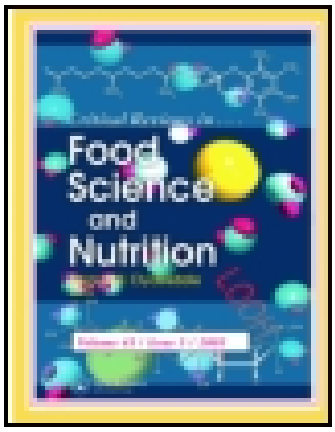

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Parisa Shokryzadan[a], Mohamed Ali Rajion[a], Goh Yong Meng[ab], Liang Juan Boo[b], Mahdi Ebrahimi[a], Maryam Royan[c], Mahbod Sahebi[b], Parisa Azizi[b], Rambod Abiri[d] & Mohammad Faseleh Jahromi[b]

[a] Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[b] Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[c] Agriculture Biotechnology Research Institute of Iran (ABRII), North branch, Rasht, Iran

[d] Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

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Conjugated Linoleic Acid: A Potent Fatty Acid Linked to Animal and Human Health

Parisa Shokryzadan[1], Mohamed Ali Rajion[1*], Goh Yong Meng[1,2], Liang Juan Boo[2], Mahdi Ebrahimi[1], Maryam Royan[3], Mahbod Sahebi[2], Parisa Azizi[2], Rambod Abiri[4], and Mohammad Faseleh Jahromi[2]

[1]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2]Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3]Agriculture Biotechnology Research Institute of Iran (ABRII), North branch, Rasht, Iran

[4]Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[*]Corresponding author: Professor Dr. Mohamed Ali Rajion, Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia, Tel: +603 - 8609 3411, Fax: +60389471971, Email: mohdali@upm.edu.my

ABSTRACT

Conjugated linoleic acid (CLA) is a mixture of isomers of linoleic acid (C18:2 n-6), which is mostly found in the ruminant meat and dairy products. The CLA is known to have many potential health benefits, and considered a potent powerful fatty acid, which is linked to animal and human health. The present work aims to discuss the source and production, mechanism of action, and effects of CLA on humans, poultry and ruminants by reviewing the recent studies carried out on CLA. Despite most of recent studies indicating beneficial effects of CLA on improving body weight control parameters, its effects on reducing risk factors of cardiovascular

diseases (CVD), inflammation, blood glucose and insulin are still controversial, and need to be further studied in different hosts.

Keywords

conjugated linoleic acid, ruminant, poultry, human

INTRODUCTION AND BACKGROUND

Conjugated linoleic acid (CLA) which is a mixture of positional and geometric isomers of linoleic acid (LA) or octadecadienoic acid with conjugated double bonds, which has been reported to be involved in animal and human health is mostly found in the dairy products like raw milk derived from ruminants but cannot be produced by the human body. The amount of CLA in the human body seems to be directly related to the dietary intake of CLA (Jiang *et al.*, 1999). Interest in CLA comes from late 1980s when Professor Michael W. Pariza, from the University of Wisconsin, discovered a chemical form of linoleic acid, as an isolated agent in fried hamburger, which caused a reduction in the incidence of cancer in mice. Later, his team called it conjugated linoleic acid (Pariza and Hargraves, 1985; Ha *et al.*, 1987). The CLA is known to have many potential health benefits, such as reducing body fat deposits, improving immune function and preventing different types of cancer, asthma, cardiovascular diseases (CVD), high blood pressure, high cholesterol and triglycerides, osteoporosis, insulin resistance and inflammation. However, recently, some adverse effects of CLA have also been reported, which are related to the effects of CLA on oxidative processes, eicosanoid production and carcinogenesis. These effects are mostly observed in mice and with the t10,c12 isomer, and not c9,t11 isomer or the mixture of both isomers (Wahle *et al.*, 2004). In animal models, CLA, especially t10,c12 isomer, has been reported to have some pro-carcinogenic effects for some cancers, such as colon and prostate cancer, and it can increase prostaglandin production in cells (Wahle *et al.*, 2004).

In 2000, Pariza *et al.* discussed possible biochemical mechanisms for CLA physiological effects in their excellent review. Although the mechanisms of physiological actions of CLA are not fully

clear yet, two possible mechanisms have been suggested. Firstly, CLA can reduce production of eicosanoids by decreasing the amounts of arachidonic acid (C20:4 n-6). Since eicosanoids are involved in cytokine production and in turn in inflammation effects, CLA is considered as an anti-inflammatory and anti-cancer agent (Pariza *et al.*, 2000; Belury, 2002). Secondly, CLA can regulate the expression of genes involved in the apoptosis induction in the cells, and also lipid oxidation, adipocyte differentiation, energy balance and atherogenesis (Pariza *et al.*, 2000; Belury, 2002).

It has been reported that a total of 54 isomers of CLA possibly occur (Delmonte *et al.*, 2004), but only about 20 of them have been identified (Bernas *et al.*, 2002) and the c9,t11 and t10,c12 isomers are reported to be the most abundant and bioactive CLA isomers (Pariza *et al.*, 2001). Between these two isomers, c9,t11-octadecadienoic acid is known to be more characterized in terms of its effects on certain health conditions, especially related to arachidonic acid metabolism (Rosberg-Cody *et al.*, 2007) and is also incorporated into the phospholipids of cell membranes (Ip *et al.*, 1994). However, the t10,c12 has also been considered as an important bioactive agent (Pariza, 2004; Soel *et al.*, 2007) and later considered as the most potent isomer of CLA for prevention of cell proliferation, as the causal agent for apoptosis induction in cancer cells (Kim *et al.*, 2002; Ochoa *et al.*, 2004; Cho *et al.*, 2005; Cho *et al.*, 2006; Lee *et al.*, 2006) and as a factor responsible for lowering of body fat (Hornung *et al.*, 2005; Rosberg-Cody *et al.*, 2007). Figure 1 shows the structures of the parent LA and the two main isomers of CLA derived from LA.

The present review aims to provide some information on the different possible ways to produce CLA, especially using microorganisms. In addition, several published reviews on CLA which

studied the different effects of CLA on the health of different hosts showed varied results. Therefore, the present review attempts to provide an updated summary on the CLA effects by reviewing recent studies which focus on CLA effects on ruminants, poultry and humans.

