Chapter 3

Bovine Feed Manipulation, Enhancement of Conjugated Linoleic Acid and Its Bioavailability

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Abstract

Diet is a pivotal contributing factor to the onset and progression of some chronic ailments nowadays. The conjugated linoleic acid (CLA), a bioactive component of ruminant fat, introduces more elucidates what we know polyunsaturated fats and diseases. CLA, a mixture of isomers c9, t11 and t10, c12, is the most abundant ranging from 80 to 90% of total CLA isomers and account for most known health benefits. Dairy milk and meat are the major dietary sources of CLA, and its concentration is of great interest to human health. The biofunctionalities of CLA from enriched dairy products are major attributes in the context of a substance present in our everyday diet. Thus, dietary modifications in animal feed, synthetic and microbial production have been made to increase CLA intake to enhance its clinical manifestations. However, the bioavailability and distribution of enriched or supplemented CLA has not been fully elucidated because of its response variation in different animal models. This chapter deals with different dietary sources, availability, enhancement of CLA in dairy products and its positive manifestation against different maladies. In conclusion, it is feasible to produce CLA-enriched dairy products with acceptable storage and sensory characteristics while deriving its nutritional benefits.

Keywords: feed manipulation, essential fatty acids, conjugated linoleic acid, enhancement, bioavailability

1. Introduction

The consumption of dietary fat and fatty acids is still the prominent focusing on human nutrition and health research. This continuing trend in research not only leads to classify fat as saturated, unsaturated, monounsaturated, polyunsaturated and omega fatty acids but also the essential role of fairly small and relatively specific fatty acid called, conjugated linoleic acid (CLA). CLA is a mixture of isomers that are characterized by the presence of conjugated dienes on different geometric positions coming from ruminant to human diet primarily in meat and milk products [1]. The promising health effects of CLA are the major interest of research in fatty acids. CLA from dairy sources has predominant isomers such as c9, t11 and t10, c12 and has shown biological effects against modern nutritional disorders [2]. These dairy products with natural CLA concentration ranging from 0.34 to 1.07 g/100 g fat in milk and 0.12 to 0.68 g/100 g fat in meat [3, 4] but this CLA concentration is not sufficient to meet daily requirement (1.5 to 3.5 g/day) of human being [5, 6].

To meet the recommended daily CLA intake, production and sale of meat and milk products supplemented and/or enrichment with essential fatty acids, particularly CLA has increased drastically from the late 1990s due to its biofunctionalities. Several efforts have been made to increase the concentration of CLA in these dairy products for pronounced health effects. In this context, the feeding practices of dairy animals let them to change nutrients concentration, particularly the fatty acids composition in its milk and meat and its products. However, these bio-enriched dairy products do not differ in their nutrient composition as compared to the conventional foods. Animal diet modifications result in change of amount of trans fatty acids, unsaturated fatty acids, ratio of 3:6 omega fatty acids but most pronounced difference was observed in concentration of CLA while feeding on grain supplemented diet as compared to pastures diet [7, 8].

To date, there is a lot of scientific literature on animal feedings practices and effects of CLA on human health but there are relatively very few studies on the bioavailability of CLA from dairy products and more precisely, the bioavailability of CLA from these naturally bio-fortified dairy products needs to be fully explored. It is generally considered that c-9, t-11 CLA from dairy products and animal meat accounts of 90 and 75%, respectively, while plant oils have less than 50% c-9, t-1I CLA isomer in total CLA. It formulated as biologically active form that tended to become less active in processed dairy and meats products [1]. Furthermore, the comparative human health effect of CLA products from dairy products remains inconclusive. Most of the previous studies were conducted on animals, and secondly, in most of these studies, synthetic mixtures of CLA supplement were used that do not mimic the similar functions as CLA from natural food sources possess and do not confound differently with potential risk factors [9]. To our knowledge, this is the first manuscript to discuss the bioavailability of CLA from dairy products obtained by ruminant diets modification and chemically synthesized CLA.

1.1. Biosynthesis of CLA

The biosynthesis of CLA in ruminants depends on content of diet and microbial and enzymatic action. CLA isomers produce either in rumen or in the intestine as shown in **Figure 1**. For example, the major isomers of CLA, cis-9, trans-11 CLA is produced in rumen from dietary

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linoleic and linolenic acids by microbial biohydrogenation [10, 11]. The major pathway in the biosynthesis of cis-9, trans-11 CLA in cow's milk is the biohydrogenation and desaturation. After microbial biohydrogenation of cis-9, trans-11, it is further bio-hydrogenated to *trans*-11-octadecenoic acid if not absorbed directly. Bioconversion of trans-vaccenic acid to cis-9, trans-11 CLA is occurred with the help of stearoyl-CoA desaturase action in ruminants [12, 13]. The presence of *trans*-10, *cis*-12 in ruminant's milk indicates that *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA have been converted to *trans*-10-octadecenoic acid via biohydrogenation in rumen. But due to lack of delta 12 desaturase, mammal could not desaturate *trans*-10-octadecenoic acid back to *trans*-10, *cis*-12 CLA depositing *trans*-10, *cis*-12 CLA in their tissues [12, 14].

