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Review article

Potential strategies to enhance conjugated linoleic acid content of milk and dairy products: A review

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ABSTRACT

Conjugated linoleic acid (CLA) is a general term for all the geometric and positional isomers of linoleic acid. The cis-9, trans-11 CLA and trans-10 cis-12 CLA are considered to be the most abundant and essential isomers associated with health benefits. Though milk and dairy products are considered to be the major sources of CLA, the CLA content found in regular milk and dairy products is relatively low for effective health benefits in human beings. Thus, for effective health benefits, increasing the concentration of CLA in milk and dairy products is beneficial. Dietary supplementation with PUFA-rich lipid sources such as oilseeds and/or vegetable oils, fish meal, fish oil and microalgae and grass-based feeding can enhance the content of CLA in milk and dairy products. Application of CLA-producing bacterial strains during the fermentation process and ripening/storage are considered as potential strategies for enhancing the CLA content of fermented dairy products. Alternatively, the CLA content of milk and dairy products can be improved using genetic factor. In this paper, the latest scientific studies regarding CLA enrichment in milk and dairy products are reviewed, giving an overview of the effectiveness of the different CLA enrichment strategies and their combinations.

1. Introduction

Conjugated linoleic acid is a collective term that represents for all the CLA isomers, defined by two conjugated double bonds in different geometric (i.e., *cis* or *trans*) and positional locations. Up to the present time, there are about 28 known isomers of CLA [1]. Of those, *cis*-9, *trans*-11 CLA and *trans*-10 *cis*-12 CLA are the most abundant isomers representing approximately 85 % and 10 %, respectively, of all the naturally occurring CLA isomers [2]. The *cis*-9, *trans*-11 CLA and *trans*-10 *cis*-12 CLA are also considered to be the most essential isomers associated with health benefits. A number of human trials and meta analysis reported positive correlation between CLA supplementation and reduction in cardiovascular diseases. For instance, the systematic review and meta analysis by Namazi et al. [3] indicated that CLA supplementation reduced body weight and body fat mass, and improved the body mass index. In addition, CLA played an important role in reducing abdominal obesity [3] which is linked to Type 2 diabetes, cardiovascular disease and metabolic syndrome [4,5]. Likewise, other systematic reviews and meta analysis [6,7] have shown the anti-inflammatory roles of CLA supplementation. CLA supplementation can also play a vital role in protecting blood pressure and different types of cancers [8,9].

Initially, CLA was discovered in ruminants and the main dietary sources of CLA are ruminant driven food products [10]. CLA isomers are synthesized in the rumen as intermediates in the biohydrogenation of dietary linoleic acid to stearic acid [11]. In addition, the *cis*-9, *trans*-11 CLA can be synthesized from vaccenic acid (another biohydrogenation intermediate of linoleic acid and linolenic

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acid) in the mammalian tissue by delta-9 desaturase [12]. Consequently, this is considered as an essential alternative source of *cis*-9, *trans*-11 CLA.

Despite dairy-driven food products are considered as the major sources of CLA, the concentrations of CLA in dairy foods are relatively low, less than 10 mg CLA/g fat [13]. In the USA, it was estimated that the average intakes of CLA for men and women were 176 mg/day and 104 mg/day, respectively [14]. In the UK, on the other hand, the average intake of CLA was estimated to be 97.5 mg/day [13]. However, if the health benefits of CLA are found to occur in humans, increasing the concentration of CLA in foods may be beneficial. According to Huth et al. [15], about 0.42 g of CLA/day is required for effective anticarcinogenic effects in human beings. Consequently, in recent times, increasing the concentration of CLA in diary-driven foods has become the primary focus of researchers to increase the dietary intake of CLA. The CLA content of milk and dairy products can be enhanced using different strategies. In the present review, different strategies used to enhance the CLA content of milk and dairy products have been discussed. The strategies used to enhance CLA content may involve the addition of polyunsaturated fatty acids (PUFA)-rich dietary lipid sources to the diet of the ruminants [16,17], application of some strains of bacteria such as bifidobacteria and lactic acid bacteria during the fermentation of dairy products [18,19] and/or genetic manipulation (selective breeding program) [20]. For each strategy, their effects to increase CLA, particularly *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and total CLA in milk and dairy products have been discussed in detail. To the author's knowledge, no recent review is available regarding the various strategies used in increasing the CLA content of milk and dairy products.

2. Dietary enhancement of CLA content in milk and dairy products

Manipulating the diet of ruminants is one approach to modify the fatty acid (FA) profile of milk and dairy products [21]. Nowadays, grazing on pasture and supplementation with various lipid sources containing high amounts of PUFA are the main dietary approaches to enhance CLA content in milk and dairy products [22,23].

2.1. Effectiveness of lipid supplementation in enhancing CLA content of milk and dairy products

Lipid supplementation improves the growth and performance of ruminants due to its noticeable energy contribution [16]. In addition, it has been used as an effective feeding strategy to alter the fat composition of milk and dairy products as a result of its high content of essential fatty acids [21]. Oilseeds and vegetable oils, fish meal and fish oils and microalgae are the major sources of lipid supplementation that have been used in ruminant diets to enhance CLA content in milk and dairy products [16,24].

2.1.1. Oilseeds and/or vegetable oils

Linseed oil, soybean oil, sunflower oil, rubber seed oil and hemp seed are among the widely used seed and/or vegetable oils in ruminant nutrition. Those lipid supplementary sources are rich in PUFA and their incorporation into the diet of ruminants is a vital feeding strategy to improve the contents of CLA in milk and dairy products [25,26]. This feeding strategy is also more important when ruminants mainly depend on feedstuffs poor in PUFA content [23].

