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Advances in Research on cis-9, trans-11 Conjugated Linoleic Acid: A Major Functional Conjugated Linoleic Acid Isomer

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Advances in Research on *cis*-9, *trans*-11 Conjugated Linoleic Acid: A Major Functional

Conjugated Linoleic Acid Isomer

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Summary: Conjugated linoleic acid (CLA) consists of a group of positional and geometric conjugated isomers of linoleic acid. Since the identification of CLA as a factor that can inhibit mutagenesis and carcinogenesis, thousands of studies have been conducted in the last several decades. Among the many isomers discovered, *cis*-9, *trans*-11 CLA is the most intensively studied because of its multiple, isomer-specific effects in humans and animals. This paper provides an overview of the available data on *cis*-9, *trans*-11 CLA, including its isomer-specific effects, biosynthesis, *in vivo/in vitro* research models, quantification, and the factors influencing its content in ruminant products.

Keywords: conjugated linoleic acid, *cis*-9, *trans*-11 CLA, isomer-specific effects, biosynthesis, *in vivo/in vitro* research models, quantification

General introduction

Milk, one of the most natural foods, is a good source of nutrients and provides high quality protein, essential fatty acids, minerals, vitamins, and other nutrients (German and Dillard, 2006; Arvidsson, 2009). Milk is processed into numerous products, including butter, cheese, condensed milk, cream, kefir, whey protein, powdered milk, yogurt, and so on (Classification of Dairy Products, 2007). Humans, particularly westerners and nomadic people, consume milk and milk products from animals, especially cattle, goats, and sheep (Bencini and Pulina, 1997; Haug *et al.*, 2007; Jirillo *et al.*, 2010). Due to the high nutritive value and multiple health benefits of milk, global milk consumption has increased significantly, especially in developing countries (Delgado, 2006; Andrea, 2011). Although the components of milk vary according to species, it is a balanced and universally accepted nutritious food (Jensen and Thompson, 1995).

Among the many nutritional ingredients of milk, conjugated linoleic acid (CLA), which is composed of a series of positional and geometric conjugated isomers of linoleic acid (Dilzer and Park, 2011), is uniquely important. Since the identification of CLA as a compound capable of inhibiting mutagenesis and carcinogenesis (Pariza *et al.*, 1979; Ha *et al.*, 1987), thousands of relevant studies have been conducted in the subsequent decades (Bauman, 2002; Pariza, 2004).

The positive health effects of CLA obtained from experimental models include anti-atherosclerotic, anticarcinogenic, and antidiabetic effects, immune system modulation, bone composition modulation, and body fat reduction (MacDonald, 2000; Salas-Salvadó *et al.*, 2006).

The *cis*-9, *trans*-11 CLA isomer was first isolated from milk fat in 1977, and is the most predominantly studied (Pariza *et al.*, 2000; Pariza *et al.*, 2001; Khanal and Dhiman, 2004). The ratio of *cis*-9, *trans*-11 CLA to total CLA in milk and beef is 82-97% and 57-85%, respectively (Dhiman *et al.*, 2005a). The *cis*-9, *trans*-11 CLA isomer exhibits isomer-specific effects both *in vivo* and *in vitro*, including antidiabetic (Moloney *et al.*, 2007), anti-tumor (Hwang *et al.*, 2007; Igal, 2011), anti-inflammatory (Lee *et al.*, 2009), anti-atherosclerosis effects (Lee and Vanden Heuvel, 2010), and immune system modulation (Albers *et al.*, 2003).

This paper provides an overview of the research on *cis*-9, *trans*-11 CLA isomer-specific effects, biosynthesis, *in vivo/in vitro* research models, quantification, and the factors influencing its content in ruminant products.

***cis*-9, *trans*-11 CLA isomer-specific effects**

The *cis*-9, *trans*-11 CLA isomer is biologically active, and its isomer-specific effects include a less severe inflammatory response and protection against the development of atherosclerosis (de Roos *et al.*, 2005), anti-inflammatory effects in human bronchial epithelial cells, eosinophils (Jaudszus *et al.*, 2005), and white adipose tissue (Moloney *et al.*, 2007), decreased activity of atherogenesis-related genes in human umbilical vein endothelial cells, altered adhesion of

macrophages (Lee *et al.*, 2010), and enhanced proliferation of neural progenitor cells, which may be associated with the up-regulation of cyclin D1 expression (Wang *et al.*, 2011). Despite numerous descriptions of the isomer-specific effects of *cis*-9, *trans*-11 CLA, the mechanisms of action remain unclear. Another important CLA isomer, *trans*-10, *cis*-12 CLA, exhibits some effects that are distinct from those of *cis*-9, *trans*-11 CLA (Pariza *et al.*, 2001). It was identified as the isomer responsible for the inhibition of milk fat synthesis in dairy cows (Baumgard *et al.*, 2001). In mice, *trans*-10, *cis*-12 CLA increased liver lipids and decreased adipose tissue lipids, while *cis*-9, *trans*-11 CLA showed no such effects (Warren *et al.*, 2003). In healthy humans, *trans*-10, *cis*-12 CLA increased the ratios of LDL to HDL cholesterol and total to HDL cholesterol, while *cis*-9, *trans*-11 CLA decreased these ratios (Tricon *et al.*, 2004). Research has also suggested that diets rich in *trans*-10, *cis*-12 CLA may be pro-atherogenic, whereas those rich in *cis*-9, *trans*-11 CLA may be anti-atherogenic (Arbonés-Mainar *et al.*, 2006).

