Conjugated Linoleic Acid - The Natural Trans Fat: A Review

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ABSTRACT

Milk has been known as nature's most complete food for millennia, playing currently an important role in the diet of over six billion people in the world (Górska *et al*., 2019). They are daily consumption foodstuffs, considered as important source of energy and of a variety of bioactive substances positively associated with human health, such as proteins and peptides, oligosaccharides, lipids, minerals and vitamins. Milk fat is the costliest component and is mainly composed of triacylglycerols (~98%). The high concentration of saturated fatty acids (mainly that of palmitic, myristic and lauric acids) in the milk's lipid fraction has generated some concern, because of their negative effects on human health, especially in relation with the increased risk of cardiovascular diseases (Lordan *et al.,* 2018). However, milk's lipid fraction also contains mono and polyunsaturated fatty acids, such as oleic acid ($C_{18:1}$ cis-9), linoleic acid and certain fatty acids with trans configuration that own immense health benefits. These trans fats are naturally present in milk. Fatty acids that contain conjugated *trans* double bonds are considered as a separate entity and can be called as Natural Trans Fat such as conjugated linoleic acid. The review aims on highlighting the isomers of CLA in milk, factors influencing CLA content, the health benefits, presence of CLA in dairy products and the aspects in designing CLA enriched milk fat concerning nutrition and health.

Key words: CLA enrichment, CLA in dairy products, Conjugated linoleic acid, Health benefits, Natural trans fat.

CLA is a generic term used for describing the geometrical and positional isomers of linoleic acid with a conjugated double bond system (Gutierrez, 2016). Milk fat is the richest natural dietary source of CLA. Milk contains an average 4.5 mg CLA/g of fat (Kelly *et al*., 1998). There is an increasing research interest towards the CLA and its potential health benefits such as anti- carcinogenic, anti- atherogenic and anti- diabetic effects (Tyagi *et al*., 2004). It is very effective against cardiovascular disease too. Furthermore, CLAs are the only natural fatty acids accepted by the National Academy of Sciences of USA as they exhibit consistent antitumor properties at levels as low as 0.25-1.0 per cent of total fats (NRC, 1996). Many other biological activities have been reported over the past few years confirming that individual CLA isomers present in milk fat have a high health promoting potential. Their possible use in functional dairy products explains the increasing interest of the food industry in CLA research (Prandini *et al*., 2007). The CLA or the natural fatty acid group will be powerful enough to decide the value of milk in future.

History

In 1979, it was reported that grilled ground beef contained both bacterial mutagens and a substance that inhibited mutagenesis (Pariza *et al*., 1985). The finding of mutagens in grilled beef was confirmatory, but evidence of mutagenesis inhibitor was a novel discovery that had not been previously reported. The new anticarcinogen was identified as CLA (Ha, *et al*., 1987).

CLA is a collection of positional and geometrical isomers of octadecadienoic acid (linoleic acid), with conjugated double bonds ranging from 6,8 to 12,14. For every positional isomer, four geometric pairs of isomers are possible (*i.e.*, cis, trans; trans, cis; cis, cis and trans, trans). Therefore, CLA includes a total of 28 positional and geometrical isomers.

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Predominant isomers of CLA

The predominant isomers of CLA are listed below in Table 1. The cis-9, trans-11-octadecadienoic acid (C18:2 cis-9, trans-11), also known as rumenic acid, is the predominant isomer, representing between 75 and 90% of the total CLA-isomers. The second most abundant isomer is the C18:2 trans-7, cis-9, represents about 10% of the total CLA-isomers. The remaining isomers, including the trans-10, cis-12-octadecadienoic acid (C18:2 trans10, cis12), are present in small concentrations, mostly around 0.5%.

Types of trans fats (TFA)

Using the umbrella term TFA to refer to both industrial and natural TFA can be problematic, as it suggests that TFA are the same regardless of how they are made. The most recent edition of the DGA (Dietary Guidelines of America) does distinguish between artificial and natural (or ruminant) TFA, but only to indicate that TFA have two potential dietary sources.