1.2. CLA bio-fortification through diet manipulation

The presence of CLA intrigues the researchers to look at the possible ways of increasing the concentration of CLA in ruminant's milk, meat and other dairy products for its positive health promising functionalities. These products are the principle source of nutrients, minerals and vitamins. Among these, dairy milk is the major dietary source with highest concentration of CLA. All ruminants under normal physiological conditions produce only 0.2–2.0% CLA of total tissue or milk fat [16, 17]. While the consumption of 120 g beef fat having CLA concentrations from 1.2 to 12.5 mg/g of fat accounts for total recommended daily intake of 1.5 to 3.5 g of CLA [5, 6]. This naturally low level of CLA makes very difficult to consume large quantity of fat to meet daily-recommended intake of CLA. Thus, several interventions have been made to enhance CLA concentration on milk and meat. For this purpose, different animals, their breeds, diet manipulations, commercial/synthetic CLA production, use of different strains of microbes have been used as strategies. Dietary manipulation is one of the approaches to increase natural production and enhancement of CLA in dairy products [18].

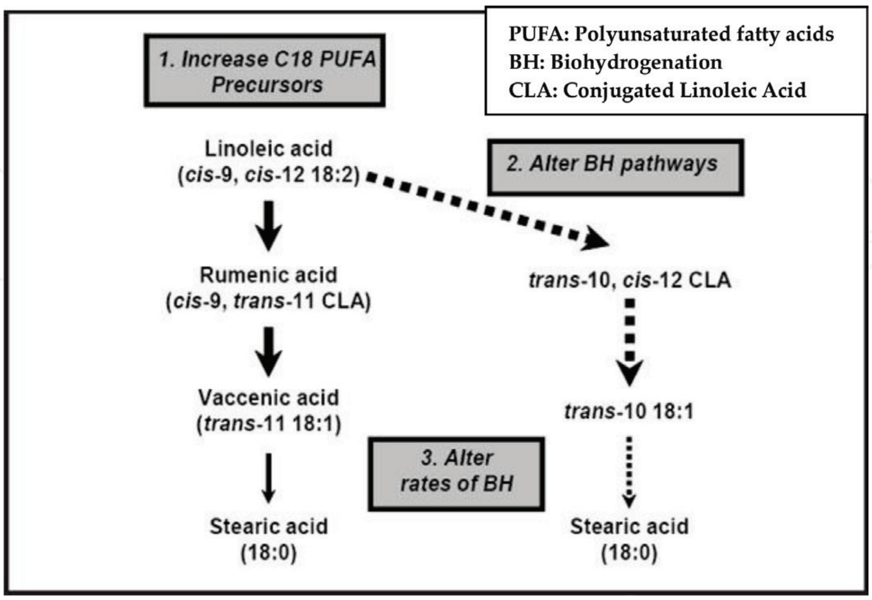

Figure 1. Schematic representation of linoleic acid in ruminants under normal (left side) and diet-induced milk fat formation (right side) [15].

