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# Effect of Dietary and Animal Factors on Milk Fatty Acids Composition of Grazing Dairy Cows: A Review

**Review Article**  **A.I. Roca Fernandez[1*] and A. Gonzalez Rodriguez[1]**

[1] Centro de Investigaciones Agrarias de Mabegondo. INGACAL, Apdo 10, 15080, A Coruna, Spain

*Correspondence E-mail: anairf@ciam.es

## ABSTRACT

In pasture-based milk production systems, using available farm resources in order to reduce feeding costs, the composition and functional properties of dairy cows' milk are of considerable importance to the dairy farmer, manufacturer and consumer. Basically, there are three options for altering the composition and/or functional properties of milk: 1) cow nutrition and grazing management, 2) cow genetics and 3) dairy manufacturing technologies. At the farm levels, manipulation of milk composition only occurs when it is perceived to be more profitable, normally, processing options are the option most considered. Nevertheless, opportunities exist for manipulation of milk composition on the farm to improve the human and physiological properties of milk and dairy products, such as enhanced concentrations of conjugated linoleic acid (CLA), or to improve its milk fatty acids (FA) composition for more efficient processing into a range of dairy products. This review considers the effect of dietary (pasture feeding, lipid feed supplements, seasonal and regional variations) and animal (breed, stage of lactation, parity and animal to animal) factors on milk FA composition of grazing dairy cows. Furthermore, it highlights the relevance that these factors, in the context of an integrative-view, might play on the sustainability of pasture-based milk production systems in humid areas in terms of milk differentiation and higher added value which profit would be directly reached for the milk producer on the farm.

**KEY WORDS** conjugated linoleic acid, dairy cattle, lipid feed supplements, milk fatty acids profile, pasture feeding, rumen biohydrogenation.

## INTRODUCTION

**Role of milk fatty acids on dairy products**

While milk from dairy cattle is widely recognized as a valuable natural food source containing beneficial amino acids and bioactive peptides with potential antihypertensive, antithrombotic and antimicrobial activities (Park *et al.* 2007), the role of fats in milk is often not well understood (Elwood *et al.* 2008). Milk has been criticized in the past for its high content of saturated fats, which are damaging to human health. Thus, it is not surprising that milk fat, containing about 70% of the fatty acids (FA) as saturated (SFA), 25% as monounsaturated (MUFA) and 5% as poly-

unsaturated (PUFA) (Grummer, 1991), has been considered as an important determining factor to humans' health for a long-time. Nevertheless, it is worth noting that probably only some of these SFA such as C12:0, C14:0 and C16:0 would be considered as cholesterol-raising saturates, while other SFA contained in milk such as C4:0, C6:0, C8:0, C10:0 and C18:0 might suppose no risk of cardiovascular disease (Parodi, 2009). Jensen (2002) has catalogued approximately 400 different FA in milk fat most which are products of ruminal microbial modification of dietary FA. The FA of ruminant milk has a dual origin. Those of chain length C4:0 to C14:0 are derived from *de novo* synthesis in the mammary gland, whereas those of C18:0 and longer are

derived from the diet. The FA of greatest proportion (C16:0) arises from both sources; the relative amount of each source can be influenced by the diet. Ashes *et al.* (1992) reported that feeding encapsulated canola seeds (60% oleic acid) significantly reduced the proportions of lauric, myristic and palmitic acid in milk fat while increasing oleic acid. The resulting fat-modified milk contained 51% of SFA, 39% of MUFA and 10% of PUFA. When men and women consumed fat-modified dairy products, their plasma total cholesterol were reduced 4.5% in comparison with the intake of conventional dairy products (Noakes *et al.* 1996). High-fat diets, especially those rich in saturated fats, can elicit detrimental effects on cardiovascular disease risk factors such as blood low density lipoprotein (LDL) cholesterol (Williams, 2000; Shingfield *et al.* 2008; Parodi, 2009). Cardiovascular risk, however, might be reduced by lowering the intake of undesirable saturated fats or by making alterations in the quality of the fat consumed due to changes on the animals' diet with the aim of increasing the proportion of unsaturated (UFA) and decreasing saturated (SFA) (Dewhurst *et al.* 2003a; Elgersma *et al.* 2003c; Khanal and Olson, 2004) by using fresh forage as the main source of nutrients for feeding dairy cattle.