SOURCES OF CLA

The presence of CLA in dairy products is due to isomerization and biohydrogenation of LA and α-linolenic acid (C18:3 n-3) in the rumen by ruminal bacteria (especially *Butyrivibrio fibrisolvens*), and also by conversion of vaccenic acid in the mammary gland (Sieber *et al.*, 2004; Rodríguez-Alcalá *et al.*, 2011). The reports on the concentration of CLA in different animal products are controversial. For example, the CLA contents of different dairy products, measured by gas chromatography have been summarized and reported to be 0.55 to 9.12 mg CLA/g fat (Lin and Lee, 1997). Khanal and Olson (2004) reported that the CLA contents (mg/g fat) ranged from 1.2 to 17.0 (ruminant meat), 3.2 to 33.0 (ruminant milk), 0.9 to 2.0 (chicken) and 3.0 to 32.0 (egg yolk of chickens receiving diets containing 10 g CLA/kg of diet). Bauman *et al.* (1999) mentioned that the concentration of CLA in dairy products and meat from ruminants typically ranged from 3 to 7 mg/g of fat. In another study, the total amounts of CLA in raw milk fat has been reported to range from 2 to 20 mg/g (Alonso *et al.*, 2003). It has been considered that fermented dairy products have higher concentrations of CLA in comparison to the non-fermented ones (Rodríguez-Alcalá *et al.*, 2011). For example, an increase in CLA concentration has been observed during cheese ripening (Colbert and Decker, 1991). Ha *et al.* (1989) reported 8.81 mg CLA/g fat for fermented cheese compared to unprocessed milk containing 0.83 mg CLA/g fat. Shantha *et al.* (1995) showed that the CLA concentration of yogurt with 0.05% fat was higher (5.3mg CLA/g fat) compared to unprocessed milk (4.4 mg CLA/g fat) but they reported no

differences in the concentration of CLA in low-fat and regular yogurts, sour cream, and cheeses in comparison to unprocessed milk. The c9,t11 isomer is the main CLA isomer of milk fat, which forms about 80 to 90 % of total CLA in milk fat. However, the t10,c12 isomer of CLA forms only one percent of total CLA of milk fat (Jensen, 2002).

PRODUCTION OF CLA

Production of CLA can be through traditional organic synthesis, which usually results in a mixture of different CLA isomers. For example, the CLA which is synthesized by alkali isomerization of LA and is commercially available in the market, contains four isomers (c8,t10-, c9,t11-, c10,t12-, and c11,t13-18:2) of CLA (Sehat *et al.*, 1998). The CLA can also be produced by microbial fermentation of LA using linoleic acid isomerase (LAI) enzyme, which results in a greater specificity of the produced isomers (Irmak *et al.*, 2006). For example, it is reported that many strains of lactic acid bacteria (LAB), especially the genus *Lactobacillus*, produce the c9,t11 isomer of CLA as a major end product of the LAI activity (Kishino *et al.*, 2011). However, some of this isomer can be further converted to the t9, t11 CLA isomer (Coakley *et al.*, 2006). Bacterial strains belonging to LAB, such as *Lactobacillus* and *Enterococcus*, and also *Bifidobacterium* genera have been reported to produce CLA in either synthetic media or milk with a strain specific profile (Sieber *et al.*, 2004). The first report on the detection of CLA in *Lactobacillus* strains was by Fairbank *et al.* (1988). After that, many researchers investigated the formation of CLA through common LAB, and found that although many of the tested strains were not able to produce CLA, many others belonging to lactobacilli, lactococci and streptococci were able to convert LA to CLA in the growth medium or in skim or whole milk (Jiang *et al.*, 1998; Lin *et al.*, 1999; Ham *et al.*, 2002; Kishino *et al.*, 2002; Alonso *et al.*, 2003; Rodríguez-

Alcalá *et al.*, 2011). Hence, production of CLA using LAB and other microbial strains containing LAI could be a good alternative for organic synthesis of CLA toward producing more specified isomers. Since LAB, especially *Lactobacillus* strains are most commonly used as probiotics (Shokryazdan *et al.*, 2014a; Shokryazdan *et al.*, 2014b) and as fermentative factors for fermentation of dairy products (Beena Divya *et al.*, 2012), the use of lactic acid bacterial strains, which are able to produce CLA from LA, as probiotic supplements or starter for fermentation of dairy products has been considered as an opportunity to increase the nutritional value of dairy products.

Many studies worked on the production of CLA in microorganisms, especially LAB strains. For example, Jiang *et al.* (1998) evaluated the ability of CLA production from free LA of 19 different strains of lactobacilli, lactococci, streptococci and propionibacteria, which were commonly used as dairy starter strains. Among the tested strains, two strains of *Propionibacterium freudenreichii* subsp. *freudenreichii* and one strain of *P. freudenreichii* subsp. *sheramnii* were able to convert LA into CLA with a maximum amount of 265 mg CLA/ml medium. The c- and t-9,11-octadecadienoic acid formed more than 70% of the total produced CLA. None of the tested LAB in their study was able to produce CLA. Lin *et al.* (1999) studied six lactic acid bacterial strains (including *L. acidophilus, L. delbrueckii subsp. bulgaricus, L. delbrueckii subsp. lactis*, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. lactis* and *Streptococcus salivarius subsp. thermophlius*) for their ability to produce CLA by adding LA in their growth media at different incubation times. They concluded that addition of LA from 1000 to 5000 mg/ml medium, and increasing the incubation time from 24 to 48 h did not affect the CLA production. Among the tested strains, *L. acidophilus* in 1000 mg LA per ml of skim milk

added-medium incubated for 24 h showed the highest amounts of CLA production. Ham *et al.* (2002) investigated CLA production of 34 LAB, isolated from human baby feces samples. Among the tested strains, only one culture of *L. fermentum* showed amounts of CLA which were detectable by HPLC analysis. Alonso *et al.* (2003) tested four different cultures of *Lactobacillus* including two strains of *L. acidophilus* and two strains of *L. casei* for their CLA production from LA in MRS broth supplemented with LA. They reported a maximum range of CLA production at 80.14 to 131.63 µg/ml. In another study, Coakley *et al.* (2003) also assessed different strains of *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Bifidobacterium* for their ability to convert free LA to CLA. They found that nine strains of *Bifidobacterium* converted free LA to isomer c9,t11 of CLA. In their study, *B. breve* was able to convert up to 65% of LA to c9,t11 of CLA. The t9,t11 isomer of CLA was also produced by some of the strains at low amounts. Rodríguez-Alcalá *et al.* (2011) tested 22 probiotic bacteria for their CLA production ability. Among the tested strains, they selected two strains belonging to *Bifidobacterium*, two *Lactobacillus* strains and one *Lactococcus* strains based on their CLA production ability, with LA as substrate. They determined the amounts of produced CLA to be in the range of 40 to 50 µg CLA/ml medium. They identified the produced CLA isomers to be in the following order: C18:2 c9,t11 (60 to 65%) > C18:2 t10,c12 (30 to 32%) > C18:2 t9,t11 and C18:2 t10,t12 (2 to 5%).