The differences reported in CLA contents of milk and dairy products as a result of oilseeds and/or vegetable oils supplementation are summarized in Table 1. Dietary supplementation studies by Bu et al. [22] and Pi et al. [23] investigated the effects of soybean oil, rubber seed oil, flaxseed oil and their mixtures on the fatty acids composition of cows' milk, and reported higher contents of *cis*-9, *trans*-11 CLA (0.54 g/100 g versus 3.25 g/100 g) and *trans*-10, *cis*-12 CLA (0.04 g/100 g versus 0.09 g/100 g) in milk obtained from the

Table 1
CLA contents of milk and dairy products reported in different studies as a result of PUFA-rich oilseeds and/or vegetable oils supplementation to ruminants.

CLA	Maximum differences reported (g/100 FA)	Species	Product	References
cis-9, *trans*-11 CLA	1.02–2.73	Bovine	Milk	[23,131]
	1.14–1.46	Ovine	Milk	[16,25]
	0.35–1.57	Caprine	Milk	[32]
	0.10–3.14	Bovine	Cheese	[33,34]
	1.3	Ovine	Cheese	[36]
	0.51	Caprine	Cheese	[38]
	0.81–1.13	Caprine	Yogurt	[132]
trans-10, *cis*-12 CLA	0.05–0.19	Bovine	Milk	[22,24]
	0.02–0.11	Ovine	Milk	[25,133]
	0.001–0.004	Caprine	Milk	[31]
	0.06–0.07	Bovine	Cheese	[34,35]
	0.10	Ovine	Cheese	[36]
	0.01	Caprine	Yogurt	[132]
Total-CLA	0.05	Ovine	Yogurt	[134]
	1.4	Ovine	Cheese	[36]
	1.17	Bovine	Cheese	[34]
	0.34–0.49	Bovine	Milk	[28,29]
	1.16–1.77	Ovine	Milk	[25]
	1.22–3.77	Caprine	Milk	[31]

supplemented cows. In addition to CLA, alpha-linolenic acid (ALA) content, another health beneficial n-3 FA, increased by 86 % in the milk of supplemented lactating cows with a concomitant reduction in the concentration of saturated fatty acids (SFA). Other studies [27,28] carried out on linseed supplementation, at its different forms (linseed oil, whole crude linseed, extruded linseed), to lactating dairy cows, similarly, showed 1.8–2.5 fold increase in total CLA content. According to those studies, supplementation could also result in 0.49–0.67, 0.03–0.05 and 0.03–0.05 g/100 g FA increases in ALA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively, with a contemporary reduction in SFA (11.45–12.20 g/100 g FA). However, the values observed for CLA content in milk obtained from supplemented cows varied depending on the forms of linseed supplementation. For instance, cows supplemented with extruded linseed had higher CLA content compared to whole crude linseed [29] which could be explained by an increase in the digestibility of extruded linseed supplements, as extrusion improves diet digestibility [30]. Thanh and Suksombat [24] also used oil mixtures (3 % linseed and fish oils at 1:1, 3 % sunflower and fish oils at 1:1 and 3 % mixture of linseed, sunflower, and fish oils at 1:1:1) as lipid supplementary sources in dairy cows and could produce milk with improved therapeutic properties. Because *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA isomers increased by 39.6–198 % and 700–950 %, respectively, whereas unhealthy fatty acids (SFA) and n-6/n-3 ratio decreased by 14.3–18.2 % and 70.2–80.4 %, respectively, in the milk from supplemented cows.

Although dietary manipulation of FAs in milk is more intensely carried out on dairy cows, several researchers, especially during the last few decades, could also effectively modify the FA profile of milk from sheep and goats by incorporating oilseeds and/or vegetable oils to the diet, primarily to raise the concentrations of health beneficial FAs [25]. In dairy ewes, considerable increases in *cis*-9, *trans*-11 CLA (1.5–4.6 fold), *trans*-10, *cis*-12 CLA (1.2 fold) and total CLA (1.0–1.6 fold) were found when linseed oil or hemp seed were included in the diet [16,25]. What's more, the authors reported an increase in ALA (1.3–8.2 fold), EPA (1.2–2.2 fold) and DHA (1.2–1.9 fold) with a concomitant reduction in SFA content (1.1–1.3 fold). Similar to the milk from dairy cows and sheep, the *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and total CLA contents of milk from dairy goats raised by 52–298 %, 13–57 % and 161–405 %, respectively as a result of sunflower oil or linseed oil supplementation [31,32], indicating that oilseeds and/or vegetable oils supplementation would be an effective tool in producing CLA enriched milk from ruminants.

Compared to milk, studies performed to investigate the effects of oilseeds and/or vegetable oils supplementation on FA composition of dairy products such as cheese and yogurt are limited heretofore. Despite limited studies carried out so far, the fatty acid profile of the products could be improved by supplementation. For instance, inclusion of sunflower oil or olive oil in the diet of dairy cows noticeably increased the contents of *cis*-9, *trans*-11 CLA (from 0.10 to 3.14 g/100 g FA), *trans*-10, *cis*-12 CLA (from 0.05 to 0.06 g/100 g FA) and total CLA (1.17 g/100 g FA) in different types of cheeses [33–35]. Likewise, inclusion of sunflower seed in the diet of lactating ewes, raised the *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12-CLA and total CLA contents of cheese by 122 %, 100 % and 190 %, respectively, [36]. Medeiros et al. [37,38] also reported an increase in *cis*-9, *trans*-11 CLA content (+0.51 g/100 g FA) of cheese made from dairy goats fed a diet supplemented with Faveleira oils. Supplementation of canola seed, sunflower seed or linseed oil to lactating dairy goats also raised *cis*-9, *trans*-11 CLA content (1.13 g/100 g FA), *trans*-10, *cis*-12 CLA (0.01 g/100 g FA) and total CLA (0.33 g/100 g FA) [39]. Similarly, supplementation of palm oil also raised the concentration of total in *cis*-9, *trans*-11 CLA by 5.3 % in yogurt produced from ewes' milk. In addition to the CLA, a significant increase in ALA and a concomitant decrease in SFA and n-6/n-3 ratio were observed in all the cheeses and yogurts made from the milk of the different ruminants species supplemented with different oil seeds and/or vegetable oils rich in PUFA.