Biosynthesis of *cis*-9, *trans*-11 CLA

Cis-9, *trans*-11 CLA is found in many animal products, especially those from ruminants, which may contribute to the unique physiological characteristics of ruminants (Bauman *et al.*, 2000). As shown in Figure 1, *cis*-9, *trans*-11 CLA is *de novo* synthesized from *trans*-11 vaccenic acid (TVA) in the mammary gland (Grinari *et al.*, 2000; Corl *et al.*, 2001; Kay *et al.*, 2004), while additional *cis*-9, *trans*-11 CLA is directly absorbed in the rumen (Bauman *et al.*, 2000). Stearoyl-CoA desaturase (SCD; Δ 9-desaturase) is a critical enzyme that plays a key role in *cis*-9, *trans*-11 CLA

synthesis from TVA in the mammary gland (Griinari *et al.*, 2000; Corl *et al.*, 2001; Kay *et al.*, 2004).

Metabolic production of cis-9, trans-11 CLA and TVA in the rumen

Ruminants possess a unique organ, the rumen, which harbors many microorganisms that can biohydrogenate dietary unsaturated fatty acids. This microbial biohydrogenation has a major influence on the fatty acid composition of ruminant products (Jenkins *et al.*, 2008). Among the many rumen bacteria, *Butyrivibrio fibrisolvens* was the first shown to be capable of biohydrogenation (Kepler *et al.*, 1966). Numerous other rumen bacteria have since been isolated, and their biohydrogenation capacity has been confirmed (Sieber *et al.*, 2004; Ogawa *et al.*, 2005). The biohydrogenation of unsaturated fatty acids in the rumen involves several steps, and no single rumen bacterial species can catalyze complete biohydrogenation (Bauman *et al.*, 2000). Bacteria involved in the metabolic production of *cis*-9, *trans*-11 CLA and TVA are shown in Table 1. These bacteria not only play an important role in the metabolism of *cis*-9, *trans*-11 CLA and TVA in the rumen, but also provide essential precursors for lipogenesis in the mammary gland.

Isomers of CLA in the rumen are synthesized by different mechanisms (Wallace *et al.*, 2007). The ratio of individual CLAs produced in the rumen is significantly affected by diet, which can regulate parameters in the rumen environment, such as pH, osmotic pressure, and rumen content, to name a few (Van Soest, 1994; Martin and Jenkins, 2002). Diet-mediated alterations of the rumen environment often result in bacterial population shifts and consequent changes in the

pattern of fermentation end products (Bauman *et al.*, 2000; Martin and Jenkins, 2002). Therefore, *cis*-9, *trans*-11 CLA content in ruminant products can be regulated by dietary alteration of the rumen environment.

De novo synthesis of cis-9, trans-11 CLA in the mammary gland

In the process of *cis*-9, *trans*-11 CLA *de novo* synthesis in the mammary gland, TVA and SCD play important roles (Bauman *et al.*, 2000). Therefore the published literatures on them were overviewed in this part.

TVA

TVA is a positional and geometric isomer of oleic acid and the precursor of *cis*-9, *trans*-11 CLA (Figure 2A) (Bauman *et al.*, 2000; Field *et al.*, 2009). TVA has received much attention because of its important role in *cis*-9, *trans*-11 CLA biosynthesis (Santora *et al.*, 2000; Turpeinen *et al.*, 2002; Field, 2009) and its health benefits beyond those associated with CLA (Wang *et al.*, 2008; Bassett *et al.*, 2010; Wang *et al.*, 2010). TVA is an intermediate of ruminal biohydrogenation. In the rumen, TVA is absorbed and transported to mammary tissue, adipose tissue, and other sites, where it is converted to *cis*-9, *trans*-11 CLA (Figure 1) (Bauman *et al.*, 2000; Kay *et al.*, 2004). TVA content in ruminant products can be regulated by altering feed (Mozaffarian *et al.*, 2006). Therefore, supplying feed containing TVA precursor is an effective means of enhancing the *cis*-9, *trans*-11 CLA concentration in ruminant products (Shen *et al.*, 2007).

