1241.

Pollard, M.R., F.D. Gunstone, A.T. James and L.J. Morris, 1980. Desaturation of positional and geometric isomers of monoenoic fatty acids by microsomal preparations from rat liver. Lipids, 15: 306-314.

Porter, S.F., 2003. Conjugated linoleic acid in tissues from beef cattle fed different lipid supplements. Master's Thesis. Utah State University, UT, USA.

Poulson, C.S., 2001. Influence of diet on the conjugated linoleic acid content and quality of beef. Master's Thesis. Utah State University, UT, USA.

Raes, K., G. Huyghebaert, S.D. Smet, L. Nollet, S. Arnouts and D. Demeyer, 2002. The deposition of conjugated linoleic acid in eggs of laying hens fed varying in fat level and fatty acid profile. J. Nutr., 132: 182-189.

Reiser, R., 1951. Hydrogenation of polyunsaturated fatty acids by the ruminant. Fed. Proc., 10: 236.

Riel, R.R., 1963. Physico-chemical characteristics of Canadian milk fat. Unsaturated fatty acids. J. Dairy Sci., 46: 102-106.

Sackman, J.R., S.K. Duckett, M.H. Gillis, C.E. Bealin, A.H. Parks and R.B. Eggelston, 2003. Effects of forage and sunflower levels on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim. Sci., 81: 3174-3181.

Santora, J.E., D.L. Palmquist and K.L. Roehrig, 2000. Trans-vaccenic acid is desaturated to conjugated linoleic acid in mice. J. Nutr., 130: 208-215.

Shorland, F.B., R.O. Weenink and A.T. Johns, 1955. Effect of the rumen on dietary fat. Nature, 175: 1129-1130.

Shorland, F.B., R.O. Weenink, A.T. Johns and I.R.C. McDonald, 1957. The effect of shee-rumen contents on unsaturated fatty acids. Biochem. J., 67: 328-333.

St. John, L.C., D.K. Lunt and S.B. Smith, 1991. Fatty acid elongation and desaturation enzyme activities of bovine liver and subcutaneous adipose tissue microsomes. J. Anim. Sci., 69: 1064-1073.

Troegeler-Meynadir, A., M.C. Nicot, C. Bayourthe, R. Moncoulon and F. Enjalbert, 2003. Effects of pH and concentrations of linoleic acids on extent and intermediates of ruminal biohydrogenation *in vitro*. J. Dairy Sci., 86: 4054-4063.

Verhulst, A., G. Janssen, G. Parmentier and H. Eyssen, 1987. Isomerization of polyunsaturated fatty acids by Propionibacteria. Syst. Appl. Microbiol., 9: 12-15.

Ward, P.F.V., T.W. Scott and R.M.C. Dawson, 1964. The hydrogenation of unsaturated fatty acids in the ovine digestive tract. Biochem. J., 92:60-68.

Watkins, B.A., Y. Li, D.R. Romsos, W.E. Hoffman, K.G.D. Allen and M.F. Seifert, 2003. CLA and bone modeling in rats. In J. Sebedio, W.W. Christie and R. Adolf (ed) Advances in Conjugated Linoleic Acid Research, Vol. 2, pp: 218-250. AOCS Press, Champaign, IL.

Wilde, P.F. and R.M.C. Dawson, 1966. The biohydrogenation of á-linolenic acid and oleic acid by rumen micro-organisms, Biochem. J., 98: 469-475.

Wolff, R.L., 1995. Content and distribution of *trans*-18:1 acids in ruminant milk and meat fats. Their importance in European diets and their effect on human milk. J. Am. Oil Chem. Soc., 72: 259-272.

Yang, L., Y. Huang, A.E. James, L.W. Lam and Z.Y. Chen, 2002. Differential incorporation of conjugated linoleic acid isomers into egg yolk lipids. J. Agri. Food Chem., 50: 4941-4946.

Yurawecz, M.P., J.A.G. Roach, N. Sehat, M.M. Mossoba, J.K.G. Kramer, J. Fritsche, H. Steinhart and Y. Ku, 1998. A new conjugated linoleic acid isomer, 7 *trans*, 9 *cis*-octadecadienoic acid, in cow milk, cheese, beef and human milk and adipose tissue. Lipids, 33: 803-809.