2.1.2. Fish meal and fish oil

Fish meal and fish oil, because of their high nutritional quality, have been widely used in ruminant nutrition to improve animal performance and product quality [30]. Those supplementary feed sources are high in quality proteins, energy and easily digestible essential amino acids and fatty acids. Moreover, fish meal and fish oil contain considerable amounts of minerals (P and Ca), vitamins (B12, A, D, E, choline and biotin) and trace elements (SeandI) [21]. Similar to oilseeds and vegetable oils, fish meal and fish oil have been used as lipid supplementary sources in manipulating the fatty acid profile of milk and dairy products [40,41]. However, the fatty acid composition of fish meal or fish oil is different from that of oilseeds and vegetable oils as fish meal or fish oil contains large quantities of health-promoting long-chain n-3 fatty acids such as eicosapentaenoic (EPA) acid, docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) [42]. Hence, fish meal or fish oil is primarily used as a dietary supplement source of EPA, DPA and DHA in

Table 2
CLA contents of milk and dairy products reported in different studies as a result of fish meal or fish oil supplementation to ruminants.

CLA	Maximum differences reported (g/100 FA)	Species	Product	References
cis-9, *trans*-11 CLA	1.73–2.25	Bovine	Milk	[43,44]
	1.02–3.80	Ovine	Milk	[47,48]
	0.18–0.73	Caprine	Milk	[50]
	0.10–1.01	Bovine	Cheese	[34,135]
trans-10, *cis*-12 CLA	0.02–0.08			[44,45]
	0.02–0.09	Ovine	Milk	[47,49]
	0.21	Caprine	Milk	[40]
	0.06	Bovine	Cheese	[34]
Total CLA	1.81–2.40	Bovine	Milk	[43,44]
	1.09–3.89	Ovine	Milk	[47,48]
	0.85–3.57	Caprine	Milk	[50,51]
	0.49–1.06	Bovine	Cheese	[34,57]

ruminants to enhance their concentrations in milk and dairy products. However, in addition to the raise in the concentration of those health-beneficial fatty acids, a concomitant increase in the concentration of another health-beneficial fatty acid (CLA), due to rumen biohydrogenation, has also been observed when fish meal or fish oil is incorporated in the diet of ruminants [43].

The maximum differences reported in CLA isomers contents of milk and dairy products, specifically *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and total CLA as a result of fish meal or fish oil supplementation are summarized in Table 2. According to the studies performed by Kupczyński et al. [43] and Bodkowski et al. (2020) [44], inclusion of fish oil into the diet of lactating cows increased the contents of *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and total CLA in milk by 267–362 %. 29–33 % and 254–364 %, respectively. Vafa et al. [45] also investigated the role of fish oil alone at 2 % DM or combined with canola oil at 1 % fish oil and 1 % canola oil on the FA composition of milk obtained from Holstein cows; and reported higher concentrations of *cis*-9, *trans*-11CLA (0.58–0.69 g/100 g of FA) and *trans*-10, *cis*-12 CLA (0.08–0.10 g/100 g FA), when fish oil included in both forms (alone or combined). However, comparing both forms of inclusion, *cis*-9, *trans*-11CLA and *trans*-10, *cis*-12 CLA contents were higher in milk of cows supplemented with fish oil combined with canola oil (1.05 g versus 1.16 g/100 g FA) and (0.13 g versus 0.15 g/100 g FA), respectively. In addition, a considerable increase in the concentrations of CLA isomers (*cis*-9, *trans*-11 CLA, *trans*-10, cis-12 CLA) in milk was observed when fish oil combined with sunflower oil or linseed oil was fed to lactating cows [24]. Moreover, a significant increase in total CLA content (0.50 g versus 3.47 g/100 g FA) has been obtained as a result of fish oil combined with sunflower oil supplementation [46]. In dairy ewes, inclusion of fish oil alone or combined with other plant oils (soybean or sunflower oil) to the diet markedly increased the concentrations of *cis*-9, *trans*-11CLA (1.02–3.8 g/100 FA), *trans*-10, cis-12 CLA (0.02–0.09 g/100 g FA) and total CLA (1.09–3.89 g/100 g FA) in milk [47–49]. Similar to the cases observed in dairy cows, the raise in CLA content varied with the manner of inclusion, in that, CLA content was higher when fish oil combined with soybean or sunflower oil was fed to the lactating ewes. Akin to dairy cows and ewes, dietary supplementation of fish oil alone or combined with other plant oils raised the concentration of CLA in milk from dairy goats. For instance, milk obtained from goats supplemented with fish oil alone, fish oil combined with sunflower oil and fish oil combined with linseed oil raised the concentration of total CLA by 100–406 %, 2167–2372 %, and 1689–1939 %, respectively and *cis*-9, *trans*-11CLA by 103–404 %, 2150–2350 % and 1589–1906 % [50]. Those authors have also reported dose (20 g versus 40 g/day) dependent increase in *cis*-9, *trans*-11 CLA (0.36 versus 0.91). and total CLA (37 versus 1.06) content in goat's milk. Another trial by Toral et al. [52] has also shown 4 fold increase in total CLA when fish oil alone was included in the diet of lactating goats.