Modifications in the milk FA profile can be obtained when animals are fed with higher rations of fresh forage (Elgersma *et al.* 2004a) or rich in dietary lipid supplemented with oilseeds which allow beneficial UFA to pass into milk (Chilliard *et al.* 2006) by increasing the conjugated linoleic acid (CLA) levels and decreasing the ratio of *n*-6: *n*-3 FA. These beneficial UFA are believed to have positive implications on human health (Pariza *et al.* 2001; Jensen, 2002; Parodi, 2009) through reducing the risk of cardiovascular disease and they may also reduce the incidence of some cancers such as asthma, diabetes and atherosclerosis, which enhanced bone mineralization and modulation of the immune system (Belury, 1995; Banni and Martin, 1998; Houseknecht *et al.* 1998). Care must be taken when introducing lipid feed supplements into rations as some of them might reduce milk yield, milk fat and protein content (Bauman and Griinari, 2001; Roca-Fernández *et al.* 2011a). An increasing understanding of animal diet specialists in UFA could play, therefore, a valuable role in creating niche markets for animal products. Nevertheless, consumer perception and acceptance will be the driving force behind the success of these dairy products.

**Rumen biohydrogenation and mammary gland lipid synthesis**

The lipid composition of fresh forages consists largely of glycolipids and phospholipids, and the major FAs are the UFA, as linolenic and linoleic acid. While, the lipid composition of seed oils used in concentrate feedstuffs is pre-dominantly triglycerides containing linoleic and oleic acid as the predominant FA. When consumed by ruminant animals, dietary lipids undergo two transformations in the rumen (Dawson and Kemp, 1970; Keeney, 1970; Dawson *et al.* 1977). The initial transformation is the hydrolysis of the ester linkages catalyzed by microbial lipases. This step is a prerequisite for the second transformation, the biohydrogenation of the UFA. Bacteria are largely responsible for biohydrogenation of UFA in the rumen; protozoa seem to be of only minor importance (Harfoot and Hazlewood, 1988). For a number of years, the only bacterium known to be capable of biohydrogenation was *Butyrivibrio fibrisolvens* (Kepler *et al.* 1966). However, nowadays a range of other rumen bacteria have been isolated that have the capacity to biohydrogenate UFA (Harfoot and Hazlewood, 1988). Investigations with pure cultures suggest that no single species of rumen bacteria catalyzes the complete biohydrogenation sequence. Kemp and Lander (1984) divided bacteria into two groups based on the reactions and the end products of biohydrogenation. Group-A bacteria were able to hydrogenate linoleic acid and α-linolenic acid, *trans-11* C18:1 being their major end product. Group-B bacteria utilized *trans-11* C18:1 as one of the main substrates with stearic acid being the end product.

The biohydrogenation sequence of linoleic acid is similar to that of linolenic acid. Isomerization of the *cis-12* double bond represents the initial step during biohydrogenation of FA containing a *cis-9*, *cis-12* double bond system. The isomerase reaction is unusual because it has no cofactor requirement and occurs in the middle of a long hydrocarbon chain remote from any activating functional groups. Linoleate isomerase is the enzyme responsible for forming conjugated double bonds from the *cis-9*, *cis-12* double bond structure of linoleic as well as α- and γ-linolenic acids. The second reaction is the conversion of *cis-9*, *trans-11* CLA into *trans-11* C18:1. Hydrogenation of the *trans-11* monoene occurred less rapidly and, thus, it's concentration increased (Tanaka and Shigeno, 1976; Singh and Hawke, 1979). Reduction of Trans-11 C18:1 seems to be rate-limiting in the biohydrogenation sequence of C18:0. As a consequence, this penultimate biohydrogenation intermediate accumulates in the rumen (Keeny, 1970) and, therefore, is more available for absorption. Besides stearic acid (C18:0), a number of other FAs can serve as substrates for $\Delta^9$-desaturase in the mammary gland and other animal tissues. In this respect, the ability of this enzyme to convert C18:1 *trans-11* to C18:2 *cis-9, trans-11* has generated interest in the scientific community (Palmquist *et al.* 2005). Because, the milk rumenic acid (RA) is estimated to obtain between 64 and 97% from endogenous synthesis in the mammary gland from *trans*-vaccenic acid (TVA) (Palmquist *et al.* 2005; Mouriot *et al.* 2009). In addition,

