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Conjugated linoleic acid (CLA): Implications for human health and animal production

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Abstract
Conjugated linolenic acid are isomers refer to a group of positional and geometric isomers of the omega-3 essential fatty acid α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3, ALA). Its isomers can be either *cis*- and/or *trans*- and their double bonds are separated by a single bond. Food products from ruminants and some plant products are the major sources of CLA. The isomers in ruminants arise as a result of bacterial isomerization of ALA in the rumen. The concentration of CLA isomers in seed oils is higher than in milk and edible parts of ruminant carcass. The CLA isomers from the plant sources are in the form of conjugated trienes, whereas those in ruminant products are of conjugated diene type. Some plant seed oils are the richest natural sources of CLA isomers. So, searching for methods of increasing the CLA isomer content in food of animal origin not exhibiting negative effects on animal welfare and physiology is very important for researchers. A presence of long-chain and very long-chain conjugated unsaturated fatty acids was also confirmed in some ruminant tissues. Clinical studies documented that health-promoting properties have been attributed to CLA isomers. It was also evidenced that animal diet may influence the CLA synthesis. The proper identification of geometric and positional isomers of conjugated unsaturated fatty acids in biological samples is a great challenge in research field.

Keywords: Conjugated linolenic acid, fatty acids, isomers, ruminants and seed oils

1. Introduction
Studies reported that grilled ground beef contained both bacterial mutagens and a substance that inhibited mutagenesis [15]. The finding of mutagens in grilled beef was confirmatory, but evidence of a mutagenesis inhibitor was a novel discovery that had not been previously reported. That study concluded with a speculative prediction: “It may also be found that the mutagenic inhibitory activity inhibits carcinogens”. Subsequently, this speculation was indeed the case [14] and the new anticarcinogen was identified as conjugated linoleic acid (CLA) [15]. The term CLA consists of a collection of positional and geometrical isomers of octadecadienoic 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). The term CLA therefore includes a total of 28 positional and geometrical isomers.

2. Synthesis of CLA
The presence of CLA in milk fat in dairy animals relates to the isomerization and biohydrogenation of unsaturated fatty acids (FAs) by rumen bacteria as well as the D9-desaturase activity in the mammary gland. The cis-9, trans-11 CLA comprises 75–90% of total CLA and is derived from linoleic acid and linolenic acid [8]. Linoleic acid (cis-9, cis- 12, 18:2) is first isomerized to the CLA cis-9, trans-11 by cis-12, trans-11 isomerase and then hydrogenated by *Butyrivibrio fibrisolvens* to vaccenic acid (VA, trans-11 18:1) in the rumen. These initial steps occur rapidly. There is a strong positive correlation between the trans isomers of 18:1 (VA, trans-13–14, trans-15, and trans-16) in milk fat and the level of linoleic acid in the diet. The hydrogenation of VA to stearic acid appears to involve a different group of organisms and occurs at a slow rate. For this reason, VA typically accumulates in the rumen. This main trans FA is responsible for the formation of the CLA isomer cis-9, trans-11, which occurs by desaturation (D9-desaturase) of the ruminally derived VA in the mammary gland [6]. The pathway for the formation of the CLA is presented in Fig 1.