The milk of all ruminant species (bovine, ovine and caprine) contains higher concentrations of EPA, DPA and DHA when fish oil alone is included in the diet, whereas higher concentration of CLA has been obtained when fish oil combined with other plant oils (sunflower oil, linseed oil, soybean and canola oil) are incorporated in the diet; indicating that fish oil is rich in long chain n-3 PUFA (≥20 carbon) while sunflower oil, linseed oil, soybean and canola oil are rich in 18 carbon PUFA. In addition, this observation could be supported by the fact that fish oil plays a vital role in inhibiting the rumen biohydrogenation of trans 18:1 (an important precursor for CLA synthesis) to stearic acid (C18:0) [53].

Fish meal is another form of fish-related dietary supplement that can be used in ruminant nutrition primarily to modify the fatty acid profile of animal-driven foods. In dairy cows, incorporation of fish meal alone or combined with extruded soybean to the diet raised the concentrations of *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and total CLA in milk [54]. Based on the findings of those author, supplementation of fish meal alone increased the contents of *cis*-9, *trans*-11 CLA and total CLA by 42 % and 40 % respectively, but not *trans*-10, *cis*-12 CLA, whereas, supplementation of fish meal combined with extruded soybean increased the contents of *cis*-9, *trans*-11CLA, *trans*-10, *cis*-12 CLA and total CLA by 321 %, 400 % and298 %, respectively. Moreover, the *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and total CLA contents obtained from cows supplemented with fish meal combined with extruded soybean were higher than the contents obtained from cows supplemented with extruded soybean alone, indicating that combining fish meal with extruded soybean is more effective to achieve higher CLA content in milk from dairy cows. In lactating dairy ewes, similarly, incorporation of fish meal to the diet raised the concentration of total CLA in colostrum and milk by 22 % and 43 %, respectively [55]. In both ruminant species (bovine and ovine), in addition to CLA, fish meal supplementation raised the concentrations of the health-beneficial long chain n-3 PUFA (EPA, 0.03–0.09 g/100 g FA, and DHA, 0.07–0.28 g/100 g FA) and reduced the SFA content (−1.05 to −7.21 g/100 g FA) and n-6/n-3 ratio ((−0.19 to −0.47), indicating that incorporating fish meal to the diet of ruminants is crucial to produce healthier ruminant driven food products.

Unlike milk, the studies performed to investigate the effect of fish meal or fish oil supplementation on the FA composition of other dairy products, so far, are limited. To the author's observation, there are only a few studies performed [34,56,57] to evaluate the FA composition of cheese made from the milk of cows supplemented with fish oil. According to the results disclosed by those authors, cheeses made from the milk of cows supplemented with fish oil contained higher *cis*-9, *trans*-11 CLA (0.11–0.64 versus 0.21–1.62 g/100 g FA), *trans*-10, *cis*-12 CLA (0.11 versus 0.17 g/100 g FA) and total CLA (0.58–0.99 versus 1.07–2.05) contents compared to that of made from the milk of cows not supplemented with fish oil. In addition to the increase in CLA, those authors also reported an increase in EPA and DHA and a decrease in SFA and n-6/n-3 ratio.

2.1.3. Microalgae

Microalgae, in its different forms (full fatted biomass, defatted biomass and algal oil), can be incorporated into the diet of ruminants [58,59]. Microalgae is rich in long-chain PUFA such as EPA and DHA which are either low or absent in most other ruminant feed sources [58,60]. As a result, those long-chain PUFA are found in a very low amount or absent in milk and dairy products [61]. Therefore, inclusion of microalgae in the diet of ruminants would be an opportunity primarily to increase the quantity of those long-chain PUFA in dairy-driven food products [16,62]. In addition to those health-beneficial long-chain PUFA, its inclusion in the diet of ruminants increased the concentrations of CLA in milk and dairy products [59,63] that is expected to be due to the role it plays in inhibiting the rumen biohydrogenation of trans 11, 18:1 (an important precursor to CLA synthesis) to stearic acid (18:0) [53].

The maximum differences reported in CLA isomers contents of milk and dairy products as a result of microalgae supplementation are summarized in Table 3. Inclusion of full fatted microalgae biomass to the diet of lactating cows raised the concentration of *cis*-9, *trans*-11-CLA ranging from 0.19 to 0.87 g/100 g FA [64–66] and total CLA ranging from 0.66 to 2.25 g/100 g of FA [67]. Moreover, the results of those authors showed a raise in Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) with a concomitant reduction in SFA and n-6/n-3 ratio. Milk obtained from cows fed with algal oil has also shown a 19 % increase in *cis*-9, *trans*-11-CLA and a 100 % increase in *trans*-10, *cis*-12-CLA content [68]. A study by Moate et al. [69] evaluated the effects of algal meal supplementation at different doses (0, 125, 250 and 375 g/cow/day) on FA composition of milk from dairy cows. Accordingly, the authors reported 3–5 fold increase in *cis*-9, *trans*-11-CLA content as the dose increased from 125 to 375 g/cow/day and 2 fold increase in *trans*-10, *cis*-12-CLA when cows were supplemented with 250 or 375 g/cow/day. In addition to the CLA isomers, particularly at the highest dose of inclusion (375 g/cow/day), DHA, Docosapentaenoic acid (DPA) and EPA increased at 23 fold, 7 fold and 2 fold, respectively.