Mechanisms of CLA production

So far, two groups of enzymes introducing conjugated double bonds in fatty acids are known. These groups are the special desaturases or conjugases, which are mainly responsible for conversion of LA into linolenic acid isomers containing three conjugated double bonds and the polyenoic fatty acid isomerases (PAI), which have been reported from *Butyrivibrio fibrisolvens*

(Kepler *et al.*, 1966). The LAI is included in the second group (Hornung *et al.*, 2005). The activity of LAI, the catalyzing enzyme for conversion of LA into CLA, has first been described by Tove's team for production of c9,t11 isomer from LA by *B. fibrisolvens* (Kepler *et al.*, 1966), a rumen bacteria involved in the biohydrogenation process (Kepler and Tove, 1967). They explained that first LA is rapidly converted to c9,t11 CLA by LAI of the rumen bacteria, and after that c9,t11 CLA is converted to C 18:1,t11 vaccenic acid by a slower rate (Kepler *et al.*, 1966; Kepler and Tove, 1967). To complete the biohydrogenation process of LA, vaccenic acid is then reduced to form stearic acid as the end-product. Figure 2 shows the biochemical pathway for biohydrogenation of linoleic acid by rumen microorganisms. Vaccenic acid has also been reported to be transformed into c9,t11 CLA in the mammary glands by the delta-9 desaturase enzyme. This is considered as the second mechanism for the presence of c9,t11 CLA in milk fat (Griinari and Bauman, 1999; Coakley *et al.*, 2003). In 2001, Ogawa *et al.* explained a mechanism of CLA production from LA and suggested that presence of LA in the growth medium of microorganisms induced the enzyme system for CLA production. Based on their report, the conversion of LA to CLA involved the production of hydroxy fatty acids (10-hydroxy-*t*-12-octadecaenoic acid and 10-hydroxy-*c*-12-octadecaenoic acid) as intermediate factors (Ogawa *et al.*, 2001). They used washed cells of *L. acidophilus* AKU 1137 to produce CLA from LA in microaerobic conditions, and reported a conversion of over 95% of added LA in the growth medium to CLA, which caused the concentration of CLA to be more than 80% (w/w) of total fatty acids. Figure 3 presents their proposed pathway of CLA production from linoleic acid using washed cells of *L. acidophilus* AKU 1137.

Enzyme purification for production of CLA

Purification of LAI from microorganisms and using it for manufacturing CLA is another possible way to produce considerable amounts of CLA. To date, three LAI from *Lactobacillus reuteri* (producing c9,t11 CLA), *Clostridium sporogenes* (producing c9,t11 CLA) and *Propionibacterium acnes* (producing t10,c12 CLA) have been fully characterized (Zhang *et al.*, 2012). The LAI has been found in two forms of soluble enzyme and as a membrane-bound protein. In terms of using purified LAI enzyme to produce CLA, the soluble form of LAI from *P. acnes* has been intensely studied because of its stability and ease of purification (Hornung *et al.*, 2005; Rosberg-Cody *et al.*, 2007). However, the *P. acnes* is a pathogenic strain for human and its usage for fermentation of foods and dairy products is impossible. On the other hand, the LAI is presented in LAB in a membrane-associated form, and produce the c9,t11 isomer of CLA as the main product (Irmak *et al.*, 2006; Macouzet *et al.*, 2010). Hence, LAB containing LAI has been considered as a good candidate to be used as a starter for fermentation of dairy products or as a probiotic supplement for humans and animals to take advantage of the produced CLA. However, the LAI of LAB is unstable during its recovery in a soluble form making it difficult to be studied (Irmak *et al.*, 2006).

Biotechnological techniques in microbial production of CLA

Nowadays, new biotechnological techniques can be used to obtain transformed microbial strains that are able to produce higher amounts of CLA than the ordinary existing strains (Irmak *et al.*, 2006). Some studies employed genetic engineering of microorganisms to enhance CLA production. For example, the LAI derived from *P. acnes* has been expressed in *Saccharomyces cerevisiae* (Hornung *et al.*, 2005), *E. coli* and *Lactococcus lactis* (Rosberg-Cody *et al.*, 2007) to produce CLA by the transformed organisms. Hornung *et al.* (2005) expressed PAI from *P. acnes*

in *E. coli* and characterized it biochemically. This enzyme catalyzes the isomerization of a methylene-interrupted double bond to a conjugated double bond, resulting in the 10E,12Z isomer of CLA from a wide range of free polyunsaturated fatty acids (PUFA) as substrates. Rosberg-Cody *et al.* (2007) cloned and overexpressed the LAI from *P. acnes* into *Lactococcus lactis* and *E. coli*. They found between 30 and 50% conversion rates of LA to t10,c12 CLA. Zhang *et al.* (2012) expressed the LAI gene from *P. acnes* into *Yarrowia lipolytica* Polh improving the expression of the enzyme in *Yarrowia lipolytica* by codon usage optimization and multi-copy integration. They found that the yeast containing the codon-optimized gene produced six time higher amounts of t10,c12 CLA than the yeast containing the native gene, which increased to about 30 times higher by a combination of multi-copy integration. In another study, the same microorganism (*Y. lipolytica*) was used to enhance its production of CLA by using co-expression of the delta 12-desaturase gene from *Mortierella alpina* together with the codon-optimized LAI gene which enhanced the production of CLA by the transformed *Y. lipolytica* (Zhang *et al.*, 2013).

THE CLA IN RUMINANTS

The CLA has been studied intensively in ruminants. Some studies investigated the CLA content of ruminant milk fat and meat, and some others evaluated other effects of CLA on ruminants, such as effects of CLA on milk production and composition, and also on metabolic key parameters of blood, lipid metabolism, inflammation, etc, which will be discussed in the following sub-sections. However, most of the studies on CLA in ruminants focused on milk fat.