SCD

SCD is an enzyme located in the endoplasmic reticulum (ER) that contains four transmembrane domains with the N and C termini oriented toward the cytosol (Figure 2B). It catalyzes Δ^9 -*cis* desaturation of a range of fatty acyl-CoA substrates (Man *et al.*, 2006; Paton and Ntambi, 2009). Two SCD isoforms, SCD1 and SCD5, have been discovered in ruminant species; SCD1 predominates in mammary and adipose tissue, while SCD5 is highly expressed in the brain (Lengi and Corl, 2007). In the mammary gland, SCD plays a critical role in the *de novo* synthesis of *cis*-9, *trans*-11 CLA, which accounts for 78% of this isomer in milk fat (Griinari *et al.*, 2000; Corl *et al.*, 2001; Kay *et al.*, 2004). SCD is generally regulated at the transcriptional and translational level by numerous effectors, including hormones and dietary factors (Ntambi and Miyazaki, 2004; Ntambi *et al.*, 2004; Mauvoisin and Mounier, 2011). In addition, some synthetic materials can also regulate SCD activity (Liu, 2009). Altering the factors that regulate SCD expression/activity will theoretically modify the content of *cis*-9, *trans*-11 CLA.

***In vivo* research models of *cis*-9, *trans*-11 CLA biosynthesis**

Ruminants

In *cis*-9, *trans*-11 CLA biosynthesis research, ruminants are widely employed as *in vivo* models. Because of the unique physiological characteristics of ruminants (Bauman *et al.*, 2000), *cis*-9, *trans*-11 CLA predominantly exists in ruminant products like milk, beef, and lamb meat (Chin *et al.*, 1992). The *cis*-9, *trans*-11 CLA in milk is produced by the mammary gland (Griinari *et al.*,

2000; Corl *et al.*, 2001; Kay *et al.*, 2004) and the rumen (Bauman *et al.*, 2000; Jenkins *et al.*, 2008).

The individual quantitative importance of these two sources was unknown until data from experiments using lactating cows were reported (Griinari *et al.*, 2000; Corl *et al.*, 2001; Kay *et al.*, 2004). The results indicated that *de novo* synthesis in the mammary gland is the primary source of *cis*-9, *trans*-11 CLA in milk fat, accounting for 78% of the isomer. Several experiments aimed at investigating the amount of supplementations that can enhance *cis*-9, *trans*-11 CLA content in milk were undertaken in dairy sheep (Gomez-Cortes *et al.*, 2008; Gomez-Cortes *et al.*, 2009; Gomez-Cortes *et al.*, 2011). These studies suggested that individual and breed differences could affect the CLA content in milk (Mihailov and Odjakov, 2011; Mierlita *et al.*, 2011). The *cis*-9, *trans*-11 CLA and total fatty acid contents differ between sheep and goats consuming the same diet, which is partially attributable to differences in the mRNA of SCD and lipogenic genes in the mammary gland (Tsiplakou and Zervas, 2008; Tsiplakou *et al.*, 2009). In addition, in a proteomic analysis of lactating goat mammary gland tissue, candidate proteins involved in *cis*-9, *trans*-11 CLA *de novo* synthesis were discovered (Jin, 2011).

Non-ruminants

Murine animals are used in *cis*-9, *trans*-11 CLA biosynthesis research not only because they can be treated simultaneously and easily handled, but also because the testing expenses are quite low. The well-known process of TVA conversion to *cis*-9, *trans*-11 CLA *via* Δ 9-desaturase in ruminants has also been identified in mice (Loor *et al.*, 2002; Santora *et al.*, 2000) and rats (Banni

et al., 2001). During the treatment with TVA, three differently expressed proteins, protein disulfide-isomerase precursor (PDI) and laminin receptor 1 (LAMR1) in the rat mammary gland, and peroxiredoxin-2 (PRDX2) in the mouse mammary gland epithelia cells (HC11) were suggested may be related to CLA biosynthesis (Jin *et al.*, 2010). But further studies are needed to confirm these findings.

***In vitro* research models of *cis*-9, *trans*-11 CLA biosynthesis**

For reasons of feasibility and efficacy, *cis*-9, *trans*-11 CLA biosynthesis research using large ruminants is difficult, especially when tissue sampling is needed. Therefore, alternative use of *in vitro* models, including cultured bovine mammary epithelial cell lines, milk somatic cells, and milk epithelial cells has been explored.

Cultured bovine mammary epithelial cell lines

Two bovine mammary epithelial cell lines frequently used in *cis*-9, *trans*-11 CLA related research are reviewed here. A bovine mammary alveolar cell line, MAC-T, characterized by immortality, the ability to uniformly differentiate, and casein protein secretion, has often been used (Huynh *et al.*, 1991). Through using MAC-T cell, the lipid depression of *trans*-10, *cis*-12 CLA, but not *cis*-9, *trans*-11 CLA, was identified through inhibition of the proteolytic activation of SREBP-1 (Peterson *et al.*, 2004); and the transcriptional responses of bovine mammary cell to acetate, linoleic acid (*cis*-9, *cis*-12-18:2) or α -linolenic acid (*cis*-9, *cis*-12, *cis*-15-18:3) treatment were investigated (Jacobs, 2011). When MAC-T cells were treated with t11 C18:1 at 50 μ M for

0-6 h, t11 C18:1 was continually absorbed and converted into *cis*-9, *trans*-11 CLA (Jin, 2011). In addition, at high doses, both *cis*-9, *trans*-11 CLA (30 μ M) and *trans*-10, *cis*-12 CLA (30 μ M) had detrimental effects on the growth and survival of MAC-T cell (Keating *et al.*, 2008). In BME-UV cells (Bovine Mammary Epithelial Clone-University of Vermont) (Zavizion *et al.*, 1996), the milk fat depression effects were confirmed to be related to *trans*-10, *cis*-12 CLA, but not *cis*-9, *trans*-11 CLA through inhibition of basal- and PRL-induced IDH1 mRNA expression (Liu *et al.*, 2006). Generally, these two mammary epithelial cell lines simulate the normal physiological status of the bovine mammary gland. They can be treated simultaneously and sampled easily, enabling large-scale experiments with precise aims. The results appear to be comparable to the real-life situation in animals at a fraction of the cost. Because of these advantages, mammary epithelial cell lines have become a valuable tool for functional studies of the mammary gland.