1.3. Bio-fortified CLA in bovine's milk

Different diet manipulations, seasonal effects and farm characteristics (e.g., organic vs. traditional) have been used to enhance CLA concentration in milk of cow and buffalo. The diet-manipulating strategies and their effects on CLA enhancement in milk have been summarized in **Table 1**. It was observed the dietary substrates of CLA in animal feed results result in an increase of CLA in milk, most variation in cow milk [19–21]. It has been shown that the concentration of milk CLA is as result of interaction between the diet composition and fatty acid profile of diet supplement. This complex interaction greatly influences greatly the biohydrogenation of supplemented fat in rumen and the formation of CLA. For example, Holstein cow fed with high concentrate and forage at the ratio of 65:35 along with 5 g/100 g dry matter of sunflower oil, 5 g/100 g linseed oil or 2.5 g/100 g fish oil drained out greater CLA in milk as compared to control without fat supplementation. The cow fed on sunflower oil drained out greater total CLA (8.3 g/day vs. 4.0 g/day) as compared to feed consist of fish oil while linseed oil feeding results in 6.9 g/day of total CLA. The cis9, trans11-CLA (0.22 g/100 g total fatty acids) was higher in case of feeding on sunflower oil compared to linseed oil (0.13 g/100 g) and fish oil (0.06 g/100 g) [22]. Another study conducted to evaluate the effects of sunflower oil in dairy rations for vaccenic (trans-11-18:1) and rumenic acids (cis-9, trans-11-18:2) production in milk, the animal were fed with forage and concentrate of barley/alfalfa/hay barley-alfalfa-hay silage and corn/barley grain. They reported that there was linear increase in total trans-18:1 (5.2, 9.1, 14.1, and 21.3%) and total CLA (0.7, 1.9, 2.4, and 3.9%), respectively. The rumenic acid concentration also increased in linear pattern from 0.43, 1.5, 1.9, and 3.4% for 10 days feeding period and 0.42, 2.15, 2.09, and 2.78% for 38 days feeding period, respectively. Rumenic acid increased from 66 to 85% using sunflower, linseed and fish oil supplement in cow's feed. CLA enhancement of 4.5-fold by feeding 3% sunflower, oil/fish oil appears to be most promising in trans-11-18:1 and cis-9, trans-11-18:2. While total saturated fatty acids declined to 18%. A good and healthy composition of fatty acids including 4% vaccenic and 2% rumenic acids was achieved by feeding 3% sunflower oil and 0.5% fish oil in animal diet dry matter [23, 24]. Bell et al. adopted three dietary strategies to enhance the flow of CLA in cow milk. The Holstein cows were fed for 2 weeks with control diet of forage and dry matter while 6%, monensin, safflower and safflower oil as experimental diet. The cis-9, trans-11 CLA in milk increased from 0.45 to 5.15% of total fatty acids for control or experimental diet. Furthermore, the addition of vitamin E supplementation resulted in retained the CLA content in milk of cow [25]. The high corn and corn silage dietary feeding strategy was also observed in increase of cow milk from 3.8 and 3.9 mg/g total fatty acids, respectively. The Alfalfa hay and concentrates replacing all pasture by one-third, two-thirds resulted in increase of milk CLA to 8.9, 14.3, and 22.1 mg/g. Grazing pasture only led to 500% more CLA as compared to feeding on typical dairy diets. Gairn with alfalfa with fish oil and monensin supplement resulted in 6.8 mg/g of milk total fatty acids [14]. Beside fatty acids dietary modification, other diet components modification also resulted in increase the concentration of cow milk CLA. For example, studies of Morales et al. have shown that tannin diet contents can modify the milk CLA concentration. The tannin supplementation in feeding cow diet cow feed affects the microfloura of rumen resulting in unsaturated fatty acids biohydrogenation and hence influencing linolenic acid (c9, c12, c15–18:3), vaccenic acid (t11–18:1) and rumenic acid (c9, t11–18:2) [26]. Tyagi studied the effects of green fodder feeding on CLA in milk fat of buffaloes. There was and reported that there was

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Feed type	Feed specialty	CLA (control)	CLA (treatment)	Reference
Grass hay, concentrates	Fish oil	0.72 mg/g	2.83 mg/g	[30]
Grass hay, concentrates	Sunflower oil and wheat starch	0.72 mg/g	1.33 mg/g	[30]
Hay forage, concentrate	CLA (38%) + EPA + DHA 36.5% and humic-mineral carrier	2.33 mg/g	2.78 mg/g	[31]
Egyptian clover, sorghum forage	Rice straw	0.433 mg/g	1.0.4 mg/g	[32]
Egyptian clover, sorghum forage	*Pleurotus ostreatus*	0.27 mg/g	0.80 mg/g	[32]
Corn silage, 27.7% dietary starch	Fish oil (0.80%)	0.48 mg/g fat	0.76 mg/g fat	[33]
Forage, concentrate, palm oil (300 g/day)	Linseed oil (300 g/day)	0.82 mg/g fat	1.90 mg/g fat	[34]
Forage, concentrate, palm oil (300 g/day)	Top dressed whole linseed (688 g/day)	0.82 mg/g fat	2.05 mg/g fat	[34]
Concentrate	Pasture and extruded soybeans	15.4 percentage FA	24.2 percentage FA	[35]
Alfalfa, corn silage, concentrate	Fish oil (2%)	2.86 mg/g fat	3.14 mg/g fat	[36]
Alfalfa, corn silage, concentrate	Canola oil + fish oil (1:1)	2.86 mg/g fat	3.32 mg/g fat	[36]
Alfalfa, corn silage, concentrate	Canola oil (2%)	2.86 mg/g fat	3.16 mg/g fat	[36]
Concentrate, maize ground, soybean, cane molasses, alfalfa	Whole cottonseed	7.59 percentage FA	9.36 percentage FA	[22]
Alfalfa hay forage	Sunflower oil	0.65 g/100 g fatty acids	0.80 g/100 g fatty acids	[37]
Alfalfa hay forage	Hydrogenated palm oil	0.65 g/100 g fatty acids	0.71 g/100 g fatty acids	[37]
Indoor concentrate	Pasture	4.3 mg/g fat	6.80 mg/g fat	[38]
Grass forage	Sunflower oil (255 g/day)	1.76 mg/g fat	1.87 mg/g fat	[39]
Grass forage	Sunflower oil: fish oil (255:52.5 g/day)	1.76 mg/g fat	2.36 mg/g fat	[39]
Grass forage	Fish oil (105 g/day)	1.76 mg/g fat	2.16 mg/g fat	[39]
Animal fat (400 g)	Fish oil: sunflower oil (100 g:300 g)	2.02 mg/g fat	3.41 mg/g fat	[40]
Typical indoor concentrate	Pasture + sunflower oil (100 g/ kg.day)	0.46 mg/g fat	2.22 mg/g fat	[41]
Wheat straw, concentrate	Sunflower seed supplemented (11.2%)	0.54 mg/g fat	2.0 mg/g fat	[23]