Likewise, supplementation of microalgae positively affected the contents of CLA in sheep and goats' milk [48,51]. In sheep, inclusion of microalgae raised the *cis*-9, *trans*-11-CLA content of milk from 2 to 8 folds [16,70] and doubled the total CLA content [71]. Similarly, inclusion of microalgae in the diet of dairy goats showed 1.3–3 fold increase in *cis*-9, *trans*-11-CLA [66,72,73], 1 fold increase in *trans*-10, *cis*-12CLA [72] and 1–3 fold increase in total CLA [66,72] content in milk. Akin to what could be observed in dairy cows, the concentrations of DHA, EPA and DPA increased in ewes' and goats' milk as a result of algal supplementation.

Investigations performed associated with the influence of microalgae supplementation on the CLA content of dairy products other than milk are limited so far. To the author's observation, there is a study carried out by Till et al. [75] on cheddar-type cheese made from cows supplemented with different doses of microalgae (0, 50, 100 and 150 g/cow/day). According to the findings of the authors, the contents of *cis*-9, *trans*-11-CLA, *trans*-10, *cis*-12-CLA and total CLA increased from 0.10 to 0.27, 0.01 to 0.02 and 0.11 to 0.28 (g/100 g of FA), respectively as the dose of inclusion increased from 50 to 150 g/cow/day.

Overall, inclusion of microalgae in the diet of ruminants either in the form of algal oil, or full-fatted microalgae biomass enhanced the concentrations of CLA and other health beneficial long chain PUFA (DHA, DPA and EPA) in milk and dairy products, showing that such milk and dairy products are more healthy for human consumption.

In general, during lipid sources supplementation in ruminant nutrition, combining oilseeds and vegetable oils with fish oil or microalgae has been found to be an effective feeding strategy to enhance the CLA content of dairy-driven food products even above that attainable on oilseeds and vegetable oils or fish oil and microalgae alone. Thus, this supplementation approach would be promising in the production of milk and dairy products with a better CLA content.

Despite lipid supplementation also play an important role in enhancing CLA content in milk and dairy products, inclusion of lipid source rich in PUFA in the diet of ruminants has often been associated with negative impacts on milk yield and fat content when incorporated at high levels [48,66]. Thus, determining an appropriate level of supplementation to diminish the possible adverse effects, while also acquiring the advantages of CLA enrichment in milk and dairy products can be an active area of future research.

2.2. Effectiveness of grass or pasture based feeding in enhancing CLA content of milk and dairy products

The total FA content of grasses is relatively low and ranges from 20 to 50 g/kg dry matter [76]. Despite grasses contain relatively low FA content, they are considered as the cheapest sources of FA in ruminant nutrition. ALA is the major FA in grasses, representing 50–75 % of the total FAs [77]. Though they are found at low levels, the two main FAs in grasses, next to ALA, are linoleic acid (LA) and palmitic acid (PA) and those three FAs (ALA, LA and PA) comprised about 95 % of the total FA in grasses [78], indicating that grasses are among the potential feed sources for the production of CLA isomers in milk and dairy products as CLA isomers are mainly synthesized from rumen biohydrogenation of ALA and LA [79].

In ruminants, grass-based feeding, either in the form of fresh or conserved (hay and silage), positively affected CLA content in milk and dairy products compared with total mixed ration (TMR) and/or concentrate-based feeding [80,81]. Compared with TMR-based feeding, in dairy cows, grass-based feeding increased the contents of *cis*-9, *trans*-11 CLA (0.99 versus 2.01 g/100 g of FA), *trans*-10, *cis*-12 CLA (0.07 versus 0.08 g/100 g of FA) and total CLA (1.07 versus 2.10 g/100 g of FA) in milk [82]. The total CLA content of milk obtained from ewes raised on grazing pasture was also about 3 fold higher compared to the total CLA content of milk obtained from

Table 3
CLA contents of milk and dairy products reported in different studies as a result of microalgae supplementation to ruminants.

CLA	Maximum differences reported (g/100 FA)	Species	Product	References
cis-9, *trans*-11 CLA	0.19–1.49	Bovine	Milk	[65,66,69]
	1.57–2.36	Ovine	Milk	[16,71]
	0.34–1.70	Caprine	Milk	[64,66,72]
	0.10–0.27	Bovine	Cheese	[74]
trans-10, *cis*-12 CLA	0.01–0.001	Bovine	Milk	[66,69]
	0.02	Ovine	Milk	[71]
	0.03	Caprine	Milk	[72]
	0.01–0.02	Bovine	Cheese	[74]
Total CLA	0.66–2.25	Bovine	Milk	[67,136]
	1.75	Ovine	Milk	[71]
	0.05–1.76	Caprine	Milk	[66,72]
	0.11–0.28	Bovine	Cheese	[74]

ewes raised on feedlot [83]. In addition to CLA, grass-based feeding, compared with TMR or concentrate-based feeding, produced higher contents of ALA (0.58 versus 1.33 g/100 g of FA), EPA (0.08 versus 0.14 g/100 g of FA) and DPA (0.12 versus 0.19 g/100 g of FA) in milk obtained from dairy cows [82]. However, grass-based feeding showed minor effects on DHA contents in milk and dairy products of ruminants, because all the cited studies did not detect DHA when grasses were incorporated to the diet.

The form (fresh versus conserved) and proportion of inclusion of grasses in ruminant feeding affected the contents of CLA in ruminant-driven foods. Compared with conserved grasses, fresh grasses were more effective in enhancing CLA content (0.69 versus 2.39 g/100 g of FA) in milk obtained from lactating dairy cows [84], which could be due to the oxidative loss of ALA during the conservation process (wilting or fermentation). For instance, wilting ryegrass in a glasshouse for 24 h decreased ALA content by 33 % compared with the un-wilted ryegrass [85].