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biohydrogenation of γ-linolenic acid, *cis-6*, *cis-9* and *cis-12* octadecatrienoic acid also results in formation of *trans-11* C18:1 (Harfoot and Hazelwood, 1988; Griinari and Bauman, 1999). Reduction in rumen pH often results in bacterial population shifts and consequent changes in the pattern of fermentation end products (Van Soest, 1994). Leat *et al.* (1977) showed that changes in rumen bacteria populations are associated with modifications in the biohydrogenation pathways in consistent with the altered *trans*-octadecenoic acid profile found in ruminal digesta and tissue lipids. Moreover, Griinari *et al.* (1998) demonstrated that an altered rumen environment induced by feeding high-concentrate, low-fiber diets is associated with a change in the *trans*-octadecenoic acid profile of milk fat. During this situation, *trans-10* octadecenoic acid replaced *trans-11* C18:1 as the predominant *trans* C18:1 isomer in milk fat. Putative pathways for the production of *trans-10* octadecenoic acid have been proposed (Griinari and Bauman, 1999). These involve a specific *cis-9*, *trans-10* isomerase in rumen bacteria with the formation of *trans-10, cis-12* conjugated double bond structure as the first intermediate. Further supportive evidence to specific bacterial *cis-9*, *trans-10* isomerase is provided by the observations that low-fiber diets increase the proportion of *trans-10*, *cis-12* CLA isomer in milk fat (Griinari *et al.* 1999). *Trans-10, cis-12* CLA has also been observed as one of the three major isomers of CLA in rumen digesta obtained from continuous flow-through fermenters (Fellner *et al.* 1997).

When the dairy cow is in negative energy balance, the relative contribution of FA from adipose tissue for milk fat synthesis is high (Dunshea *et al.* 1989; 1990), and *de novo* synthesis of the FA in the mammary gland is reduced (Christie, 1981; Palmquist *et al.* 1993). As a result, the concentrations of long chain fatty acids (LCFA), but particularly oleic, are at their highest level, and concentrations of short chain fatty acids (SCFA) and medium chain fatty acids (MCFA), but particularly palmitic, are at their lowest level in the milk fat produced by cows in early lactation (Christie, 1979; Palmquist *et al.* 1993; Auldist *et al.* 1998). As the concentration of solid fat in milk fat between 0 and 20 ˚C is well correlated with the ratio of oleic to palmitic acids, milk fat from cows in early lactation can be softer than that of cows in mid- and late lactation (Rowney and Christian, 1996).

## Saturated, unsaturated and trans-fatty acids
## Saturated fatty acids (SFA)
Milk is often described as having 70% of SFA. Nevertheless, this is inaccurate and exaggerated (Palmquist, 2010). Such accounting includes the SCFA (<12 C) that are largely oxidized in the liver upon absorption and have never been implicated in cardiovascular disease. These constitute

up to 15% of milk FA. Further, stearic acid is 9 to 15% of milk FA, and has been shown not to be atherogenic. Those that have been shown to have atherogenic properties are lauric, myristic and palmitic acids (Mensink *et al.* 2003). In most milk fats, the sum of these SFA is about 45% of the total milk FA. In healthy, well-balanced diets, atherogenicity even of these may be questioned. Lauric acid does increase plasma cholesterol concentrations, but most of the increase is in high density lipoproteins (HDL), with increase in HDL/LDL ratio, which is considered to be a positive marker for cardiovascular health (Fernández and Webb, 2008). The atherogenicity of palmitic acid has long been uncertain, because when included in diets that contain adequate quantities of UFA, no negative effects of C16:0 are evident (Clandinin, 2000).