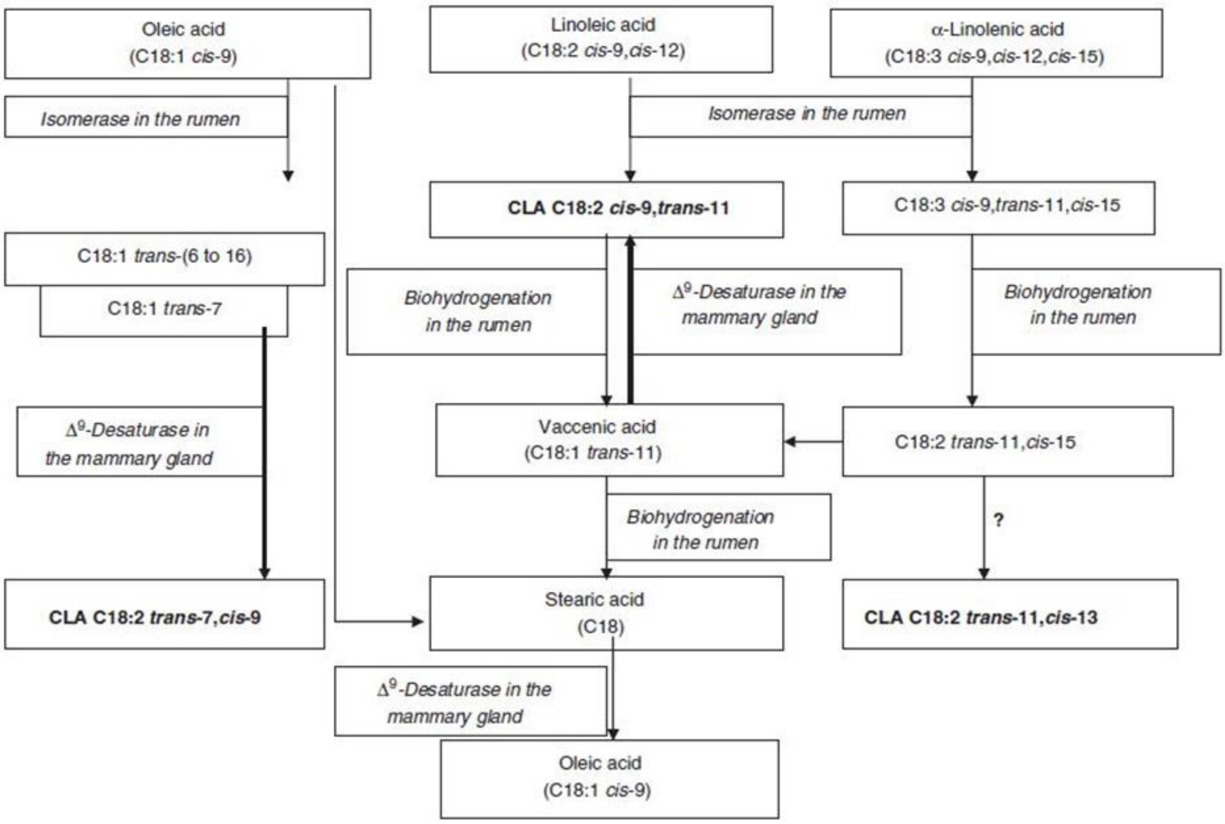

Fig 1: Synthesis pathways of CLA [6]

3. CLA content in milk and factors affecting CLA content in milk

The milk is the richest source of CLA and its content may vary greatly with the diets, fed to the dairy animals. It may range from 4 to 54 mg/g of fatty acids. [3, 17]

There are many factors affecting the milk CLA concentration. The major one is dietary factors. It contributes the major variation in the milk CLA contents. The various factors affecting milk CLA content are 1. Dietary factors, 2. Physiological factors, 3. Tissue Δ9-Desaturase activity 4. Rumen microflora and environment, 5. Seasonal effects

3.1. Dietary factors

Different dietary factors and supplements that are affecting variation in milk CLA are described in detail below.

3.1.1. Pasture feeding

Pasture feeding can increase in the short-term milk fat CLA concentrations in lactating dairy cows when changed from indoor winter feeding and that milk fat CLA content increases with increasing proportions of pasture in the diet [7]. 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.

With rising altitude, which is accompanied by a decrease in the proportion of grasses and a corresponding increase in dicotyledonous species, there was an increase in CLA levels from lowlands to mountains and highlands [6].

Animals fed with fresh cut pasture than conserved fodder produce more CLA in milk. It because wilting of the grasses causes oxidative loss of PUFA in grasses. Maturity of the pasture has a negative effect on CLA content of animal tissue [11]. Deceased leaf stem ratio, maturation of leaves, initiation of flowering and leaf senescence can cause a decreased total FA and LNA content in grasses.