Milk somatic cells

Generally milk somatic cells include leukocytes (polymorphonuclear neutrophils, macrophages, and lymphocytes) and a small percentage of epithelial cells exfoliated from mammary alveolar tissue (Boutinaud and Jammes, 2002; Sarikaya *et al.*, 2006). These cells can be distinguished by their size and shape utilizing flow cytometry (Keys *et al.*, 1997; Sarikaya, 2006) or modified Pappenheim method (Sarikaya, 2006). In different animal species, the number and type of these cells in milk varies, which partially contributes to distinctive milk secretions (Wooding *et al.*, 1977; Boutinaud and Jammes, 2002) (Table 2). In the same species under normal healthy

conditions, the total somatic cells in milk change with variations in numerous factors, including seasonal, diurnal, and milk volume variations, lactation stage, parity, and management practices (Boutinaud and James, 2002). Moreover, variations in somatic cell count were observed in different breeds of cow (Rupp *et al.*, 2000). In addition, storage temperature and time influence the viability of somatic cells in milk samples. In normal practice, somatic cells should be extracted from fresh milk or milk stored at 4°C in less than 30 hours (Feng *et al.*, 2007).

Somatic cells are present in milk throughout the lactation period (Boutinaud *et al.*, 2002). Originally, the number of somatic cells was used as an indicator of milk quality (Moon *et al.*, 2007). Subsequent research found that somatic cells extracted from fresh milk could be used as a model for the study of mammary gland lactogenesis (Boutinaud and Jammes, 2002; Boutinaud *et al.*, 2002; Murrieta *et al.*, 2006). To study SCD expression in dairy cows, milk somatic cells was used as a source of mRNA, which offers a non-invasive alternative to mammary tissue samples obtained by biopsy (Feng *et al.*, 2007; Jacobs, 2011). In a functional study of the proteins involved in *cis*-9, *trans*-11 CLA *de novo* synthesis, goat milk somatic cells were employed, and six candidate proteins with various functions were found (Jin, 2011). The use of somatic cells is a significant improvement over the use of biopsy samples, and somatic cell use allows for easy and repetitive sampling in large-scale studies without damage to the mammary gland.

Mammary epithelial cells in milk

Alveoli epithelial cells of the mammary gland are subject to turnover, and must be continuously replaced by new cells. These displaced cells are partially discharged into the milk and form a small part of the resident somatic cells (Sarıkaya, 2006). The number of epithelial cells in milk from normal healthy animals is affected by milk secretion, lactation stage, and milking methods (Boutinaud and Jammes, 2002). Most of the epithelial cells in milk are viable and exhibit the characteristics of fully differentiated alveolar cells (Boutinaud and Jammes, 2002). In contrast to milk secretion in goat and human, bovine milk secretion is merocrine, which makes the number of epithelial cells in bovine milk very low (Boutinaud and Jammes, 2002). Therefore, an immunomagnetic method was used to separate epithelial cells from bovine milk, and these purified cells had better total RNA quality and seemed to be more suitable for bovine mammary transcripts research than total milk somatic cells (Boutinaud *et al.*, 2008). Although there is little data on the use of milk epithelial cells in *cis*-9, *trans*-11 CLA research, in our lab, studies using bovine milk epithelial cells to explore the proteins involved in *cis*-9, *trans*-11 CLA *de novo* synthesis are current underway.

Quantification of *cis*-9, *trans*-11 CLA

The identification of the various beneficial effects of *cis*-9, *trans*-11 CLA has heightened interest in the manufacture of *cis*-9, *trans*-11 CLA-fortified products (Dhiman *et al.*, 2005a; Rodríguez-Alcalá and Fontecha, 2007). Therefore, reliable and precise techniques to identify and

quantify *cis*-9, *trans*-11 CLA and its precursor, TVA, are necessary (Table 3). Among the many methods, gas chromatography (GC; Rodríguez-Alcalá and Fontecha, 2007; Petrović *et al.*, 2010), silver-ion high-performance liquid chromatography (Ag^+ -HPLC; Sehat *et al.*, 1998a; Rodríguez-Castañedas *et al.*, 2011), and nuclear magnetic resonance spectroscopy (NMR; Lie Ken Jie, 2001) are routinely used.