Artificial and natural trans fats

Sources of artificial trans fatty acids include partially hydrogenated vegetable oils used in processed foods such as microwave popcorn, frozen pizza, some margarines and coffee creamer. Natural trans fats are present naturally in foods that come from ruminant animals (*e.g.*, cattle and sheep) and pass on to their produce such as milk and milk products, beef and lamb. They are naturally produced in the rumen. *e.g.* CLA.

History of artificial trans fat

In 1911, Procter and Gamble introduced Crisco (Crystalized cotton seed oil), the first hard shortening made entirely from vegetable oil (Pendleton *et al*., 1999). Crisco was initially marketed as a cheap alternative to lard and butter for the manufacture of processed foods, for frying and to use in home cooking. For many years the food industry has used partially hydrogenated vegetable oils extensively for deep frying, because they improve the stability and the longevity of oils subjected to high temperatures and they make French fries (chips) and other deep-fried foods appealingly crunchy. Processed food producers quickly realized the artificial trans fat's potential to delay rancidity and lengthen shelf life. In its prime, artificial trans fat often went by the identity, partially hydrogenated oil and developed the growing international appetite for processed food. From the late 1950s, a number of human and animal studies found that when vegetable oils high in polyunsaturated fat were hydrogenated, they lost their ability to lower cholesterol levels, although some other studies have failed to confirm this relationship. It is noted in 1957 and 1961 that hydrogenated vegetable fats raised cholesterol levels and decreased the amount of polyunsaturated fats in oils (Page *et al*., 1957).

Table 1: Predominant Isomers of CLA.

Isomer	Percentage of the total isomers
$C_{18:2}$ cis-9, trans-11	72.6-91.2
$C_{18:2}$ trans-7, cis-9	1.2-8.9
$C_{18:2}$ trans-9, trans-11	0.8-2.0
$C_{18:2}$ trans-11, trans-13	0.3-4.2
$C_{18:2}$ trans-12, trans-14	0.3-2.8
$C_{18:2}$ trans-10, trans-12	0.3-1.3
$C_{18:2}$ cis-11, trans-13	0.2-4.7
$C_{18:2}$ trans-8, trans-10	0.2-0.4
$C_{18:2}$ trans-11, cis-13	0.1-8.0
$C_{18:2}$ trans-9, trans-9	<0.1-2.4
$C_{18:2}$ trans-8, cis-10	<0.1-1.5
$C_{18:2}$ trans-10, cis-12	<0.1-1.5
$C_{18:2}$ cis-12, trans-14	<0.01-0.8
Other cis-cis	0.1-4.8

(Bauman and Lock, 2006).

Artificial trans-fat and regulatory bodies

Partially hydrogenated oils or PHOs are the primary dietary source of artificial trans fats in processed foods. In 2015, FDA released its final determination that partially hydrogenated oils (PHOs) are not Generally Recognised as Safe (GRAS). The determination is based on extensive research into the effects of PHOs, as well as input from stakeholders during the public comment period (Grossman *et al*., 2015). The FSSAI has limited trans-fat content to 5% in 2016. The Food Safety and Standards Authority of India or FSSAI has published two draft regulations to limit TFA. One issued in December 2018 proposes to lower the TFA limit to 2% by January 2022, but it applies only to fats and oils. The second draft regulation issued in August 2019 proposes a 2% TFA limit, for foods "in which edible oils and fats are used as an ingredient (Dhaka *et al*., 2011). After determining in June 2015 that partially hydrogenated oils (PHOs) were no longer "Generally Recognized as Safe" for use in human food, the United States Food and Drug Administration (FDA) requested food manufacturers to remove them from products by June 2018. WHO

Table 2: Content of CLA in different food types.

Product	mg/g fat	Product	mg/g fat
Dairy product		**Meat/fish**	
Homogenized milk	5.5	Fresh ground beef	4.3
2% milk	4.1	Veal	2.7
Butter fat	6.1	Lamb	5.8
Condensed milk	7.0	Pork	0.6
Cultured butter milk	5.4	Chicken	0.9
Butter	4.7	Fresh ground turkey	2.6
Sour cream	4.6	Salmon	0.3
Ice cream	3.6	Egg yolk	0.6
Low fat yoghurt	4.4	**Vegetable oils**	
Custard style yoghurt	4.8	Safflower oil	0.7
Plain yoghurt	4.8	Sunflower oil	0.4
Frozen yoghurt	2.8		

(Bawa, 2003).

recommends that the total trans-fat intake be limited to less than 1% of total energy intake, which translates to less than 2.2 g/day with a 2,000-calorie diet.