A recent meta-analysis of prospective epidemiologic studies (Siri-Torino *et al.* 2010) showed that there is no significant evidence for concluding that dietary saturated fat is associated with an increased risk of cardiovascular disease. Parodi (2009) has disputed the collective perception that reduced saturated FA intake decreases the risk of coronary heart disease due to not all saturated fats behave in the same way and since most works are based on epidemiological studies, concerted and targeted research effort would be necessary to unravel the potential relationship between particular SFA and human disease (Elwood *et al.* 2008; Parodi, 2009). The recognized beneficial effect of milk and dairy products on human health (Elwood *et al.* 2008) provides further support for the importance of recognizing that dairy SFA are not consumed as a single dietary entity but along with other milk nutrients that appear to counteract any potential negative effect (Parodi, 2009).

## Unsaturated fatty acids (UFA)
The mono-unsaturated fatty acids (MUFA) in milk consist mainly of C18:1 *cis-9* (oleic acid) and also C18:1 *trans-11* (TVA). Oleic acid, considered universally to be a desirable component of dietary fat, is the second most abundant FA in milk fat. Its content is variable, influenced by the dietary fat content, and the extents of ruminal biohydrogenation and mammary $\Delta^9$-desaturase activity. With higher dietary fat content, significant amounts of oleic acid have been reported by Palmquist and Griinari (2006). Rumenic and vaccenic acid are both *trans-11* FA produced by rumen microorganisms and are unique for ruminant fat. They could also be termed omega-7 *trans* FA (Ellen and Elgersma, 2004), to distinguish them from *trans* FA in general, which have a negative health effect.

The content of "beneficial" poly-unsaturated fatty acids (PUFA) in cow milk fat is low. Linoleic acid (C18:2n-6) ranging from 1 to 3% and linolenic acid (18:3n-3) is 0.5-2% of the total milk FA, being the main isomer of CLA in milk

fat the RA (C18:2 *cis-9 trans-11*). In addition, the balance of *n-6:n-3* FA in the diet is a health determinant, and a ratio lower than 4 is related to a reduction in the risk of highly prevalent chronic diseases, including stroke, cancer, inflammatory and autoimmune diseases (Simopoulos, 2002). In some Western diets, where the average *n-6:n-3* FA ratio is higher than 15, both the increase of *n-3* FA and the reduction of *n-6* FA would be necessary to achieve a healthier diet (Simopoulos, 2002). So the supply of *n-6* FA in milk is not an issue; however, intake of the desirable *n-3* FA and linolenic acid remain low. The CLA contents in dairy products, calculated on the fat fraction, are comparable with those of the milk fat in the milk from which these products are obtained (Lavillonière *et al.* 1998; Dhiman *et al.* 1999a). The content of linolenic acid in milk fat can be increased by the selected feeding practices, most directly by grazing cattle on high-quality pastures (Dewhurst *et al.* 2006). There has been also considerable research effort to increase the LCFA by feeding oilseeds (cottonseeds or linseeds). Milk produced by dairy farmers, therefore, plays a key role. As milk FA profile is related to the FA composition of the feed in the dairy cows diet (Khanal and Olson, 2004), effects of forage, lipid feed supplements and feeding systems must be also taken into account.