GC

GC is a common type of chromatography used in analytical chemistry to separate and analyze compounds that can be vaporized without decomposition (Pavia *et al.*, 2006). This method has been used extensively for *cis*-9, *trans*-11 CLA quantification because of its high precision, simple pretreatment and high sensitivity with small sample size (Morrison *et al.*, 1964; Wang *et al.*, 2006). It can sensitively detect *cis*-9, *trans*-11 CLA in milk fat (Kramer *et al.*, 2004), rumen bacteria/fluid (Chand *et al.*, 2001), muscle tissues (Aldai *et al.*, 2006), and cheese (Sehat *et al.*, 1998b; Prandini *et al.*, 2011). In common practice, GC analysis must be preceded by selection of a good internal and non-interfering standard, conversion of fatty acids into detectable methyl esters, and selection of the appropriate column to separate the fat mixture. In addition, careful attention should be paid on the requirements of splitless injections because the contents and compositions of fatty acids are various among tissues. Otherwise, overload or underload of the phase maybe happen.

A multidimensional separation technique of 2-dimensional GC (GC*GC) was developed to analyze complex milk fat (Vlaeminck *et al.*, 2007). In this technique, a milk complex is separated by two combined columns (nonpolar/polar or polar/nonpolar arrangement) with an intervening modulator. Then, the chromatographic data are collected and analyzed by transform software (Fortner Software LLC, Sterling, VA, USA). Finally, well-ordered structures of fatty acids are displayed as a 2-dimensional contour plot according to the number of carbon atoms and the degree of unsaturation (Vlaeminck *et al.*, 2007). However, the separation efficiency and stability of this technique needs further refinement to separate CLA isomers and *trans* 18:1 isomers. Another method, gas chromatography-mass spectrometry (GC-MS) was developed to simultaneously determinate *cis*-9, *trans*-11 CLA and other important biological compounds like cholesterol and cholesterol oxidation products (COPs) (Yen *et al.*, 2010).

Ag⁺-HPLC

Ag⁺-HPLC is often used to separate compounds that can be dissolved in solution. The instrumentation usually consists of a mobile phase reservoir, pump, injector, separation column, and detector. *Ag⁺*-HPLC has been validated as reliable for the quantification of CLA isomers in foods (Sehat *et al.*, 1998a), cow milk fat (Collomb *et al.*, 2004), human milk fat (Luna *et al.*, 2007), beef fat (Dance *et al.*, 2010), and ewe milk fat (Rodríguez-Castañedas *et al.*, 2011). With respect to HPLC the main shortcoming is its inability on the detection of coelution which may lead to inaccurate compound categorization.

NMR

NMR is an extremely important analytical technique for the organic chemist. The first report on the application of this technique for fatty compounds analysis was published in 1959 (Hopkins and Bernstein, 1959; Knothe, 2010). Nowadays, NMR is considered an ideal technique for determining the position of double bonds and the configuration of the conjugated diene system of CLA (Lie Ken Jie, 2001; Maria *et al.*, 2010). Two types of NMR, ^1H -NMR and ^{13}C -NMR, are frequently used in the structural analyses and quantification of fatty acids and their derivatives. General speaking, NMR has a higher capacity and faster speed compared with GC. Moreover, it is simple and does not require sophisticated sample preparation (Maria *et al.*, 2010). But an inevitable consequence of carrying out NMR investigations is the need to work in a high-magnetic-field environment which can affect equipment routinely used in lab (Chatham and Blackband, 2001).

Overall, the analytical techniques mentioned above were widely employed in the determination of CLA and TVA profiles in ruminant products. But milk fat contains about 400 different fatty acids (Jensen, 2002; Lindmark-Månsson, 2008). Among these fatty acids, CLA and TVA provide many challenges to lipid analysts because of their unique physical properties and various positional and geometric isomers (Kramer *et al.*, 2004). So far, no single method can simultaneously separate all milk fats; therefore, combined methods are applied, which show high separation efficiency. The combination of GC and Ag^+ -HPLC can analyze CLA isomers in dairy

fats and animal tissues (Kraft *et al.*, 2003; Rodríguez-Alcalá and Fontecha, 2007), while the combination of gas liquid chromatography and argentation thin-layer chromatography (Ag-TLC) can efficiently analyze *trans* 18:1 isomers in beef meat, cow colostrum, and milk fat (Precht and Molkentin, 1996; Paszczyk *et al.*, 2005).

Factors influencing *cis*-9, *trans*-11 CLA content in raw ruminant products

Normally, *cis*-9, *trans*-11 CLA is relatively abundant in raw ruminant products, including milk, beef, and lamb meat (Pariza *et al.*, 1990; Bauman *et al.*, 2000; Jenkins *et al.*, 2008). However, the average consumption of CLA from ruminant products by adults is much less than the amount needed to reduce cancer in animal studies (Dhiman *et al.*, 2005a; Rodríguez-Alcalá and Fontecha, 2007). So it is necessary to enhance CLA intake, but this can be impractical. Therefore, increasing *cis*-9, *trans*-11 CLA content in ruminant products seems to be a good choice; it would not only increase the effective dose, but also improve the nutritive and therapeutic value of ruminant products. Various factors can regulate/affect the *cis*-9, *trans*-11 CLA content in ruminant products (Dhiman *et al.*, 1999; Dhiman *et al.*, 2005a; Slots *et al.*, 2008), and *cis*-9, *trans*-11 CLA precursor supplements, lactation period, and seasonal variations will be discussed in the following sections.