Natural trans fat

CLA that is having eighteen carbon atoms and with trans configuration is naturally produced in the ruminant animals (cows, sheep, buffalo *etc*.) by rumen bacteria from the feed they take and is termed as natural trans-fat. Milk and dairy products are rich sources of CLA.Table 2 depicts the content of CLA in different types of food.

Production of CLA by biohydrogenation

Ruminants consume fodder and forages. They rely on microbial digestion of forages and supplementary feed materials in the rumen. Rumen bacteria play a major role in biohydrogenation. They convert C18 unsaturated fatty acids to stearic acid (SA), *via* a number of intermediates (Fig 1).

Biohydrogenation of unsaturated fatty acid by rumen bacteria

Rumen fluid contains bacteria, fungi and protozoa. Small amount of contribution is made by fungi and protozoa in biohydrogenation. Among which the major source of biohydrogenation is bacteria when compared to fungi and protozoa.

Bacteria involved in biohydrogenation process can be divided into two: Group A and B:

Group A bacteria

They hydrogenate linoleic and linolenic acid to trans-11 octadecadienoic acid and are not able to or incapable of hydrogenating octadecadienoic acid. The final step of biohydrogenation (vaccenic acid to stearic acid) cannot be performed by group A bacteria, whereas group B bacteria can perform all steps in the biohydrogenation pathway.

Examples are *Butyrovibrio*, *Micrococcus*, *Ruminococcus* and *Lactobacillus.*

Group B bacteria

They are capable of hydrogenating a wide range of octadecadienoic acids, including cis-9 (oleic) and trans-11 (trans vaccenic) acids as well as linoleic acid to stearic acid. Examples are *Fusocillus* sp. and gram-negative rods.

Factors influencing CLA content and designing the milk fat with enriched CLA

Milk fat is a good source of natural trans-fat which has immense health benefits. Research has shown that it is possible to affect the extent of ruminal biohydrogenation and the concentration of CLA absorbed and incorporated into milk fat.

The different means of increasing CLA in milk is by :

1. Providing PUFA substrates for rumen

Reports suggest that feeding lipid sources rich in linoleic and linolenic acids either as seeds or free oil increases the CLA content of milk when oil is accessible to the rumen microorganisms for biohydrogenation. The scientists found that supplementing the dietary dry matter with 2% or 4% soybean resulted in a 237% or 314% increase in CLA content of milk compared with the control (Dhiman *et al.,* 2000).

2. Pasture feeding

Numerous studies have confirmed that pasture feeding can increase the CLA concentrations in lactating dairy cows (Kelly *et al*., 1998 and Stanton *et al*., 1997). The CLA-enriching effect of pasture has been attributed to the effects on biohydrogenation and the provision of α-*linolenic* acid as a lipid substrate for the formation of VA in the rumen and its subsequent desaturation to cis-9, trans-11 CLA in the mammary gland. Cows receiving all of their daily feed as pasture produced higher milk fat CLA content (22.1 mg/g fat) than cows receiving only one-third (8.9 mg/g fat) or two-thirds (14.3 mg/g) of their daily diet as pasture (Dhiman *et al.,* 1999).