Feed type	Feed specialty	CLA (control)	CLA (treatment)	Reference
Corn silage-based rations	Fish oil (45 g) + sunflower oil (45 g)	0.50 mg/g fat	3.47 mg/g fat	[42]
Hay supplemented with tallow	Fresh forage supplemented with tallow	0.93 mg/g fat	1.07 mg/g fat	[43]
Hay supplemented with tallow	Fresh forage + ground solin seed	0.93 mg/g fat	1.30 mg/g fat	[43]
Concentrate (50%), corn silage (25%), alfalfa hay (25%)	Fish oil (0.5%)	0.33 mg/g fat	0.47 mg/g fat	[44]
Concentrate (50%), corn silage (25%), alfalfa hay (25%)	Soybean oil (2.5%)	0.33 mg/g fat	0.79 mg/g fat	[44]
Concentrate (50%), corn silage (25%), alfalfa hay (25%)	Fish oil: soybean oil (0.5%:2%)	0.33 mg/g fat	1.39 mg/g fat	[44]
Grass silage	Fish oil	0.2–0.6 mg/g fat	1.5–2.7 mg/g fat	[45]
Grass silage	Fish oil	0.2–0.6 mg/g fat	1.5–2.7 mg/g fat	[45]
Forage and grain (TMR)	Pasture feeding	3.8 g/100 g fatty acids	22.1 g/100 g fatty acids	[14]
Dry matter with blood meal, feather meal and corn gluten	Sunflower oil (53 g/kg)	3.55 g/100 g fatty acids	24.4 g/100 g fatty acids	[46]
Dry matter with blood meal, feather meal and corn gluten	Linseed oil (53 g/kg)	3.55 g/100 g fatty acids	16.7 g/100 g fatty acids	[46]
Dry matter with blood meal, feather meal and corn gluten	Peanut oil (53 g/kg)	3.55 g/100 g fatty acids	13.3 g/100 g fatty acids	[46]
DM + 4% calcium salts	Canola oil	3.5 g/100 g fatty acids	13.0 g/100 g fatty acids	[47]
DM + 4% calcium salts	Soybean oil	3.5 g/100 g fatty acids	22.0 g/100 g fatty acids	[47]
DM + 4% calcium salts	Linseed oil	3.5 g/100 g fatty acids	19.0 g/100 g fatty acids	[47]

CLA represents two major CLA isomers of C18:2 cis-9, trans-11 and trans-10, cis-12 isomer, TMR: total mixed ration, DM: dry matter, FA: fatty acid.

Table 1. Effect of feed modification on CLA content in bovine's milk.

no change in milk composition of buffaloes with respect to dietary treatments while 310% increase in CLA contents were increased was observed by feeding buffalo on green fodder [27]. The starch diet containing high proportions of polyunsaturated fatty acid promotes shifts in biohydrogenation mechanism, which results in major intermediate trans isomers [28, 29].

Griinari et al. made an attempt by modifying the endogenous activity of D9-desaturase, which involves in synthesis of CLA from trans-11 18:1 in ruminal biohydrogenation [12]. They observed that infusion of trans-11 18:1 resulted in a 31% increase of concentration of cis-9, trans-11 CLA in milk fat. While induction of D9-desaturase inhibitor in cow abdomen resulted in a 45% decrease of 45% in the concentration of CLA. Overall, they concluded that endogenous synthesis of CLA is the primary source of milk CLA in ruminants.