Regarding the proportion-based inclusion of grasses in the diet of ruminants, Ward et al. [86] investigated the effects of fresh grass proportion on the FA profile of milk from lactating dairy cows and reported an increase in *cis*-9, *trans*-11 CLA contents of milk (from 1.57 to 1.90 g/100 g of FA) as the proportion of fresh grass increased from 50 to 80 % of the diet. Another study by Patel et al. [87] has also shown an increase in *cis*-9, *trans*-11 CLA content in milk (from 0.67 to 0.90 g/100 g of FA) as the proportion of grass-silage increased from 50 to 85 % of the diet. In addition to the CLA, those proportion-based studies reported an increase in ALA content (from 0.37 to 0.90 g/100 g of FA) and a decrease in n-6/n-3 ratio (from 5.2 to 1.6) as the proportion increased, which could be attributed to an increase in ALA intake. On the other hand, in cows' milk, decrease in total CLA content (from 0.62 to 0.56 g/100 g of FA) and ALA content (from 0.57 to 0.39 g/100 g of FA) and increase in n-6/n-3 ratio (from 2.3 to 4.4) were observed as grass-silage was substituted by maize-silage, indicating that grass silage contains higher ALA content compared to maize-silage [88].

Supplementation of grass with oilseeds or vegetable oils rich in linoleic or linolenic acid and fish oil or microalgae would appear also to be an effective feeding strategy to enhance the CLA content of dairy-driven food products even above that attainable on grasses alone [89,90]. Overall, grass-based feeding in ruminants, particularly when fed fresh and/or supplemented with vegetable, fish or algal oils could effectively improve CLA and ALA contents in milk and dairy products.

3. Technological processes enhancement of CLA in fermented dairy products

Different technological processes are normally applied, worldwide, during the manufacturing of various fermented dairy products. Of the various technological processes applied, application of CLA-producing bacterial strains during fermentation and ripening/storage are considered as potential strategies for enhancing the CLA content of fermented dairy products [18,91,92].

3.1. Effectiveness of bacterial cultures in enhancing CLA content of fermented dairy products

Apart from rumen bacteria, several *in vitro* studies have screened strains of *Bifidobacterium, Lactobacillus, Enterococcus, Streptococcus, Lactococcus, Enterococc and Propionibacterium* as potential producers of CLA [18,19]. According to the *in vitro* studies, 10–84 % of linoleic acid could be converted to CLA using various bacterial strains [93]. Despite most of the *in vitro* studied bacterial strains are normally used as starter cultures in the production of different dairy products, it is vital to note that the results obtained *in vitro* essays in MRS medium may not be extrapolated to the manufacturing processes of dairy products due to the changes and multiple interactions characterizing the milk-based substrates.

The maximum differences reported as a result of CLA-producing bacterial cultures application during the production of different fermented dairy products are summarized in Table 4. Several studies have used the CLA-producing strains as a starter culture in the production of different dairy products to investigate their role in enhancing the CLA content of the manufactured products. For instance, while manufacturing Dahi, a fermented milk product similar to yogurt, using *L. casi* and *L. acidophilus* as starter cultures, about 2 fold increase in CLA content was achieved [94,95]. Different strains of *B. animalis* ssp. *lactis* and *S. thermophilus* used to ferment organic milks could also raise the CLA content of the fermented milk by 12–20 % compared to the starting milk [91,96]. Mohan et al. [34] used CLA nonproducing commercial direct vat set (DVS) cheese culture, DVS 850 (Chr. Hansen Inc., Milwaukee, WI), consisting of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* and CLA-producing culture (CI4b) in the production of Cheddar cheese and evaluated their effect on the CLA content of Cheddar cheese during ripening. According to the authors, Cheddar cheese produced using CI4b showed about 1.5 fold increase in total CLA content compared with the Cheddar cheese produced using DVS 850. During the production of Miniature cheese, employing *Lb. plantarum L200* (CLA-forming) as adjunct culture increased *cis*-9, *trans*-11 CLA

Table 4
CLA contents of milk and dairy products reported in different studies as a result of CLA-producing starter culture application during processing.

CLA	Maximum differences reported (g/100 FA)	Type of CLA producing starter culture used	Product	References
cis-9, *trans*-11 CLA	0.2–0.28	Bb-12, and Cesaka-star Y508	Fermented Milk	[91,96]
	0.20–0.40	CI4b and Lb. plantarum L200	Cheese	[18,34]
	0.04–0.38	B. animalis (Bb-12)	Yogurt	[97,100]
	0.17–0.50	L. acidophilus and L. paracasei	Kefir	[92]
trans-10, *cis*-12 CLA	0.11–0.16	CI4b	Cheese	[34]
	0.02–0.25	B. animalis (Bb-12) and 4b (Lactococcus lactis)	Yogurt	[97,99]
Total CLA	1.15–1.62	CI4b	Cheese	[34]
	0.40–0.71	B. animalis (Bb-12) and 4a (Lactococcus sp.)	Yogurt	[97,99]
	0.01–0.03	ABY-2 and B. bifidum	Cream	[103,104]

content by 58 % compared to the control cheese (cheese produced using a commercial starter culture containing *L. lactis* subsp. *lactis* and *Lc. lactis subsp. cremoris*) [18]. Inclusion of different CLA-producing bacterial strains in the production of yogurt from milk of various ruminants increased the contents of *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and total CLA from 0.04 to 0.38, 0.02–0.25 and 0.40–0.71 (g/100 g fatty acids), respectively [97–102]. During the production of Kefir, Pourbaba et al. [92] used *L. acidophilus LA-5*, *L. paracasei 431*and *B. lactis BB-12* as complementary probiotics to evaluate their effect on the CLA content, and found 1.48–1.77 ppm increase in *cis*-9, *trans*-11 CLA in Kefir samples manufactured using the probiotics compared to the control Kefir. Similar to the aforementioned dairy products, application of CLA producing bacterial strains in the production of cream increased the *cis*-9, *trans*-11 CLA content from 0.09 to 0.32 mg/100 g fatty acids [103,104].