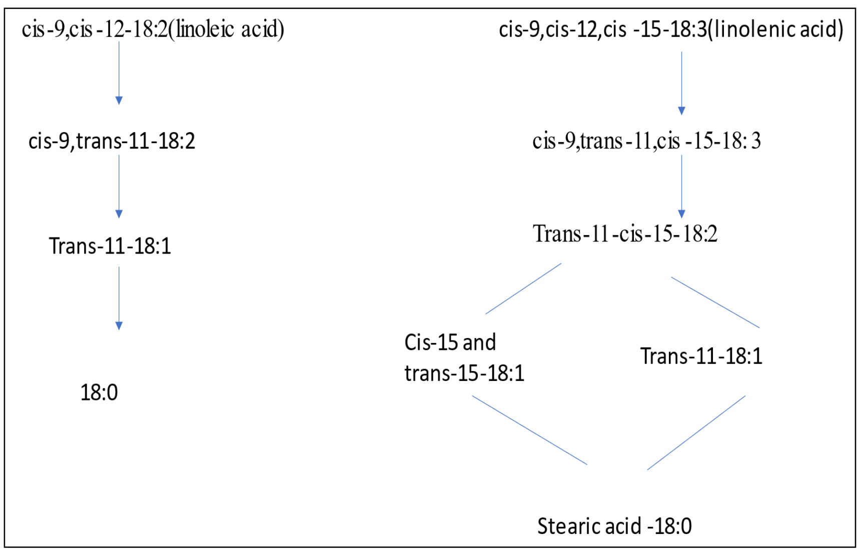

Fig 1: Biohydrogenation pathways of linoleic acid and linolenic acid (Adapted from Harfoot and Hazlewood (1997).

3. Blend of vegetable oils

The substantial variation in content of CLA in milk fat between herds suggests that diet has a major influence. Kelly *et al*., (1998) demonstrated that dietary supplementation of vegetable oils high in linoleic acid gave the greatest response and there is a clear dose-dependent increase in milk fat content of CLA. Secretion of unsaturated fatty acids and rumen-derived *trans* $C_{18:1}$ in milk may be increased by inclusion of high-oil feed ingredients in dairy diets. It was found that feeding lactating dairy cows a blend of fish oil and MUFA and PUFA resulted in an increase in the concentrations and yields of CLA in milk (Cieślak *et al*., 2015) and the greatest increase being with a blend of a high LA source (*e.g.,* regular sunflower seeds).

4. Supplementation of CLA to the diet

Supplementation of c-9, t -11 and t- 10, c-12-CLA during the first four weeks of lactation resulted in an increase of these specific CLA isomers provided during treatment. Therefore, all the CLA isomers were taken up by the mammary gland and incorporated into milk fat.

Potential health benefits of CLA

The biological properties of dietary CLA are currently attracting considerable interest because of its diverse physiological outcomes in animal studies. Beyond its nutritional value, dietary CLA is effective in suppressing tumour development during initiation, promotion and progression phases of carcinogenesis (Belury, 1999).

CLA also effectively reduces severity of atherosclerosis, improves cardiovascular health, enhances bone health, reduces obesity and also can target diabetes. Not only CLA is a powerful anticarcinogen, but it also has antiatherogenic and antidiabetic properties.

Ant carcinogenicity of CLA

It has been assumed that CLA is involved in various steps in all three stages of carcinogenesis (initiation, promotion, progression) and that the effect differs according to CLA isomer, type and site of the cell/organ and stage of carcinogenesis. CLA are included in the phospholipids of membranes and replace other polyunsaturated fatty acids. Thus, cell metabolism and signal transduction may be influenced in several ways: modulation of cell proliferation and apoptosis, regulation of gene expression, influence on eicosanoid synthesis and metabolism and antioxidative mechanisms.

CLA and atherosclerosis

Atherosclerosis is a complex inflammatory disease that is characterized by the progressive formation of lipid laden fibrous plaques within the arterial wall (Galkina *et al*., 2009). This chronic disease arises from a maladaptive inflammatory response, an impaired resolution process and a defective lipid metabolism. Progressive damage to the vessel wall culminates in arterial occlusion resulting in stenosis or lesion rupture triggering thrombosis. Atherosclerosis is the underlying cause of ischaemic events and often the first clinical manifestation of atherosclerosis is myocardial infarction or stroke. The two most abundant CLA isomers have been shown to have anti-atherogenic effects in an experimental model of atherosclerosis when administered in an 80: 20 blends. CLA has been shown to induce the regression of atherosclerosis in mice, rabbits and hamsters. However, it was demonstrated that different CLA isomers have different atherogenic effects. Mice fed on a Western diet (0.15% cholesterol) were supplemented with either c9, t11-CLA, t10, c12-CLA or linoleic acid (control diet) for 12