***cis*-9, *trans*-11 CLA precursor supplements**

Because ruminant diet provides substrates for *cis*-9, *trans*-11 CLA biosynthesis, it is the most important factor regulating *cis*-9, *trans*-11 CLA content in ruminant products. α -Linolenic acid and linoleic acid are the two major precursors of *cis*-9, *trans*-11 CLA, which are typically found in

plant oilseeds. With supplements that are rich in these two precursors, the *cis*-9, *trans*-11 CLA content of ruminant products can theoretically be improved. The α -linolenic acid and linoleic acid contents in the major oilseeds are shown in Table 4. As a common practice, supplying one or more of these oilseeds can increase *cis*-9, *trans*-11 CLA content in beef (Dhiman *et al.*, 2005b) and milk (Kelly *et al.*, 1998; Liu *et al.*, 2007; Hervás *et al.*, 2008). However, reduced milk fat synthesis is also observed, which may be attributed to the isomer-specific effect of *trans*-10, *cis*-12 CLA (Baumgard *et al.*, 2001). CLA mixture supplementation or postruminal infusion can also effectively alter *cis*-9, *trans*-11 CLA content in ruminant products (Bell and Kennelly, 2003; Sigl *et al.*, 2010). However, physical or chemical modification of CLA should be done to enhance their oxidative stability and improve their post-ruminal bioavailability (de Veth *et al.*, 2005; Gervais *et al.*, 2005; Moon *et al.*, 2008).

Lactation period

Lactation stage has an effect on *cis*-9, *trans*-11 CLA content in milk (Kay *et al.*, 2005; Stoop *et al.*, 2009). During early lactation (before 100 days) in Canadian Jersey cows, *cis*-9, *trans*-11 CLA content is significantly lower than that in a later stage (after 200 days) (Kgwatalala *et al.*, 2009). A similar monitoring study in our lab also found that *cis*-9, *trans*-11 CLA content in the milk of Holstein dairy cows continually increased from the early lactation period to the late lactation period (0.35% vs. 0.52%; Oh, 2012). In addition, the total CLA content in Holstein-Friesian cow

milk increased considerably, and remained high from five to eight months of lactation (Borowiec *et al.*, 2010).

Seasonal variations

The seasonal variations of *cis*-9, *trans*-11 CLA and TVA content in milk have been investigated in different countries (Elgersma *et al.*, 2006). In Nordic countries, the concentration of *cis*-9, *trans*-11 CLA in milk during summer was higher than during winter, and it seemed lower than that in European countries, which may due to the shorter summers and longer winters in Nordic countries (Thorsdottir *et al.*, 2004). In the Azores, a higher proportion of *cis*-9, *trans*-11 CLA (1.43% vs. 0.96% fatty acid) and TVA (3.2% vs. 2.2% fatty acid) was observed in spring milk compared to that in winter milk (Rego *et al.*, 2008). In Japan, a study documented a 3-fold higher concentration of *cis*-9, *trans*-11 CLA (1.96% vs. 0.57% FA) and TVA (4.22% vs. 1.47% FA) in summer milk (June) than those in winter milk (December) from Holstein dairy cows that grazed for whole days (Zunong *et al.*, 2008). In a study conducted in Holland, the ratios of both *cis*-9, *trans*-11 CLA (0.76% vs. 0.38% FA) and TVA (1.6% vs. 0.76% FA) to total fatty acids were about twice as high in summer milk (August) than in winter milk (February) (Heck *et al.*, 2009). Generally, these seasonal variations in *cis*-9, *trans*-11 CLA and TVA content in milk are believed to be caused by seasonal variations in α -linolenic acid content in the diet, which is higher in the summer diet (Lock and Garnsworthy, 2003; Meřuchová *et al.*, 2008; Zunong *et al.*, 2008; Butler *et al.*, 2009).

Conclusions and future prospects

The present review provides a better understanding of the recent advances in research on *cis*-9, *trans*-11 CLA, including its isomer-specific effects, biosynthesis, *in vivo/in vitro* research models, quantification protocols, and factors influencing its content in ruminant products. However, proteins involved in *cis*-9, *trans*-11 CLA *de novo* synthesis and the pathways *via* SCD in the ruminant mammary gland are still not clearly understood. Further research is needed, particularly on the diversified molecular signaling pathways controlling *cis*-9, *trans*-11 CLA synthesis and the crosstalk among these pathways.

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Table 1. Bacterial strains in the metabolic production of *cis*-9, *trans*-11 CLA and *trans*-11 vaccenic acid.