Concentration of CLA in ruminant meat

The c9,t11 isomer of CLA is the predominant isomer in both meat and milk of ruminants, but its concentration in meat is less than that in milk of ruminants. It may be related to effects of the diet, so that for cattle that received a traditional high-concentrate, low-fiber diets in the United States, the concentration of c9,t11 isomer of CLA in their meat was lower when compared with that of milk (Bauman *et al.*, 1999). However, the same isomer constituted more than 90% of the total CLA in subcutaneous and intramuscular fat of German Simmental cattle, which received corn-silage-based diets with a moderate level of grain supplement (Fritsche and Fritsche, 1998). It is important to note that the content of CLA from ruminant meat is largely dependent on the concentration of CLA in the raw products because CLA is relatively stable during processing and storage.

Concentration of CLA in ruminant milk fat

It is believed that grazing, in comparison to intensive husbandry, increased the amounts of CLA in milk fat. For example, Tsiplakou *et al.* (2006) who investigated the CLA content of milk fat of grazing sheep and goats found that the CLA concentration of sheep milk fat was much higher than that of goats, with a negative correlation between sheep milk fat and its CLA content. Pajor *et al.* (2009) who evaluated the effects of grazing on the fatty acid profile of goat milk and cheese reported that grazing considerably increased the total CLA content in milk (0.59 vs. 0.77%) and cheese (0.52 vs. 0.84%) of the grazing goats compared to the control group, which were kept indoors. Tudisco *et al.* (2014) who evaluated the effect of pasture on the fatty acid profile of goat milk reported that goats on pasture showed higher amounts of CLA in their milk in comparison with the control animals.

Effects of CLA on milk fat

It is well documented that although dietary supplementation of CLA for dairy ruminants can increase the amounts of CLA in their milk, CLA has a reducing effect on milk fat of dairy ruminants, especially cows and sheep (Bauman *et al.*, 2008) which has been mostly attributed to a decrease in biosynthesis of lipids. For example, in a study by Vyas *et al.* (2013), abomasal infusion of CLA reduced de novo synthesized fatty acid concentration in dairy cows. Perfield *et al.* (2007) who investigated the effect of CLA on milk fat of cows reported that the increase in t9,c11 CLA corresponded to a decrease in milk fat yield. The t10,c12 CLA also decreased de novo synthesized fatty acids and desaturation of 18:0 via Δ9-desaturase. However, t9,t11 CLA had no effect on milk fat yield.

In addition, the t10,c12 isomer of CLA has been reported to decrease milk fat in both ruminants and non-ruminants (Bauman *et al.*, 2008). Three studies on lactating sheep showed that t10,c12 CLA supplementation reduced milk fat production like that observed in dairy cows (Lock *et al.*, 2006; Sinclair *et al.*, 2007; Lock *et al.*, 2008). Hussein *et al.* (2013) also reported that the effect of CLA on milk fat depression is associated with less expression of mammary genes involved in lipid synthesis in dairy ewes. In their study, CLA reduced the milk fat percentage and milk fat yield by about 23%. Lock *et al.* (2008) also reported a reduction in milk fat of lactating goats by supplementation of a lipid-encapsulated t10,c12 CLA. In their study, although CLA supplementation at 30 and 60 g/d CLA did not affect milk yield, milk protein yield and dry matter intake of the goats, it reduced milk fat yield by 8 and 21%, respectively. This reduction in milk fat yield was attributed to reduction in both de novo fatty acid synthesis and uptake of offered fatty acids. They concluded that t10,c12 CLA decreased milk fat synthesis in dairy goats like that observed for dairy cows and sheep, but the reduction was lesser for goats compared to

cows and sheep. In contrast, some other studies on goats reported that t10,cis12 CLA had no effect or trace effect on milk fat yield (Erasmus *et al.*, 2004; Schmidely and Morand-Fehr, 2004; De Andrade and Schmidely, 2006). As mentioned earlier, the t10,c12 isomer of CLA has been reported to also reduce milk fat in non-ruminants (Bauman *et al.*, 2008). For example, exogenous t10,c12 CLA decreased lipid synthesis in murine adipose and mammary tissues (Kadegowda *et al.*, 2013). They showed that by supplementing with 37 mg CLA/d, the milk fat concentration of the mice was 44% lower on day 10 postpartum compared to day 6 postpartum, and concluded that CLA affected the mammary tissues mostly by alterations in cellular signaling pathways and phospholipid biosynthesis (Kadegowda *et al.*, 2013).

Other aspects of CLA effects on ruminants

Effects of CLA on ruminants are not limited to milk production and composition. Other effects on metabolic key parameters of blood, lipid metabolism, inflammation, etc, have also been investigated. Sigl *et al.* (2010) fed a CLA (50% c9,t11 and 50% t10,c12 CLA) supplemented diet to dairy cows in their first lactation month. They detected higher amounts of CLA in the milk fat of CLA-supplemented cows in comparison with the control group. They also detected a reduction in saturated fatty acids (SFA) of milk fat with an increase in monounsaturated and trans SFA in cows that received CLA. However, CLA did not have any significant effects on milk yield and composition, metabolic key parameters of blood, or gene expression of peroxisome proliferator-activated receptor (PPAR)-α, PPAR-γ, sterol regulatory element-binding protein-1and tumor necrosis factor-alpha (TNF-α) in liver tissues. Schlegel *et al.*, (2012) who fed cows with a rumen-protected CLA (c9,t11 and t10,c12) fat to investigate its effect on milk and hepatic lipid metabolism of the animals at 5th week of lactation reported that while total milk

yield increased, milk fat yield and content decreased, and energy balance improved. The regulation of 107 genes involved in hepatic lipid metabolism and expression of key enzymes of lipogenesis, β-oxidation, and ketogenesis, as well as concentrations of triacylglycerols and cholesterol in liver and plasma were not affected by CLA. Saremi *et al.* (2012) investigated potential anti-inflammatory effects of long-term supplementation of CLA in cows using the haptoglobin assessment. Although the CLA showed no anti-inflammatory effects on serum Hp and liver Hp mRNA, the CLA showed anti-inflammatory effects on Hp in omental and subcutaneous fat, indicating its tissue-specific effects. Kramer *et al.* (2013) investigated the effect of feeding a diet supplemented with a rumen-protected CLA containing c9,t11 and t10,c12 CLA on fatty acid distribution of lipids in several body tissues compared to their distribution in milk fat in early lactating heifers. They reported a milk fat depression by a reduction of de novo synthesis of fatty acids without any considerable effect on tissue lipids. Higher amounts of fed CLA isomers were detected in the milk fat of cows receiving CLA than the control animals. The distribution of fatty acids in mammary glands was similar to that of the milk fat; however, it was mainly because of residual of milk in the mammary gland, not influencing of gene expression. Fatty acids of the liver did not show any differences except for an increase in trans-octadecenoic acids. Adipose tissue and longissimus were only marginally affected by the CLA supplementation.