While using the CLA-producing strains of bacteria as a starter culture in the production of dairy products, modifying the fatty acid composition of the starting milk by dietary supplementation with diets rich in PUFA or the addition of vegetable oils rich in PUFA and prebiotics is more effective in elevating the CLA content of the final product. For instance, despite the same CLA-producing starter culture (CI4b) was used in the production of Cheddar cheese, cheese produced from cow milk that was modified its fatty acid composition as result of dietary fish oil supplementation showed higher total CLA content (1.44 versus 2.61 g/100 g of fat) compared to the cheese made from the control milk [34]. Addition of sunflower oil (0.1 mg/L) to the starting milk increased the total CLA content (9 versus 5.5 mg/g of fat) in the fermented milk made using *Lc. lactis I-01* [105]. Addition of hydrolyzed soybean oil to the starting milk also increased the concentrations of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA when cultured with *P. freudenreichii* ssp. *freudenreichii 23*, *P. freudenreichii* ssp. *shermanii 56*, *P. freudenreichii* ssp. *shermanii 51*, *L. rhamnosus* and yogurt starter cultures (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) [106]. Despite the increase in CLA depends on the type of strain, the combination of CLA-producing strains and yogurt starter cultures led to higher concentrations of CLA than those obtained without CLA-producing strains [95]. Furthermore, the total CLA content of yogurts fermented with a yogurt starter culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus)* and strains of *Lb. acidophilus L10*, *Lb. acidophilus La-5, B. lactis Bb-12*, *B. lactis BL04*, *B94*, *B. lactis HN019* or *B. longum BL05* was enhanced (from 20.5 to 66 %) by the addition of fructooligosaccharide, açai pulp and passion fruit by-product to the starting milk [97, 107,108]. Likewise, addition of lactulose powder to the starting milk raised the *cis*-9, *trans*-11 CLA content of Kefir (by 39.6 %) when CLA-producing strains (*L. acidophilus LA-5*, *L. paracasei 431* and *B. lactis BB-12*) were used as complementary probiotics in the production of Kefir [92]. Overall, application of CLA-producing bacterial strains in the production of various fermented dairy products would be a potential alternative in enhancing the CLA content of dairy products, especially, if optimum production conditions and high-yielding strains could be properly identified.

3.2. *Effectiveness of ripening/storage in enhancing CLA content of dairy products*

Some studies have evaluated the influence of ripening/storage on the CLA content of dairy products, and the reported results showed inconsistency. While most of the studies reported that ripening/storage can increase the CLA content of fermented dairy products, others reported that ripening/storage does not influence the CLA content of the products. For instance, Pourbaba et al. [92] evaluated the *cis*-9, *trans*-11 CLA content of Kefir produced with and without CLA-producing bacterial strains during refrigerated storage, and found a considerable increase in *cis*-9, trans-11 CLA content in a time-dependent manner in both Kefir samples (control and samples produced using CLA producing bacterial strains) despite a larger increase was obtained from the Kefir samples produced using CLA producing bacterial strains (2.34 versus 4.56 ppm). Likewise, several authors have reported an increase in CLA content in cheeses as ripening time progressed [109–111]. In Contrast, Cheddar cheese manufactured using direct vat set (DVS) commercial starter culture and CI4b and sausage fermented using L. plantarum AB20–961 and L. plantarum DSM2601 showed similar CLA content with an increase in time of storage/ripening [34]. The variations observed could be due to, on one hand, a longer time for the bacteria to act may cause an increase in CLA content, whereas, on the other, oxidizing reactions may destroy the double bonds, thus reducing the CLA content [112].

Based on the reports of some studies [91,104,106,113], an increase in CLA content during ripening/storage time seems to depend on the strain of bacteria used as starter culture in the production of the fermented products. For instance, Xu et al. [106] used propionibacteria strains (PFS-56, PFS-51 and PFF-23) and Lactic acid bacteria (LB) alone or in co-culture with traditional yogurt cultures to evaluate the contents of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12-CLA in yogurt during 14 days of storage period. The results of those authors exhibited an increase in *cis*-9, *trans*-11 CLA (0.29–0.64 mg/g lipid) and *trans*-10, *cis*-12 CLA (0.36–0.38 mg/g lipid) content after 14 days of storage when only traditional yogurt cultures + LB and LB alone were used to produce the yogurt, whereas, remained similar throughout the storage period when the other strains were used alone or in co-culture with traditional yogurt cultures. Likewise, Sady and Najgebauer-lejko [104]and Beata et al. [91] reported an increase in *cis*-9, *trans*-11 CLA content during storage when ABY-2 and Ceska-star Y508 (CSK Food Enrichment, Poland), respectively, were used with traditional yogurt cultures in the production of cream and yogurt. This variation may indicate the difference in the ability to tolerate the storage conditions and CLA production among bacterial strains. Thus, it needs to be studied further to determine optimum storage conditions that can suite the various CLA-producing bacterial strains and identify high CLA-yielding bacterial strains during ripening/storage.