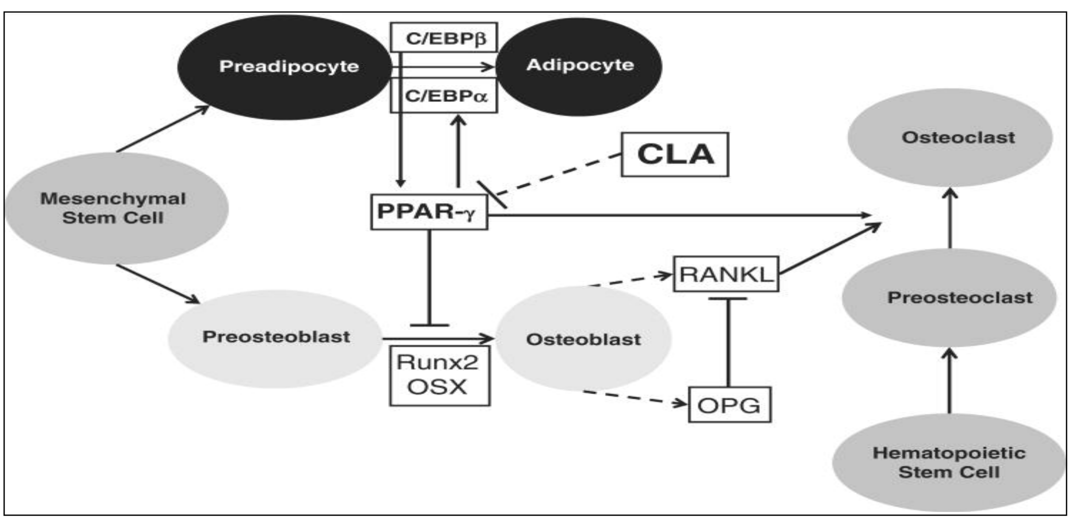

Fig 2: Possible differentiation of mesenchymal stem cells toward an adipocytic or osteoblastic lineage.

Abbreviations: PPAR-γ - Peroxisome proliferator-activated receptor gamma; C/EBP-CCAAT/enhancer-binding proteins; Cbfa1-Core-binding factor A1; Runx2-Runt-related transcription factor 2-RANKL, Receptor activator of nuclear transcription factor κβ ligand, OPG-Osteoprotegerin.

weeks. Development of atherosclerotic lesions was impaired in mice fed with c9, t11-CLA (Bruen *et al*., 2017).

CLA promotes bone health

Conjugated linoleic acid (CLA) supplementation causes increases bone mass in mice.

Potential mechanisms of conjugated linoleic acid action on bone

Dietary CLA led to differences in CLA enrichment of bone marrow and periosteum. Periosteum has a major role in bone growth and bone repair and has an impact on the blood supply of bone as well as skeletal muscle. Enrichment of chondrocytes with CLA affected collagen .

Anti-obesity effects of CLA

Due to the substantial rise in obesity prevalence over the past 30 years, interest in CLA afor weight loss treatment has been increasing. Supplementation with a CLA mixture (*i.e.*, equal concentrations of the 10,12 and 9,11 isomers) or the 10,12 isomers decreases body fat mass (BFM) in many animals and some human studies (Wang, 2004). Of the two major isomers of CLA, the 10,12 isomers specifically are responsible for the anti-obesity effects (Kennedy *et al*., 2010).

CLA regulates body weight

CLA has the ability to regulate bodyweight which is good news to the obese people across the world. Park *et al.,* (1999) were the first to demonstrate that CLA modulated body composition. In this study, male and female mice given a 0.5% (w/w) CLA mixture had 57% and 60% lower BFM, respectively, compared to controls. Other researchers have subsequently demonstrated that CLA supplementation consistently reduces BFM in mice, rats and pigs. For example, dietary supplementation with 1% (wt/wt) CLA mixture for 28 days decreased body weight and white adipose tissue (WAT) mass in C57BL/6J mice. Strong evidence from animal trials supports an influence of CLA on body composition, *i.e.,* lowering of body weight and fat mass and a relative increase in lean body mass (Poirier *et al*., 2005, Roche *et al*., 2001).