3.1.2. Feeding plant oils and seeds

Plant oils from different oilseeds have quite different FA compositions and accordingly would be expected to have different effects on milk fat CLA concentrations. Comparisons between different types of plant oils suggest that those rich in linoleic acid increase CLA concentration most effectively [6]. Different dietary oil treatments (peanut oil, high in oleic acid; sunflower oil, high in linoleic acid; linseed oil and flaxseed, high in α-linolenic acid) have been shown to exert different degrees of enrichment of milk fat with CLA. Feeding sunflower oil (53 g per kg of diet dry matter (DM) for 2 weeks resulted in a CLA concentration in milk fat of 24.4 mg per g fat which was significantly greater than that achieved with similar inclusions of peanut oil (13.3mg per g fat) or linseed oil (16.7 mg per g fat). In milk fat of cows fed a high concentrate diet supplemented with linseed oil the total conjugated 18:2 FAs were higher than without supplemental oil or low concentrate without and with linseed oil [11]. Feeding soybean oil, also rich in linoleic acid, was more effective in increasing the CLA content of the milk fat than feeding linseed oil.

The calcium salt of oils also increased the CLA content in milk fat. Feeding Ca salts of FAs from soybean oil caused the highest level of CLA compared with canola and linseed oil [3].

Processing of seeds has a definite effect on CLA. Inclusion of Extruded seed in diet of animals increases CLA content in milk [10].

3.1.3. Feeding marine oils

Equivalent amount of dietary fish oils is more effective than plant oils for increasing milk CLA content due to increased rumen output of Vaccenic Acid (VA). Supplementation of plant oils along with fish oil can produce increased milk CLA than feeding fish oil alone [2]. Feeding mixtures of plant and marine oils have an effect on CLA content in milk fat and observed a three- to four-fold increase in CLA content.

3.1.4. Supplementing ionophores
Ionophores appear to have slight positive effect in milk CLA when it is supplementing along with diet. In a study it is observed that monensin supplementation increase CLA synthesis after 12 hr incubation of rumen fluid culture in *invitro* study [19].

3.2. Physiological factors
Holsteins have a higher milk fat content of CLA than Brown Swiss. Also shows HF have higher milk CLA than both Brown Swiss and Jersey. In another study a French breed, Montbeliardes have higher milk CLA than Irish Holstein Friesian and Dutch Holstein Friesian and a least value obtained for Normand's [10].

3.3. Δ^9-desaturase activity
Δ^9-Desaturase adds a double bond across carbons 9 and 10 in fatty acids starting from carboxylic end. In a study to know the importance of this enzyme, Δ^9-Desaturase was inhibited by Sterculic acid administration and observed a decreases in the milk content of the enzyme products (*cis*-9 18:1, and *cis*-9, *trans*-11 18:2) and increases in its substrates (18:0, and *trans*-11 18:1) [1]. This indicates its role in milk CAL synthesis. The values of Δ^9-desaturase indices observed for Jersey and Brown-Swiss cows were lower compared with Holstein [18].

3.4. Effect of rumen microflora environment
Ruminal biohydrogenation is carried out mostly by bacteria belongs to *Butyrivibrio* genus. Rumen pH had a marked influence on biohydrogenation in rumen. Rumen bacteria from high fiber diet fed cows produced mostly cis-9, trans-11 CLA at pH higher than 6.2. Production of cis-9, trans-11 CLA positively correlated and trans-10, cis-12 CLA inversely correlated to rumen pH [4].

3.5. Seasonal variation
α-linolenic (cis-9, cis-12, cis-15 C18:3) and linoleic (cis-9, cis-12 C18:2) acids are predominant unsaturated fatty acids in forages. A significant difference in chemical composition of botanical families as well as individual plant species occurs during different season [5].

4. CLA in beef
Beef contains cis-9, trans-10 and trans-10, cis-12 isomers. Pasture fed beef animals has high CLA content in beef than those fed with grains or silage. Concentration of CLA depends on both systems used for the production of beef and the diet used for finishing the beef cattle. High CLA content in longissimus and supraspinatus muscle in pasture finished cattle, because they are the most tenderous muscles in the body [16]. Supplementation of safflower oil increases CLA content in various lamb tissues [13]. Increase in intramuscular proportions of vaccenic acid and CLA, observed when sunflower seed included in diet in beef cattle [12]. In another study it is observed that feeding ground flax seed increases CLA content in body fat [9]. Breed of animal can affect the CLA content in beef [12]. Breed with high fat in muscle, shows high CLA content in meat.

5. Enhancing CLA in meat and eggs
Enhancement of CLA content in egg yolk, broiler meat or pork can be achieved by dietary supplementation of CLA. CLA content in egg yolk and meat increases linearly with the increasing concentration of CLA isomers in the diet [5,18].