4. Genetic manipulation as a potential tool in enhancing CLA content of milk and dairy products

Apart from production system, the fatty acid profile of milk and dairy products can be improved through genetic selection [114]. Compared with production systems or management-related approaches, genetic selection can provide a more sustainable solution. Thus, understanding and estimating genetic parameters influencing the FA content of milk from ruminants, particularly the content of PUFA beneficial to human health, has become an active area of research over the last a few decades [115,116]. Heritability is among the genetic parameters that highlight the possibility of manipulating the FA composition of ruminant food products using genetic factor [117,118]. Up to the present time, several authors [115–117] reported low to moderate heritability for *cis*-9, *trans*-11 CLA (the major CLA in milk and dairy products) in dairy cattle (Table 5). Similarly, the findings of some authors on sheep [118] and goats [119] revealed low to moderate heritability for CLA in milk. Another trial by, Tonhati et al. [120] has also shown high heritability value for CLA in buffalo milk, indicating the possibility of enhancing the CLA content in milk and dairy products using selective breeding program. Another essential type of genetic parameter in addition to heritability, is genetic correlation. Stearoyl-CoA desaturase 1 (SCD1) is considered as an important candidate gene that regulates the endogenous synthesis of CLA in ruminants [121]. Studies performed to investigate the association between this encoding gene and CLA in milk of cattle [121], milk of goats [122] and milk of sheep [20] reported that single nucleotide polymorphism (SNP) markers within SCD1 were associated with CLA. Therefore, using these potential genetic markers, future research can be undertaken to investigate the specific relationships between combining genetics and other environmental strategies such as dietary supplementation for enhancing CLA in milk and dairy products.

5. Sensory characteristics of CLA enriched milk and dairy products

Sensory characteristics of milk and dairy products are affected by the fatty acid composition of their fat. Fats that contain high contents of unsaturated fatty acids are expected to be more susceptible to oxidation. Oxidation can also cause a considerable reduction in the sensory and nutritional quality of food products. However, sensory quality reports on milk and dairy products enriched with CLA, both using dietary supplementation and fortification, are inconsistent. For instance, Hurtaud et al. [123] reported a rancid aroma for a butter produced from CLA enriched milk as a result of linseed oil supplementation. On the other hand, milk and butter obtained from dairy cows supplemented with microalgae showed similar sensory properties, despite the supplemented milk and butter contained higher CLA contents [124]. What's more, a similar flavor was reported by Jones et al. [125] for cheese and butter samples produced from CLA enriched milk, due to fish oil supplementation, and control milk. Likewise, Nelson & Martini [126] reported that milk obtained from cows supplemented with fish oil had organoleptic properties similar to that of milk obtained from cows not supplemented with fish oil.

Similar to the dietary CLA enrichment of milk and dairy products, fortification of milk and dairy products with CLA showed inconsistent effect on the sensory quality of milk and dairy products. For instance, the overall acceptability of fluid milk fortified with CLA was lower compared to the control samples [127]. In contrast, Soliman & Elaaser [128] who investigated the effect of fortification of yogurt with CLA found a similar acceptability (sensory quality) between fortified and non-fortified yogurt samples. Moreover, yogurt fortified with three different levels of CLA (1 %, 2 % and 3 %) exhibited higher overall acceptability scores compared to the non-fortified yogurt samples, and overall acceptability increased as the amount of CLA used for fortification increased [129]. Likewise, Cheddar cheese fortified with safflower oil derived CLA had similar flavor with the control cheese samples after 90 days of ripening [130]. In general, despite there exist an inconsistency among findings, regarding the sensory quality or acceptability of milk and dairy products enriched with CLA, majority of the studies indicated similar sensory quality or acceptability among the CLA enriched and control samples.

6. Conclusion

The CLA content of milk and dairy products can be improved using different strategies such as dietary supplementation with PUFA-rich diets, grass-based feeding, technological processes and selective breeding program. The major PUFA-rich lipid supplements include oilseed or vegetable oil rich in linoleic or linolenic acid (linseed oil, soybean oil, sunflower oil and rubber seed oil), fish meal or fish oil and microalgae. Despite it is possible to noticeably increase the contents of CLA in milk and dairy products under all PUFA-rich supplementation conditions, the levels of increase are more effective when plant oil combined with fish oil and/or microalgae is supplemented to the ruminants. Moreover, incorporation of oilseeds and plant oils rich in linoleic or linolenic acid, fish oil or microalgae into grass-based feeding is an effective feeding strategy to enhance the CLA content of milk and dairy products even above that attainable on grasses alone. Fermenting the milk obtained from PUFA-rich supplemented ruminants using CLA-producing starter cultures during the production of fermented dairy products is also an effective approach to increase the CLA content in fermented dairy products. Considering the high consumption of milk and dairy products, substantial increase in CLA content of milk and dairy products by dietary manipulation, fermentation with CLA-producing starter cultures and genetic manipulation or their combinations would be

Table 5
Heritability estimates of *cis*-9, *trans*-11 CLA in different breeds of cattle, sheep and goat.

Breed	Species	Unit	Heritability	References
Crossbred (HF X Jersey)	Bovine	%FA	0.21	[116]
Montbeliard	Bovine	%fat	0.18	[137]
Normande	Bovine	%fat	0.15	[137]
Holstein	Bovine	%fat	0.14	[137]
Holstein-Friesians	Bovine	%FA	0.21–0.42	[115]
Buffalo	–	%FA	0.35	[120]
Sarda	Ovine	%FA	0.14	[118]
Saanan	Caprine	%fat	0.18	[119]
Alpine	Caprine	%fat	0.17	[119]

an important approach to increase the intake of CLA and reduce the risk of cardiometabolic and cardiovascular diseases.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Solomon Gebereyowhans: Writing – review & editing, Writing – original draft, Resources, Conceptualization.

Declaration of competing interest

I declare that there is no competing interest.

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