THE CLA IN POULTRY

Unlike ruminants, dietary fatty acids do not undergo changes before absorption in birds. Therefore, CLA supplementation of the diet is a widely used strategy to increase the CLA content of the tissues

in poultry (Du and Ahn, 2002; Sirri *et al.*, 2003; Royan *et al.*, 2013). Many studies on the effects of dietary CLA on poultry mostly focused on the performance, lipid metabolism and immune system.

Effects of CLA on poultry performance

Studies have shown that CLA can affect the performance of broilers where the growth rate is more sensitive to CLA than other performance traits, so that the dietary CLA levels higher than 1% (10 g/kg) decreased the growth rate of broilers (Szymczyk *et al.*, 2001; Badinga *et al.*, 2003). Royan *et al.* (2011b) changed the CLA dose, bird age and fat composition of the diet so that chicks fed diets containing a lower CLA level in the finisher phases showed an acceptable body weight gain compared to those receiving higher CLA levels. Results on the CLA effects on weight gain of chickens have been inconsistent. A linear increase of daily weight gain (Thiel-Cooper *et al.*, 2001), moderate weight loss (Cook *et al.*, 1993), and adverse effects on weight gain using up to 1.5% CLA supplementation (Suksombat *et al.*, 2007) and 1% CLA (Buccioni *et al.*, 2009) have been reported. Royan *et al.* (2011b) reported that the adverse effects of CLA on body weight gain of chickens is dose related, so that broilers fed a high dietary CLA dose (4.2%) have lower weight gains than those fed diets containing 2.1% CLA. Many studies reported no adverse effect on feed intake by incorporating different CLA levels in the diet (Szymczyk *et al.*, 2001; Du and Ahn, 2003; Sirri *et al.*, 2003; Takahashi *et al.*, 2003; Denli *et al.*, 2004; Bolukbasi, 2006; Suksombat *et al.*, 2007; Buccioni *et al.*, 2009). Du and Ahn (2002) showed that although up to 1% dietary CLA had no effect on feed consumption of broilers, 2 or 3% CLA reduced feed consumption. Javadi *et al.* (2007) reported that 1% dietary CLA was enough to show adverse effects on broiler feed intake. On the other hand, one occasional positive effect of CLA on feed intake has been reported by Bolukbasi *et al.* (2006) where there was an increased feed intake in broiler chickens fed diets containing 1% CLA compared with

the control group with no difference shown between dietary supplementation of 2 or 3% CLA and the control diet. Some other studies have shown that the ability of chickens to use CLA is increased with age. It means that although CLA may reduce the feed intake of the birds in the starter or grower phases of the rearing period, it recovered later in the finisher phase (Suksombat *et al.*, 2007; Royan *et al.*, 2011b). The reported effects of CLA on feed conversion ratio (FCR) of chickens are also controversial which included unfavorable increased FCR (Royan *et al.*, 2011b), no effect on FCR (Szymczyk *et al.*, 2001; Du and Ahn, 2002; Javadi *et al.*, 2007) and reduced FCR (Bolukbasi, 2006). A meta-analysis study by Cho *et al.* (2013) on the effects of CLA feeding on growth performance and fatty acid profile of chicken meat revealed that CLA does not have beneficial effects on growth performance but may be effective in modulating n-6/n-3 fatty acid ratios in the thigh meat. Adverse effects have also been observed on the performance of layer hens following CLA addition to the diet. Szymczyk and Pisulewski (2003) reported a reduced feed intake and egg mass when using CLA-enriched diets for layer hens.

Effects of CLA on poultry lipid metabolism

Reduction of carcass fatness and mainly abdominal fat pad is a goal of the poultry industry, which can be achieved by CLA supplementation in the diet. However, reports on the effects of CLA on abdominal fat pad alteration are conflicting. Some studies showed that diets containing CLA increased the abdominal fat pad content in chickens (Du and Ahn, 2002; Javadi *et al.*, 2007), while others showed a reduction (Szymczyk *et al.*, 2001; Badinga *et al.*, 2003). Buccioni *et al.* (2009) found positive effects of CLA (1%) on the carcass yield, and claimed that these effects were related to the significant decrease in abdominal fat pad and were attributed to the ability of CLA to reduce body fat accumulation. On the other hand, Suksombat *et al.* (2007) reported that the reduced

abdominal fat pad in birds fed dietary CLA was not accompanied by any increase in carcass, breast or thigh composition. Royan *et al.* (2011a) confirmed the reduction in abdominal fat by a down-regulation of PPAR-γ mRNA expression in the abdominal adipose tissue. The PPAR-γ is the most important transcription factor which regulates lipid metabolism and adipocyte differentiation, and is strongly associated with the abdominal fat deposition in avian species (Sato *et al.*, 2009; Xiong *et al.*, 2010). Royan *et al.* (2011b) showed that dietary CLA, through the effect on PPAR-γ, also reduced the fat content of the breast muscle in broiler chickens. Kawahara *et al.* (2009) reported that feeding broilers with 1 to 2% CLA in the diet reduced the total lipid and triglyceride concentration in breast meat. An increase in liver weight in CLA fed chicken has also been reported (Du and Ahn, 2002; Badinga *et al.*, 2003; Suksombat *et al.*, 2007; Royan *et al.*, 2011b). Based on animal studies, the incidence of fatty liver is the most important concern related to CLA consumption (Pariza, 2004). Fatty livers may occur due to the CLA effects on body fat mobilization as well as an increased fatty acid synthesis in the liver (Tsuboyama-Kasaoka *et al.*, 2000; Clément *et al.*, 2002; Yanagita *et al.*, 2005).

Increased plasma triglyceride concentration in broiler chickens has been observed following CLA administration (Du and Ahn, 2003). This may be due to alterations in activities of enzymes involved in hepatic lipid metabolism. Avian lipid synthesis takes place mainly in the liver, with the adipose tissue as the lipid storage organ. Park *et al.* (1997) attributed this CLA enhancing effects on serum triglycerides as a result of the simultaneous inhibitory role of CLA on lipoprotein lipase and stimulation of lipolysis in the adipose tissue. Hence a reduction in fat deposits and increased lipolysis in adipocytes could be the reasons for the elevated serum triglyceride levels observed in broiler chickens. Du and Ahn (2003) confirmed that dietary CLA increased the liver fatty acid synthase

enzyme, one of the main enzymes regulating fatty acid synthesis. Therefore, the increased plasma triglyceride levels could be due to the higher liver fatty acid synthase activity. Total cholesterol and LDL cholesterol coordinated changes (Bhattacharya *et al.*, 2006; Feitoza *et al.*, 2009) and an increase in serum HDL level (Du and Ahn, 2003; Bolukbasi, 2006) have

also been reported in birds fed CLA.