6. CLA levels in different human foods
The estimated levels of CLA in various human foods are illustrated in Table 1.

Table 1: CLA levels in various human foods [5, 8, 11]

Dairy products	CLA(mg/g of fat)
Homogenized milk	5.5
Low fat milk	4.1
Butter fat	6.1
Condensed milk	7.0
Cultured buttermilk	5.4
Butter	4.7
Sour cream	4.6
Ice cream	3.6
Low fat yogurt	4.4
Custard style yogurt	4.8
Plain yogurt	4.8
Frozen yogurt	2.8
Medium Cheddar	4.1
American processed	5.0
Meat/fish/egg	**CLA(mg/g of fat)**
Fresh ground beef	4.3
Veal	2.7
Lamb	5.8
Pork	0.6
Chicken	0.9
Fresh ground turkey	2.6
Salmon	0.3
Egg yolk	0.6
Vegetable oils	**CLA(mg/g of fat)**
Safflower oil	0.7
Sunflower oil	0.4

7. Health effects of CLA
1. Anti-carcinogenic action
CLA is inhibitory to cancer cells. About 30 -40% of cancer cases can be prevented by modification of nutritional factors and food consumption patterns [11]. CLA was found inhibitory to cancer cell growth like human malignant melanoma, colorectal cancer cells and human breast cancer cells [18]. Different mechanisms of anti-carcinogenic action include reduction of cell proliferation, alterations in the components of the cell cycle, induction of apoptosis, modulates immunity and modulating gene expressions. A recent study revealed that, LXR (Liver X receptors) activation by t9, t11-CLA isomer leads to cholesterol efflux from cell, which in turn leads to inhibition of cell proliferation and stimulation of apoptosis in mammary tumor [8].

2. Inhibition of atherosclerosis
Researchers observed less plasma triglyceride, less plasma LDL cholesterol, less LDL c/HDL c ratio and fewer aortic fatty lesion in rabbits when fed with CLA. CLA intake via diet lead to reduced atherosclerosis, reduced fatty streaks [10].

3. Enhancing immune response
Anti-cancer activity of CLA can be due to enhanced immune response. An increased IL-10 receptor expression, phosphorylation of STAT3, and downstream target gene

expression in the aorta and increase in serum IL-10 observed in regression of atherosclerosis, which can be attributed to the anti-inflammatory response [20].

8. Body fat reduction

The trans-10, cis-12 isomer is the isomer responsible for this activity. Studies conducted in older mice revealed that CLA can reduce existing body fat [7].

Different mechanisms include,

1. Increased energy expenditure by increased oxygen consumption and by increased expression of uncoupling proteins by CLA. [16].
2. Reduced adipose cell mass or cell numbers by inhibiting lipoprotein lipase at adipose cells [20].
3. Inhibiting stearoyl-CoA desaturase activities [5].
4. Enhancing apoptosis of preadipocytes and adipocytes [4].
5. Modulating lipolysis [21].

9. Effect on bone health

CLA improve overall bone mass when calcium is included as a co-supplement. CLA can significantly reduce femur Tartrate resistant acid phosphatase (TRAP) activity suggests potential reduction of osteoclastogenesis [18]. So CLA can be used to prevent bone loss and weight gain associated with menopause.

10. Anti-diabetic action

Feeding of CLA to rats prone to developing diabetes shows normalized glucose tolerance [3]. CLAs specifically activate the cell surface receptor FFA1 (Free Fatty Acid receptor 1) [16]. FFA 1 is considered as an emerging therapeutic target for diabetes mellitus. Thus, it increases glucose-stimulated insulin secretion in diabetes mellitus.

11. Conclusion

CLA can be effectively increased in milk and meat by pasture feeding. Supplementation with plant oils and fish oil can also increase CLA content in milk and meat. Dietary supplementation of CLA can enhance CLA content in non-ruminants. Ruminant animal products are the important source of CLA for human especially milk and meat from ruminants. CLA can be effectively used as nutraceuticals especially against cancer and diabetes mellitus. Thus, CLA can be considered as a cure for some deadly diseases like cancer and diabetes mellitus.

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