Strain	Substrate	Production	References
<i>Bifidobacterium breve</i>	linoleic acid	<i>cis</i> -9, <i>trans</i> -11 CLA	Park <i>et al.</i> , 2011
<i>Butyrivibrio fibrisolvens</i>	<i>cis</i> -9, <i>trans</i> -11 CLA	<i>trans</i> -11 TVA	Kepler <i>et al.</i> , 1966
<i>Lactobacillus acidophilus</i>	linoleic acid	<i>cis</i> -9, <i>trans</i> -11 CLA	Irmak <i>et al.</i> , 2006
<i>Lactobacillus brevis</i>	sunflower oil	CLA	Puniya <i>et al.</i> , 2008
<i>Lactobacillus lactis</i>	sunflower oil	CLA	Puniya <i>et al.</i> , 2008; Rodríguez-Alcalá <i>et al.</i> , 2010
<i>Lactobacillus plantarum</i> AKU 1009a	linoleic acid	<i>cis</i> -9, <i>trans</i> -11 CLA	Kishino <i>et al.</i> , 2002; Ogawa <i>et al.</i> , 2005
<i>Lactobacillus reuteri</i>	linoleic acid	<i>cis</i> -9, <i>trans</i> -11 CLA	Lee <i>et al.</i> , 2003
<i>Propionibacterium</i> <i>freudenreichi</i>	linoleic acid	<i>cis</i> -9, <i>trans</i> -11 CLA	Jiang <i>et al.</i> , 1998

Table 2. Types and distribution of somatic cell in milk of animals without mastitis.

Species	Milk secretions	Breed	Cell distributions (%)				References
			Epithelial cells	PMN	Macrophages	Lymphocytes	
Bovine	Merocrine	NM	NM	28	7.61	64.39	Gargouri <i>et al.</i> , 2008
Sheep	Merocrine	Comisana ewes	2.2	30.6	57.3	8.2	Morgante <i>et al.</i> , 1996
Goat	Apocrine	Verata ewes	<13	63	16.2	7.2	Rota <i>et al.</i> , 1993

PMN, polymorphonuclear neutrophils; NM, not mentioned

Table 3. Quantification methods for *cis*-9, *trans*-11 CLA and *trans*-11 vaccenic acid.

Items	Samples	References
GC	Milk fat;	Kramer <i>et al.</i> , 2004
	Rumen bacteria/fluid;	Chand <i>et al.</i> , 2001
	Muscular tissues;	Aldai <i>et al.</i> , 2006
	Cheese	Prandini <i>et al.</i> , 2011
Ag ⁺ -HPLC	Foods;	Sehat <i>et al.</i> , 1998a
	Cow milk fat;	Collomb <i>et al.</i> , 2004
	Human milk fat;	Luna <i>et al.</i> , 2007
	Beef fat;	Dance <i>et al.</i> , 2010
2-D GC	Ewe milk fat	Rodri'guez-Castan'edas <i>et al.</i> , 2011
GC-MS	Milk fat	Vlaeminck <i>et al.</i> , 2007
	Fatty acid	Yen <i>et al.</i> , 2010
GC + Ag ⁺ -HPLC	Dairy fats, animal tissues	Kraft <i>et al.</i> , 2003; Rodri'guez-Alcala' and Fontecha, 2007
GLC + Ag-TLC	Cow colostrum, Milk fat	Precht and Molkentin, 1996; Paszczyk <i>et al.</i> , 2005
NMR	Castor oil, Beef	Lie Ken Jie <i>et al.</i> , 1997; Maria <i>et al.</i> , 2010

GC: Gas chromatography; Ag⁺-HPLC: Ag⁺-high-performance liquid chromatography; 2-D GC: Two-Dimensional Gas Chromatography; GC-MS: gas chromatography-mass spectrometry; GC + Ag⁺-HPLC: Gas chromatography + Ag⁺-high-performance liquid chromatography; GLC + Ag-TLC: Gas liquid chromatography + argentation thin-layer chromatography; NMR: Nuclear magnetic resonance spectroscopy

Table 4. α -Linolenic acid and linoleic acid content (%) in major oilseeds.

Items	α -Linolenic acid	Linoleic acid	References
Canola oil	11	21	Comparison of Dietary Fats Chart, 2008
Corn oil	0.7	57.1	Dupont <i>et al.</i> , 1990
Cotton oil	0-0.4	46.7-58.3	O'Brien <i>et al.</i> , 2005
Linseed oil	45-71	12-24	O'Brien <i>et al.</i> , 2005
Olive oil	0-1.5	3.5-21	Kiritsakis <i>et al.</i> , 1998
Peanut oil	0.02-0.04	13.9-35.13	Worthington and Holley, 1967
Safflower oil	1	14	Comparison of Dietary Fats Chart, 2008
Sesame oil	<1	34.6-54.9	Kamal-Eldin, 1995
Soybean oil	8	54	Comparison of Dietary Fats Chart, 2008
Sunflower oil	0-1	48.3-74	Kelly <i>et al.</i> , 1998; Warner <i>et al.</i> , 2003