Effects of CLA on poultry immune system

The CLA can beneficially stimulate the immune response in broiler chickens (Zhang *et al.*, 2005). Dietary CLA induced anti-sheep red blood cell (SRBC) antibody production in broilers (Takahashi *et al.*, 2003). Long *et al.* (2011) reported the CLA enhancing role in chickens, particularly during the Infectious Bursal Disease Virus- (IBDV) immunosuppressive status. They attributed the immunoregulatory functions of CLA on broiler chickens mainly to the anti-inflammatory effects of CLA that were mediated by suppressing the IBDV-specific proinflammatory cytokine mRNA relative expression. Investigation of immunoregulatory actions of CLA by Long *et al.* (2012) showed that CLA alleviated the immunosuppression of T lymphocytes in broiler chickens exposed to cyclosporin A by increasing peripheral blood T lymphocyte proliferation and interleukin-2 levels.

THE CLA IN HUMANS

Many researchers have investigated various effects of CLA in humans, mostly focusing on the effects of CLA on serum glucose, insulin resistance, body weight control, serum cholesterol and triglycerides, cardiovascular diseases, inflammation and blood pressure. Table 1 summarizes the results of recent studies on the effects of CLA in humans.

Effects of CLA on serum glucose and insulin sensitivity

Studies on the effects of CLA on serum glucose and insulin showed conflicting findings for different subjects. Moloney *et al.* (2004), who investigated the effects of CLA (a mixture of c9,t11 and t10,c12 isomers) consumption on markers of glucose and insulin metabolism in patients with type II diabetes, reported that the CLA had an adverse effect on insulin and glucose metabolism by increasing the fasting glucose concentrations and reducing insulin sensitivity. Thrush *et al.* (2007) also reported that CLA consumption caused a reduction in insulin sensitivity in overweight, non-diabetic subjects. However, Tricon *et al.* (2006), who studied the effects of dairy products enriched with c9,t11 CLA (and t11-18:1) on insulin resistance in healthy men, reported that CLA had no effect on serum insulin and glucose. (Racine et al., 2010) showed that CLA did not have any significant effect on plasma glucose and insulin of 6-10 yr old, obese children. However, (Colakoglu *et al.*, 2006) reported a reduction in the concentrations of serum glucose and insulin in healthy female young subjects that received CLA supplementation, combined with aerobic exercise.

Effects of CLA on lipid metabolism and body weight control

Like the effects of CLA on serum glucose and insulin resistance, results of studies on the effects of CLA on lipid metabolism and body weight control were also conflicting. Some studies reported positive and improving effects of CLA consumption on lipid metabolism and body weight. For instance, Gaullier *et al.* (2005) investigated the effects of long-term (24 months) CLA consumption on body composition, body weight, body mass index (BMI) and serum lipids, and the results showed no changes in HDL cholesterol and triglycerides of plasma although total cholesterol and low density lipoprotein (LDL) cholesterol decreased. They reported that CLA decreases body fat mass (BFM) in overweight subjects, thus helping maintain initial reductions

in BFM and weight in the long-term periods. Chen *et al.* (2012) also reported that a 12 wk supplementation of CLA in overweight subjects caused lower obesity indices, with no obvious adverse effects. In addition, Colakoglu *et al.* (2006) showed that CLA supplementation combined with aerobic exercise improved the body composition of healthy female young subjects. Besides, in the study by Racine *et al.* (2010) on the effects of CLA (a mixture of c9,t11 and t10,c12 isomers) on fat and BMI in 6-10 yr old obese children, the CLA attenuated an increase of BMI and also decreased body fatness compared to the control group. Although the CLA did not have any significant effect on LDL cholesterol in these children, it reduced HDL cholesterol significantly. Pfeuffer *et al.* (2011) showed that, in comparison with safflower oil, consumption of CLA decreased body weight without changing parameters associated with a metabolic syndrome, such as total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides.

Some studies reported that CLA had no effect on lipid metabolism and body weight. For example, Tricon *et al.* (2006) showed that consumption of dairy products enriched with c9,t11 CLA (and t11-18:1) had no effect on body weight, triacylglycerols, total cholesterol and LDL and HDL cholesterol. Nazare *et al.* (2007), who studied the effects of CLA supplementation (a mixture of c9,t11 and t10,c12) in yogurt-like dairy products on body composition and the expression of several key adipose tissue genes [PPAR-γ, lipoprotein lipase (LPL), hormone-sensitive lipase (HSL) and uncoupling protein 2 (UCP-2)], observed no significant effects on body weight, fat mass or free fat mass of healthy subjects, although the basal energy expenditure increased. The mRNA expression of PPAR-γ was increased, but mRNA levels of HSL were decreased by CLA consumption. However, the amounts of mRNA for UCP-2 and LPL did not

show any changes. Asp *et al.* (2011), who investigated the effects of CLA on blood lipids in obese, post-menopausal women with type II diabetes, also reported that CLA did not affect the tested metabolic parameters. The study by Joseph *et al.* (2011) also did not support the role of CLA as an effective body weight or a blood lipid regulator. They investigated the effects of CLA (a mixture of t10,c12 and c9,t11 CLA: Clarinol G-80, and c9,t11 isomer of CLA) consumption on body composition and blood lipids in overweight, hyperlipidemic men, and showed that CLA consumption had no effect on body weight, blood lipids, β-oxidation rate of fatty acids or other tested parameters.

Effects of CLA on inflammatory markers of CVD

Although a number of human studies reported that CLA has an anti-atherosclerotic effects on serum lipid profile (as mentioned above), most of recent studies on the inflammatory markers of CVD, especially C-reactive protein, interleukin-6 (IL-6) and TNF-α, revealed that CLA supplementation have no significant effect on the markers. For instance, in the studies on healthy men (Tricon *et al.*, 2006), patients with type II diabetes (Moloney *et al.*, 2004), healthy young men (Raff *et al.*, 2008), obese, post-menopausal women with type II diabetes (Asp *et al.*, 2011) and overweight, hyperlipidemic men (Joseph *et al.*, 2011) the consumption of CLA had no effect on inflammatory markers of CVD.