1.4. Bio-fortified CLA in bovine's meat

Recent studies on different feed resources and their influence on meat quality in the term of CLA from small ruminants showed that CLA content in meat would be increased due to chopped cactus cladodes feeding to animals. The oil supplementation in all forms of safflower soybean, sunflower, linseed and fish oil results in enhancement of CLA contents in meat of small as well as large ruminants. Furthermore, reducing anti-nutritional components in above oil sources leads to more enhancements in CLA content of meat in all ruminants [48–54]. For example, a very recent study by Fiorentini et al. reported that feeding palm oil, linseed oil, soybean grain or protected fat result in increased the meat CLA contents from 0.29 to 0.67 mg/100-gram fatty acid, respectively in Nellore steers [53]. While feeding 4.5% linseed, sunflower, or soybean enhances meat CLA content to 0.47, 0.52 and 0.54 mg/100 g fatty in Holstein Friesian bulls. Similar CLA enhancement was observed when Nellore steer were fed with cottonseed at different proportion to dry matter. However, the reducing anti-nutritional contents of linseed, soybean or sunflower, and so on further led to enhance the CLA contents from 0.73 mg/100 g to 0.91 mg/100 g fatty acid in meat [55]. Joele et al. reported that 11% supplementation of coconutor 15% palm cake enhanced 7.98 and 4.98% of CLA contents, respectively, in buffalo Red Norte meet [52]. Fish oil supplementation in concentrate-based diet of Charolais steer results in enhancement of meat CLA content to 0.57% to total fatty acids [56]. Similarly, pasture grazing of small as well as large ruminants enhances the CLA content of meat. More notably, pasture grazing leads to CLA substantial increase as a proportion of total fatty acids and is more available in the form of edible fat as compared to the CLA concentration present in raw meat. This pasturing strategy also leads to reduced total fat contents in raw meat as well as product. On the other shrubs that are rich in vitamin E, protect myoglobin from oxidation and grazing saltbush (*Atriplex* spp.), preserves lamb meat color stability, while linoleic acid contents may increase in meat fat by adding olive cake silage in ewe or lamb diets, respectively. Grazing on some novel pasture species, such as *Cichorium intybus*, *Chrisantemum coronarium*, and *Galium verum* enhanced the appearance of terpenes in goat and sheep meat. Although the dietary factors contribute significantly in the increase of CLA content in milk and meat but only marginal increases in meat is observed as compared to milk. The possible mechanisms and synthesis pathway of CLA may be different according to organ site. Furthermore, other related factors regulating the synthesis of CLA in the rumen muscles and mammary glands are poorly understood [57, 58]. **Table 2** shows the different strategies with increase CLA contents in meat of bovine.

1.5. Bio-fortified CLA milk's cheese and butter

Most of the studies show that the milk processing and cooking do not influence the CLA concentration in milk by products like such as cheese, butter, and ice cream, and so on. The cheese

Breed	CLA enhancing diet	CLA content	References
Nellore steers	Palm oil	0.29 mg/g fat	[53]
Nellore steers	Linseed oil	0.67 mg/g fat	[53]
Nellore steers	Protected fat	0.39 mg/g fat	[53]
Nellore steers	Soybean grains	0.37 mg/g fat	[53]
Steers	Pasture and extruded soybeans	25.0 g/100 g fatty acids	[35]
Rubia Gallega calves	4.5% Linseed	0.47 mg/g fat	[59]
Rubia Gallega calves	4.5% Sunflower	0.52 mg/g fat	[59]
Rubia Gallega calves	4.5% Soybean	0.54 mg/g fat	[59]
Nellore steers	Cottonseed (14.35 kg/100 kg DM)	0.28 mg/g fat	[48]
Nellore steers	Cottonseed (27.51 kg/100 kg DM)	0.29 mg/g fat	[48]
Nellore steers	Cottonseed (34.09 kg/100 kg DM)	0.24 mg/g fat	[48]
Yearling steers	Flax seed oil	0.76 mg/g fat	[49]
Yearling steers	Sunflower seed oil	0.85 mg/g fat	[49]
Yearling steers	Flax seed oil	0.79 mg/g fat	[49]
Yearling steers	Sunflower seed oil	0.86 mg/g fat	[49]
Charolais × Saler steers	Extruded linseed 4%	0.72 mg/g fat	[55]
Charolais cows	Extruded linseed 4%	0.40 mg/g fat	[55]
Holstein cows	Extruded linseed 4%	0.99 mg/g fat	[55]
Charolais bulls	Extruded linseed 4%	0.91 mg/g fat	[55]
German Holstein, bulls	Pasture	17 g/100 g fatty acids	[60]
German Simmental, bulls	Pasture	12 g/100 g fatty acids	[60]
Wagyu x Limousin, steers	whole concentrate	0.12 mg/g fat	[5]
Charolais steers	Grass silage	35 g/100 g fatty acids	[61]
Holstein calves	Megalac	15·9 g/100 g fatty acids	[62]
Holstein calves	Protected lipid supplement	14·5 g/100 g fatty acids	[62]
Holstein calves	Protected lipid supplement	10.1 g/100 g fatty acids	[62]
Angus × Hereford	Finishing diet + soy oil	0.28 mg/g fat	[63]
Limousin, steers	Sunflower oil	134 g/100 g fatty acids	[64]
Angus steers	Concentrate + soy oil (6%)	0.34 mg/g fat	[65]
Angus steers	Concentrate + extruded soybean	0.73 mg/g fat	[66]
Angus crossbred steers	Whole pasture	1.5 mg/g fat	[67]
Charolais steers	Grass based + concentrate	1.1 mg/g fat	[68]
Wagyu crossbred	Barley-based diet	1.7 mg/FA	[69]
Charolais steers	Grass silage whole linseed	36 g/100 g fatty acids	[56]

Breed	CLA enhancing diet	CLA content	References
Charolais steers	Concentrate based + linseed	0.80 mg/g fat	[56]
Holstein claves	53 g/kg Sunflower oil	24.4 g/100 g fatty acids	[46]

CLA represents two major CLA isomers of C18:2 cis-9, trans-11 and trans-10, cis-12 isomer, TMR: total mixed ration, DM: dry matter, FA: fatty acid.