CLA targets diabetes

Adult-onset diabetes is fast becoming an epidemic and is largely associated with poor diet chart and nutrition and other lifestyle issues. Conjugated linoleic acid (CLA) shows promise to improve insulin action and decrease circulating glucose levels, with rat and human studies both reporting significant benefits. Professor Jack Vanden Heuvel, co-director of Penn State's Centre of Excellence in Nutrigenomics, has suggested that incorporating CLA as a dietary supplement or from enriched foods, in addition to a balanced diet, could be a suitable way of helping diabetics control their blood glucose and insulin levels. Several studies in animals and humans have found an antidiabetic effect of CLA and suggested the trans-10, cis-12 isomer to be responsible for decreasing glucose levels and increased insulin sensitivity (Khanal, 2005).

CLA concentration in dairy products and effects on processing

Variation in CLA concentration in dairy products is essentially a function of their original concentration in raw milk.

Processing of milk to cheese appears to have no effect on the final content of CLA in cheeses; its content is primarily dependent on the CLA level of the unprocessed milk. Milk from cows fed extruded oilseed was used to produce Mozzarella cheese with no alterations in CLA content (Dhiman *et al*., 1999). Similar processing of milk from cows receiving a grass silage supplement with CLA Concentration in Cheese a cereal-based concentrate to Edam cheese showed no effect on the CLA content.

The way that cheese is made influences its CLA (conjugated linoleic acid) content. In general, the longer the cheese is aged, the lower the CLA. Thus, hard cheeses such as Parmesan and Romano tend to have less CLA than softer cheeses such as cream cheese, cottage cheese, feta, farmer's cheese, ricotta and Brie. In addition, cheese that is aged through "bacterial surface ripening" (Brick and Muenster) has more CLA than cheese that does not go through this process (Avramis *et al*., 2003).

CLA concentration in butter

The concentration of CLA in dairy products is essentially a function of the concentration in raw milk fat and this can vary widely with different nutritional schemes (Griinari *et al*., 1999). Butter from cows fed the control diet had a typical CLA concentration (5 mg/g of fatty acid), whereas butter from cows fed the sunflower oil diet averaged 41 mg/g of fatty acid (Bauman *et al*., 2000).

CLA in fermented milks

Fermented milks such as yogurt are considered healthy foods, due to their beneficial effects on human health. It is also reported that fermented milks produced out of typical strains of microorganisms are good sources of CLA.

During the manufacturing of *Dahi* (fermented product from buffalo milk, similar to yogurt) Yadav *et al*., 2007 found a significant increase (about twice) in the amount of CLA, when using *L. acidophilus* and *L. casei* as starter cultures. The refrigerated storage at 4°C did not affect the concen-tration of the CLA-isomers. On the other hand, the CLA content of organic fermented milks, produced using strains of *B. animalis* ssp. *lactis* in combination with *S. thermophilus*, was slightly higher than the CLA concentration of the starting milk. In contrast, there was no formation of CLA in fermented milks with strains of *Bifidobacterium*, despite having been chosen for their ability to pro-duce CLA *in vitro* and being cultured in substrates rich in linoleic acid (Florence *et al*., 2012).

CONCLUSION

The review has illustrated the natural presence of CLA in milk and dairy products and also seen the possibility of designing milk with enriched CLA. An important concern is whether the extent of enhancement will translate into the real benefit for the person consuming milk and dairy products. Extrapolation from animal studies has suggested that the level of CLA intake necessary to produce anti-carcinogenic effects in humans may be about 3g per day. Using the CLA percentage achieved with the HFB diet, one serving of whole milk (460 mg CLA) and a sandwich with butter (365 mg CLA) and cheddar cheese (721 mg CLA) would provide 1546 mg (1.546 g) CLA. This unveils the possibility of using CLA enriched milk and milk products as dietary CLA at levels that may potentially benefit health, without the need for unrealistic changes to eating habits. This recognizes the trend among consumers towards an increased desire to make diet choices that promote good health. Of course, consumers could increase their CLA intake by taking synthetic CLA in pill form, which is already available in health food stores. The main difference between the CLA in these products and milk CLA is the broader range of isomers in the synthetically produced CLA. The relative value for human health of this range of CLA isomers compared to the CLA found in ruminant milk fat is uncertain. Nevertheless, CLA enriched milk produced through manipulation of the dairy ration has an advantage over this type of product in that it can be promoted as a “natural” source of CLA. It may also be easier for CLA enriched milk to gain acceptance since milk already has a wide distribution and consumers are well accustomed to seeing a broad variety of dairy products in the shops.

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