Effects of CLA on blood pressure

In case of blood pressure, some researchers reported that CLA had no effect on the blood pressure of healthy young men (Raff *et al.*, 2006), in young overweight women (Diaz *et al.*,

2008) or healthy volunteers (Engberink *et al.*, 2012)**.** However, Pfeuffer *et al.* (2011) noted a reduction in blood pressure in obese male subjects relative to the baseline value.

Effects of CLA on cancer

To the best of our knowledge, very few studies have been carried out on the effects of CLA on the incidence of cancer in humans. Hence, the limited number of studies makes it difficult to ascertain whether CLA could protect humans against cancer. In an study on 55 to 69 yr old female subjects, a weak positive relationship between breast cancer incidence and the intake of CLA was reported (Voorrips *et al.*, 2002). Another study on postmenopausal women, patients with breast cancer showed lower levels of serum and dietary CLA than the control subjects (Aro *et al.*, 2000). However, the concentration of CLA in breast adipose tissue was not associated with the relative risk of breast cancer (Chajès *et al.*, 2002).

Although the number of studies on the effect of CLA on incidence of cancer in humans is limited, this approach has been intensively studied in animal models, especially rats (Kelley *et al.*, 2007). Dietary supplementation of CLA inhibited chemically induced tumors of the mammary gland, skin, colon, and the forestomach in several animal models (Kelley *et al.*, 2007). Studies on the effects of CLA on tumors induced in the mammary gland of rats (Ip *et al.*, 1999; Ip *et al.*, 2002) and forestomach of mice (Chen *et al.*, 2003), suggest that CLA had an inhibiting effect on the tumor yield or incidence. In addition, CLA was able to inhibit the metastatic lung tumor load of mice with induced mammary tumor, which spontaneously metastasizes to the lung (Hubbard *et al.*, 2003). However, results of another study, in which Min mice were fed a diet containing 1% c9,t11 or t10,c12 isomer of CLA, suggested that the t10,c12 isomer can act as a growth promoter for small intestine carcinogenesis (Rajakangas *et al.*, 2003).

Mechanisms involved in the inhibitory effects of CLA on different types of cancers have been reported to be related to alteration of lipid peroxidation and tissue fatty acid composition, eicosanoid metabolism, and expression of genes involved in regulating cell growth and apoptosis (Kelley *et al.*, 2007). Since these mechanisms of action which are associated with tumorigenetic effects of CLA are varied for different types of tumor and different stages of tumor progression, the response of different types of cancers to CLA treatments in various organs would probably be different. Hence, the comparison of the results from the studies mentioned earlier and extrapolating the results to humans may be difficult and not appropriate. Further studies with appropriate animal models, which parallel human pathogenesis, have to be carried out in order to have a more accurate assessment on the effects of CLA on cancer in humans.

Experiments on rats as a model for human physiology

Zhou *et al.* (2008) investigated the effect of CLA on insulin resistance and its molecular mechanisms in obese rats, as a model representing human physiology. They reported that the dietary CLA supplementation decreased the body weight gain and white fat pad weight, the levels of plasma free fatty acids, triglycerides, cholesterin, leptin, insulin and blood glucose concentration. The CLA improved insulin resistance by increasing mRNA expression of PPAR-γ, and its target genes such as fatty acid binding proteins, fatty acid transporter proteins and adiponectin in the adipose tissues of the obese rats. In another study, Rodrigues *et al.* (2014) used fat from goat milk naturally enriched with CLA to investigate the effects of CLA on serum lipids and glucose, body weight, and intestinal and liver histopathological parameters of male rats. They showed that CLA caused an increase in the body weight of the rats from the second to the fifth wk of the experiment, indicating its growth promoting activity in young rats. The CLA

also increased the serum levels of total cholesterol and HDL cholesterol, reduced the levels of triglycerol and triglycerol/HDL cholesterol ratio with no significant effect on LDL cholesterol and serum glucose.

CONCLUSIONS

Generally, CLA is considered as a potential health-promoting factor, which has many beneficial effects on the health of humans and animals, and can prevent many health disorders. In case of CLA application in ruminant husbandry, grazing in comparison to indoor feeding and supplementation of CLA in the diet of animals can increase the concentration of CLA in ruminants products. In addition, microbial fermentation of animal products can be used as an option to increase the concentration of CLA towards production of functional food for human. In poultry industry also, CLA can be used as a dietary supplement to increase the CLA content of tissues, and also to improve performance, lipid metabolism and the function of immune system of poultry. In case of humans, although most of the recent studies confirmed the beneficial effects of CLA such as improving the body weight control parameters in humans and inhibition of different types of cancer in animal models, the CLA effects on improving serum lipid profiles, blood glucose and insulin sensitivity, reducing blood pressure and risk factors of CVD in humans are still controversial, and need to be further investigated using different hosts with different health conditions.

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Table 1. Effects of CLA consumption on different human subjects

Subject	**CLA form**	**CLA dosage**	**Period of consumption**	**Parameter**												**Reference**
				Weight control parameter				Plasma lipid profile				PFG	IS	Inflammatory markers of CVD			
				BW	BMI	BFM	LBM	TC	TG	LDL	HDL			TNF	CRP	IL6	
Overweight and obese subjects	c9,t11 t10, c12	3.4 g/d	12 wk	↓	↓	↓	-	↑	↑	↑	↓	↑	-	-	NE	NE	(Chen *et al.*, 2012)
Overweight and obese men	isomeric mixture	4.5 g/d	4 wk	↓	-	-	-	NE	NE	NE	NE	-	NE	-	NE	-	(Pfeuffer *et al.*, 2011)
Overweight, hyperlipidemic	t10, c12 c9, t11	2.7 and 2.8	three 8-wk phases, each	NE	NE	NE	NE	NE	NE	NE	NE	-	NE	NE (TNF-α)	NE	NE	(Joseph *et al.*, 2011)

men		g/d	separated by a 4-wk washout period														
Healthy subjects	c9, t11 t10, c12	7% of energy of diet	3 consecutive periods of 3 wk	-	-	-	-	-	-	-	-	-	-	NE (TNF-RI and TNF-RII)	NE	NE	(Smit *et al.*, 2011)
Obese, post-menopausal women with diabetes	c9,t11 t10, c12	6.4 g/d	16 wk	-	-	-	-	NE	NE	NE	NE	NE	NE	-	NE	-	(Asp *et al.*, 2011)

type II																	
Obese children aged 6-10 years	c9,t11 t10, c12	3 g/d	7 mo	↓*	NE	↓*	-	-	-	NE	↓	NE	NE	-	-	-	(Racine *et al.*, 2010)
Healthy young men	c9, t11 t10, c12	11 5 g/d	5 wk	NE		-	-	NE	-	NE	NE	NE (Serum glucose)	NE (Serum Insulin)	-	NE	-	(Raff *et al.*, 2008)