Table 2. Effect of diet modification on CLA content in bovine's meat.

prepared from milk produced by diet supplementing with soybean, extruded soybean, soybean, and so on in cows, soybean oils, extruded soybean, olive oil and palm oils, linseed and extruded linseed, and flaxseed meal, flaxseed oil, castor oil and soybean, and so on in goats resulted in stable CLA content [31, 70–72]. For example, the milk cheese prepared using milk from cows fed extruded soybeans and cottonseed shows the same concentration of CLA in cheese as it was present in milk [73]. Fish oil supplement in all ruminants also resulted shows in enhancement of CLA content cheese from milk used for cheese preparation. Furthermore, the CLA enhancement effects were more predominant when starter culture was used in cheese manufacturing process [74, 75]. The pasture grazing strategy is also significant to enhance the CLA contents in cheese and butter. Other reports have shown that the quality and composition of sheep and goat milk influenced by farming and feeding systems while comparing three feeding systems based on natural pasture in the plain, on mountains and on hills for goats. Thus, milk yield was shown to be lower to some extent on mountain pasture while percentages of PUFA, protein and fat contents were high. Therefore, the terpenes were more abundant in goat milk. On the other hand, milk was richer in CLA at an early stage of natural pasture grazing. Simultaneously, the milk products like cheese, butter and ice cream prepared from goat milk produced by feeding on diet enriched with castor, sesame and faveleira vegetable oils showed enhance CLA content in these products [72, 76, 77]. **Table 3** shows the summarized results of different dietary manipulation strategies to enhance CLA contents in cheeses and butters.

1.6. Enrichment of CLA

1.6.1. Commercial/synthetic CLA

The low percentage rate of CLA conversion by ruminants was accounting as very small in total percentage of fat and oil as dietary sources. The highly bioactive importance of CLA derived the focus to develop commercial CLA. Several methods were developed by using a series of acids and bases reactions to convert polyunsaturated oils to CLA. The earlier attempt to produce commercial CLA resulted in unnatural ratios of CLA isomers. The first successful attempt to develop drying oil from linolenic acid oils using monohydric and polyhydric solution with addition of numerous alkalis as catalysts. Later, another development, a modification was made by the use of water and steam to achieve a required temperature to conjugate unsaturated fatty acids. Moreover, the successive addition of mineral acid led to the successful development of free conjugated fatty acids production method [80, 81]. Christie et al. first time reported to develop a CLA product by alkaline water isomerization (KOH and NaOH catalyst at temperature > 280°C) which have all 8, 10: 9, 11: 10, 12: 11,13 trans and cis CLA

CLA enhancing feed	CLA content	Reference
Pasture + extruded soybean	1.4 g/100 g fatty acid	[16]
Whole pasture	1.5 g/100 g fatty acid	[17]
Sunflower seed (11.2%)	400 (percentage increase)	[23]
Pasture	8.12 mg/100 g cheese	[38]
Pasture, 100 g/kg of sunflower oil/d	1.93 g/100 g fatty acid	[41]
Grass-fed animals	64·19 mg/100 g cheese	[74]
Fish oil (0.75% of dry matter)	2.41 g/100 g fatty acid	[75]
Natural spring pasture	0.76 g/100 g fatty acid	[78]
Pasture grazing	1.45 g/100 g fatty acid	[79]

CLA represents two major CLA isomers of C18:2 cis-9, trans-11 and trans-10, cis-12 isomer, TMR: total mixed ration, DM: dry matter, FA: fatty acid.

Table 3. Effect of feed modification on CLA content in milk's cheese.

with unknown geometric position. However, the two major peaks in that commercial CLA mixture were assumed the isomers c9, t11 and t10, c12. Later on, further research advances turned out to achieve the possible isomerization with specific isomers ratios [82]. Propylene glycol isomerization was another method to produce CLA from monounsaturated and polyunsaturated oil fatty acids. The propylene glycol was used with KOH as a catalyst instead of ethylene alcohol for consumer safety reasons. Later, hexane was also used instead of ethylene/propylene glycol to facilitate the purification of required CLA isomers. Thus, the mixture of CLA isomers was marketed as free acids instead of n-3 concentrates [83]. Isomerization of mono-alkyl ester is a relatively recent effective quantitative method to produce CLA isomers by isomerizing methyl and ethyl esters of linolenic acids in presence of very small quantity of catalyst and virtually no solvent. Besides, thermal sigma tropic rearrangement by preceding the reaction below 100°C results in CLA isomer production.