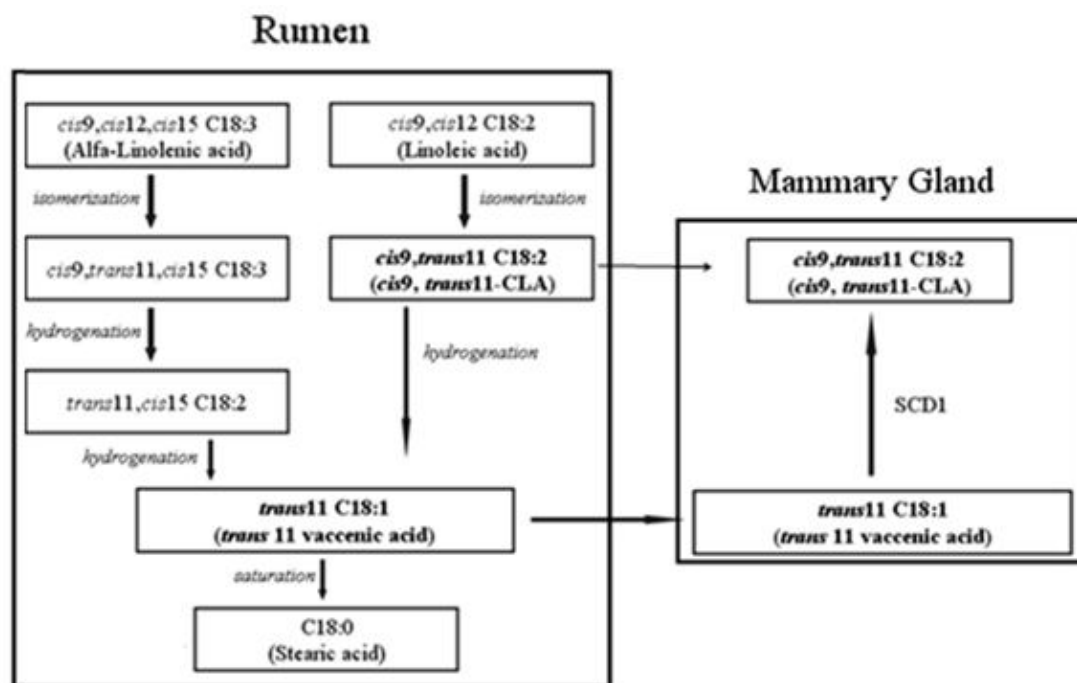
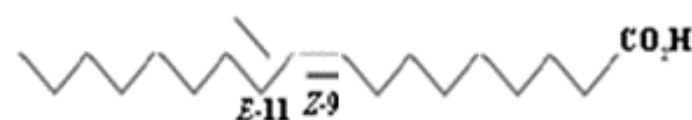


Figure 1. Biosynthesis of *cis*-9, *trans*-11 conjugated linoleic acid.

(Adapted from Bauman *et al.*, 2000; Griinari *et al.*, 2000; Corl *et al.*, 2001; Kay *et al.*, 2004; Jenkins *et al.*, 2008; McKain *et al.*, 2010)



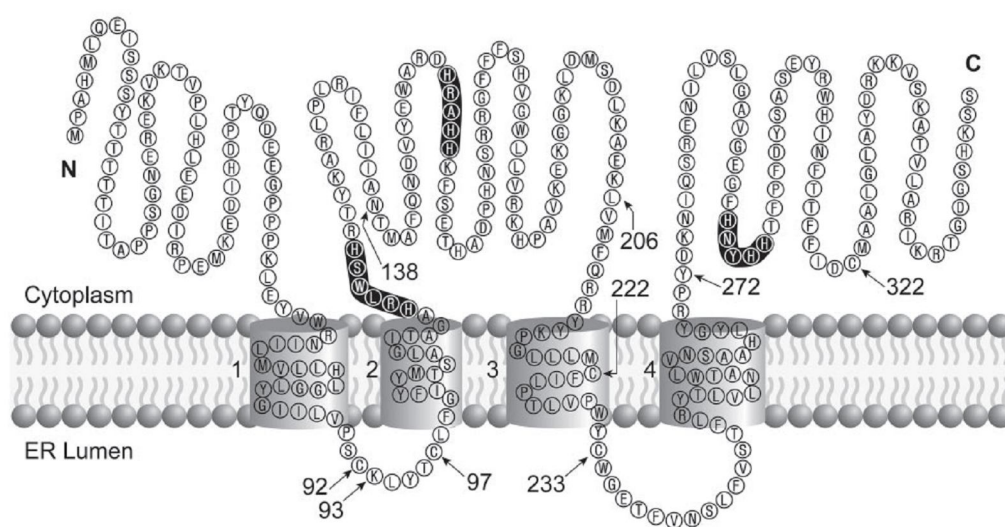
a. *trans*-11 vaccenic acid



b. *cis*-9, *trans*-11 conjugated linoleic acid

A. a. *trans*-11 vaccenic acid; b. *cis*-9, *trans*-11 conjugated linoleic acid

(Adapted from Bauman *et al.*, 2000; Field *et al.*, 2009)



B. proposed models for the membrane topology of SCD-1 (mouse)

(Adapted from Man *et al.*, 2006; have got permission from Pro. Ntambi)

Figure 2. Structure of *trans*-11 vaccenic acid, *cis*-9, *trans*-11 conjugated linoleic acid and proposed models for the membrane topology of SCD-1 (mouse)