*: CLA attenuated the increase of BMI and BFM;

BW: body weight; BMI: body mass index; BFM: body fat mass; LBM: lean body mass; TC: total cholesterol; TG: triglyceride; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; PFG: plasma fasting glucose; IS: insulin sensitivity; TNF: tumor necrosis factor; TNF-R: tumor necrosis factor receptors; CRP: C-reactive protein; IL6: interleukin 6; CVD: cardio vascular disease; ↓: decreasing effect; ↑: increasing effect; NE: no effect; -: not tested

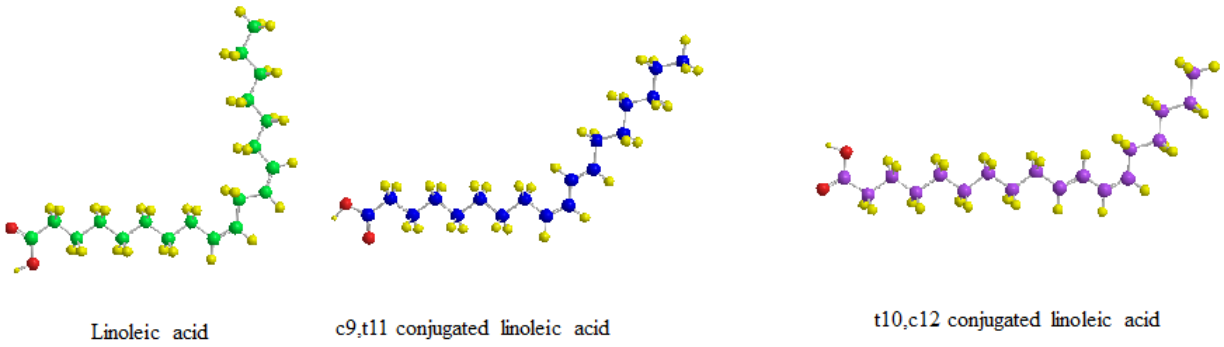

Figure 1. Chemical structure of the parent linoleic acid, and its two main derivative isomers, c9,t11 and t10,c 12 conjugated linoleic acids. The structures were constructed using the ChemBio3D software, version 14.0 from CambridgeSoft.

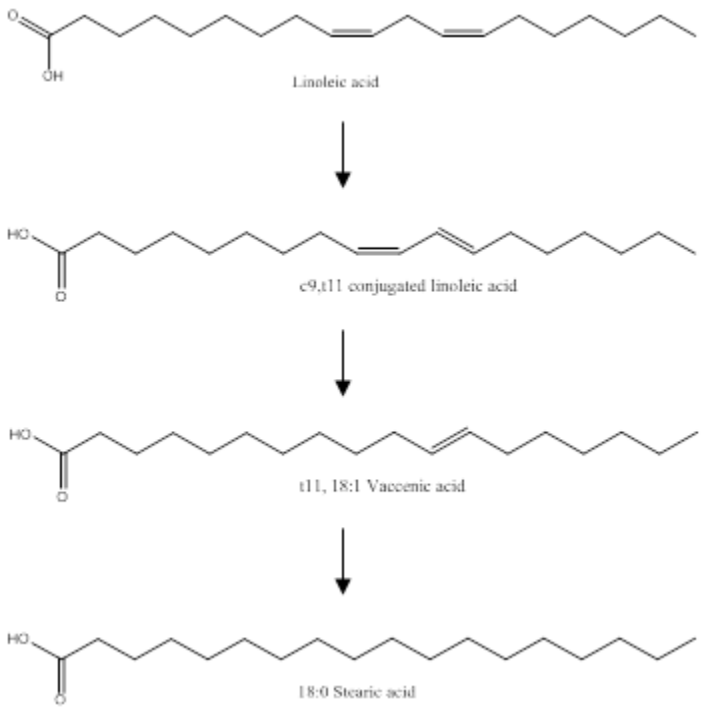

Figure 2. Biochemical pathway for biohydrogenation of linoleic acid by rumen microorganisms. The structures were constructed using the ChemBioDraw software, version 14.0 from CambridgeSoft.

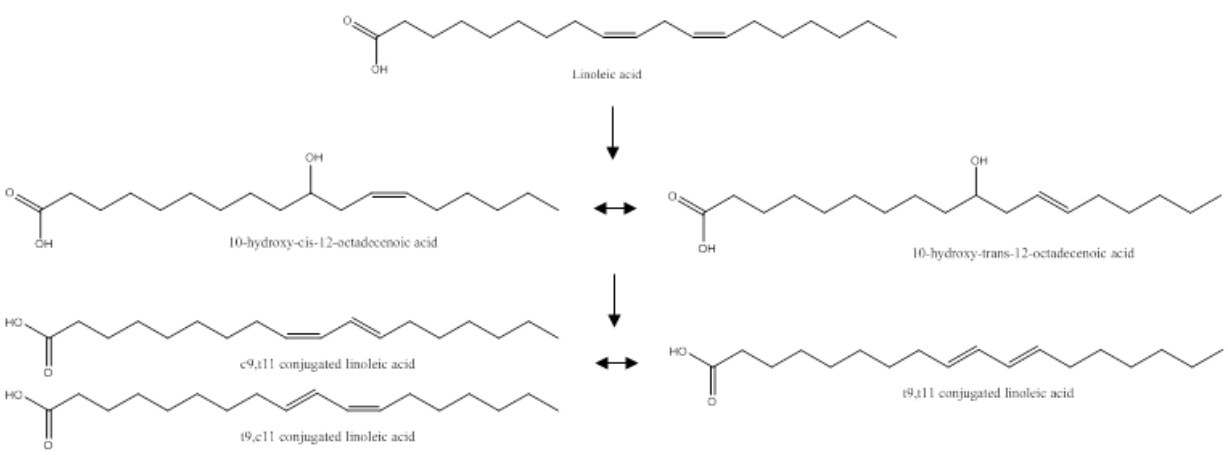

Figure 3. Proposed biochemical pathway of CLA production from linoleic acid using washed cells of *L. acidophilus* AKU 1137 (reproduced from Ogawa *et al.*, 2001, using the ChemBioDraw software, version 14.0 from CambridgeSoft).