1.6.2. Microbial CLA

Regarding the potential health effects, safe isomers selective processes are investigated for CLA production. Among these, bioprocess by microbial use is the potential method for production of CLA. Initially, the bacteria were divided into group A and group B depending on the type of reactions and the products as result of biohydrogenation. The bacteria of group A were able to hydrogenate linolenic acid and α-linolenic acid end product t11- C18:1. The group B bacteria were able to convert t11-C18:1 to end product stearic acid [84]. Besides ruminant bacteria, some bacterial strains from human/animal intestinal membrane, dairy products origins were isolated for CLA production. *Lactobacillus reuteri*, *Lactobacillus plantarum, Lactobacillus lrhamnosus, Lactobacillus brevis, Lactococcus lactis, Lactobacillus acidophilus, Propionibaterium freudenrehichii, Bifidobacterium, Streptococcus*, are capable for CLA production. Potential CLA producing strains such as bifidobacteria *Bifidobacterium*, lactobacilli, and pediococci have been selective for CLA production. The increasing interest in bifidobacteria as the natural inhabitant and

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useful in conversion of CLA fatty acid has been derived the attention of microbiologists [70]. Coakley et al. reported for the first time that the strains collection of bifidobacteria, lactobacilli, and pediococci were capable to convert LA into CLA isomers [41]. They sorted out the nine strains of bifidobacteria, which convert c9, t11 CLA from MRS culture supplemented with linoleic acids. Among these nine strains, *Bifidobacterium breve* is strong bio convertor of LA to 66% to c9, t11 CLA and 6.2% to t9, t11 CLA in only the culture supernatant [41, 85, 86]. Several studies have also reported the pivotal role of lactic acid bacteria in production of CLA from LA when grown on MRS, skim milk and cheddar cheese [75, 87, 88]. These bacteria have enzymatic conversion of LA to CLA by linoleate isomerase in their cell wall. *Lactobacillus reuteri* PYR8, *Clostridium sporogenes* and *Propionibacterium acnes* were reported to have putative polyunsaturated fatty acid PUFA linoleate isomerase function [89–91]. Several efforts were made to produce the CLA using *E. coli*, but none was capable to produce CLA. However, *Lactobacillus plantarum* AKU 1009a were found to produce t11-CLA, t10, c12-CLA and t9, t11-CLA with less known enzymatic action [85, 92, 93]. Later, the genetic mutation in linoleate isomerase enzyme machinery of strain plantrum AKU 1009a led to develop *E. coli* as catalysts to produce significant t9, t11-CLA with c9, t11-CLA [92, 94].

2. Bioavailability and clinical manifestation of enhanced CLA

The general availability of CLA from food sources has been summarized in **Table 4**. Recently, the manipulation of fatty acid profile of milk, meat, cheese and butter has been shown to confer beneficial impacts on human health. There are very few experimental studies that indicate the kinetic behavior of dietary CLA from naturally enhanced diary and meat products. The studies on kinetic behavior of polyunsaturated fatty acids (PUFAs) showed that the bioavailability and disposition of PUFA could be altered in some biologic fluids after the intake of enriched PUFA-rich food products. For example, previous studies with high-fat diet and low-fat diet containing 1% rumenic acid show higher and lower bioavailability of CLA content respectively which in return was more bioactive in reducing hyperinsulinemia [95]. The experimental rats group fed on CLA enriched butter had sixfold higher CLA content in liver compared to that of the control group, without having difference in dietary intake. The naturally enriched CLA butter consumption leads to increase the c9, t11-CLA serum concentrations and will as other PUFA without influencing the cholesterol content and blood TG [96, 97]. de Almeida et al. showed that the animals with synthetic CLA supplement diet have lower level of hyperinsulinemia, hyperglycemia and inflammatory proteins in retroperitoneal adipose tissue with high level of plasma HDL cholesterol [95]. While, other studies in which synthetic CLA mixture was used, report unhealthy effect of synthetic CLA as compared to that of naturally enriched CLA products, several authors have reported that using synthetic CLA in animal developed insulin resistance, hyperinsulinemia [97–98]. Thus, it is important to differentiate the bioavailability of CLA from different production sources, which ultimately determine the bio-functionalities of CLA from the health point of view. As commercial commercially produced CLA has predominant with mixture of 10-, 9,11-, 10,12-, and 11,13-isomers while natural CLA has 80–90% of 9,11-isomer. This difference in isomers composition is a major determinant of biological activities of CLA in diet and thus source. Furthermore,

Food Product	Fat (g)/serving	CLA(mg)/g fat	CLA (mg)/serving
Milk and milk products			
250 mL of milk (2% fat)	5.1	4.1	20.9
125 mL of condensed milk	14.1	7.0	98.7
250 mL of fermented buttermilk (2%)	5.2	5.4	28.1
175 g of plain yogurt (2–4% fat)	4.9	4.8	23.5
175 g of yogurt (1–2% fat)	2.7	4.4	11.9
50 g of processed cheese	12.3	5.0	61.5
50 g of cheddar cheese	16.6	4.1	68.1
15 mL of butter	11.7	4.7	55.0
125 mL cream	17.1	4.6	78.7
125 mL ice cream	11.8	3.6	42.5
Meat and meat products			
90 g of lamb meat	12.7	5.8	73.7
90 g of ground beef	12.3	4.3	52.9
90 g of veal	6.1	2.7	16.5
90 g of fresh ground turkey	6.5	2.6	16.9
90 g of chicken	12.1	0.9	10.9
90 g of pork	13.8	0.6	8.3
Large egg yolk	5.3	0.6	3.2
90 g of salmon	5.7	0.3	1.7
Vegetable oils			
15 mL of safflower oil	13.8	0.7	9.7
15 mL of sunflower oil	13.8	0.4	5.5

Table 4. Availability of CLA content from food products.

the bioavailability of CLA is measured either the total contents of CLA in blood circulation after ingestion or the total contents of CLA disposition in the liver, mammary fat, peritoneal fat and plasma. The composition of CLA isomers mixture can also influence the incorporation and bioavailability of CLA to tissue and organs. To date, most of the human studies evaluated by only blood measurements, which are different from animal models regarding the bioavailability and distribution modes of CLA according organs, model of dietary supplementation or enrichment [97, 99].

A very recent study by Rodriguez-Alcala et al. (2015) was conducted on oral absorption and disposition of CLA isomers from naturally enriched goat milk cheese conducted to evaluate the bioavailability of CLA. The oral doses of 153 mg vaccenic acid, 46 mg rumenic acid were fed to rats on kilogram body weight basis. The maximum concentration value of vaccenic acid and rumenic acid were 18.76 and 63.42 μg/mL in plasma and 7.60 and 26.66 μg/g in erythrocytes, respectively, suggesting that CLA-enriched dairy fat produced by dietary manipulation may be well absorbed for better health effects in humans as compared to synthetic CLA [9]. It was observed that the rats fed on butter selectively absorbed more CLA. The accumulation was fourfold more higher for total CLA in mammary gland and tissues compared to rats fed on synthetic free fatty acids while taking the same dietary intake [100]. The studies on transfer efficiency of a CLA showed that bioavailability of the 9, 11-CLA isomer was twofold more as compared to 10, 12-CLA isomer [97]. While feeding of commercial CLA to pigs showed that c9, t11-isomer preferentially incorporate to liver and c11, t13-isomer into heart. Another study showed that the serum bioavailability of synthetic CLA was low when was inducted to pig feed for 15 days dietary treatments. The results were in increase of 10–30-fold mono-CLA isomers (c9, t11-c15-18:3 + c9-t13-c15-18:3) in the heart, kidneys and liver indicating the trend of incorporation of conjugated CLA isomers, which depends on the structure and source of the conjugated fatty acids [47]. The rats fed during the mammary gland development period show that naturally enriched CLA was more potent in reducing 22% epithelial mass, 30% terminal end bud density, 30% proliferation and 53% tumor yield of mammary glands. The consumers have the options for choosing CLA to increase CLA intake from synthetic CLA pill or produced by diet modification in dairy and meat products. The relative health value benefits of CLA from ruminant, microbes, and synthetic sources are uncertain regarding the bioavailability and functional disposition in human [100, 101].

3. Conclusion

Enhanced or enriched dairy fats produced by dietary manipulation are considered well-absorbed and bioactive source of essential fatty acids with beneficial health effects in humans. However, most of the studies related to effect of CLA on human health were conducted on commercial CLA that was differentially available and dispersed in body organs and tissues and hence have different health effects. Further, there are not conclusive studies on the moderation of CLA and its dietary source on average human intake. The extrapolation from rat animal models to human intake must be taken with cautions due to difference in dietary requirements. To meet recommended intake of CLA, several efforts have been made to enhance the level of CLA naturally and natural enhanced CLA in dairy food is more health effective. The manipulation of dairy ration type and composition seems to be one of the most suitable strategies for enhancement of CLA in enriched dairy products. Manipulation of the animal's diet can result in up to 8- to 10-fold increase in the concentration of CLA in milk. As consumers become more conscious of the link between diet and health, milk designed to have enhanced levels of CLA may provide new market opportunities for milk and milk products such as butter and cheese.

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Conflict of interest

All authors declare no conflict of interest for any purpose.

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