**Trans-fatty acids**

The European Food Safety Authority defines *trans*-FA as UFA with at least one double bond in the *trans*-configuration (EFSA, 2004). Although this definition would include PUFA with conjugated double bonds (i. e. CLA), legislation in some countries (i. e. Denmark, EEUU and Canada) explicitly excludes this class of FA (Kühlsen *et al.* 2005). There are two major sources of dietary *trans*-FA partially hydrogenated vegetable oils found in many industrially prepared foods and ruminant-derived products, mainly dairy fats, with *trans*-C18:1 isomers being quantitatively the predominant *trans*-FA in both cases (Craig-Schmidt, 2006; Shingfield *et al.* 2008). Whereas FAs from hydrogenated vegetable oils are clearly related to increase in the LDL cholesterol/HDL ratio, no such link has been demonstrated for the *trans*-FA of ruminant fats. This is explained by the differences in distribution of the *trans*-monoenes between hydrogenated vegetable oils and ruminant fats. In the first group the *trans*-monoenes show a Gaussian distribution of the double bond around the $\Delta^9$ double bond, whereas the predominant *trans*-double bond in ruminant fat is $\Delta^{11}$.

Legislation or advice on limiting *trans*-FA has, in many instances, been restricted to those from industrial foods (Kühlsen *et al.* 2005). Since their consumption has been consistently associated with cardiovascular disease, the

natural *trans*-FA in dairy products have been considered innocuous (Mozaffarian *et al.* 2006; Chardigny *et al.* 2008). Furthermore, ruminant and industrial fats contain the same *trans*-C18:1 isomer, but their relative proportion is significantly different (Kühlsen *et al.* 2005), which is of crucial importance regarding the unfavorable effects mentioned for particular FA (Gutiérrez-Toral, 2010). For instance, studies with animal models suggest that *trans-10* C18:1, a relatively abundant isomer in industrial fats, may contribute to an increased risk of cardiovascular disease (Roy *et al.* 2007), while *trans-11* C18:1 (TVA), the major *trans*-C18:1 in dairy foods, might be protective (Tyburczy *et al.* 2009). This later FA has also been reported to have anti-carcinogenic properties, through its conversion to RA, while the association of other *trans*- FA with cancer is poorly understood and remains controversial (Smith *et al.* 2009).

**Conjugated linoleic acid**

The term conjugated linoleic acid (CLA) refers to a mixture of positional and geometric isomers of linoleic acid (*cis-9*, *cis-12* octadecadienoic acid) that contain conjugated unsaturated double bonds. The predominant CLA isomer in ruminant fats is C18:2 *cis-9*, *trans-11* (RA); the majority (up to 95%) of milk CLA is derived by *de novo* synthesis in the mammary glands (Griinari *et al.* 2000) by $\Delta^9$ desaturation of TVA (*trans-11* C18:1, arising from ruminal biohydrogenation) (Palmquist *et al.* 2005). RA is particularly rich in ruminant milk from grazing ruminants or those fed certain high fat diets. CLA has received a considerable interest during the past decade, because it has been shown to promote various beneficial health-related effects in experimental animals including anti-carcinogenic and anti-atherogenic effects on body composition and fat metabolism (Benjamin and Spener, 2009). Nevertheless, clinical evidences for health benefits in humans are very few (Palmquist, 2010). If these positive effects on health are found to happen in humans, the increase in the concentration of CLA would raise the nutritive and therapeutic value of milk and dairy products.

Average CLA content in milk varies between 0.3 and 0.6% of total FA (Kelly *et al.* 1998a, b). Typical CLA consumption by humans is lower (on an equivalent body weight basis) than the effective dose in reducing tumors in animal models (Ip *et al.* 1994). Intake of CLA can be increased either by enhancing the consumption of foods from ruminant origin or increasing the CLA content of foods derived from ruminant origin. The latter approach is more practical. In the rumen, dietary lipids are hydrolyzed resulting in conversion of UFA into SFA by the rumen microorganisms (Harfoot and Hazlewood, 1988).

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When the dietary supply of UFA is high, or the biohydrogenation process may be incomplete, the CLA can escape the rumen and become available for absorption in the lower digestive tract, thus, providing a source of CLA to the mammary gland. Dhiman *et al.* (1999b) have shown that cattle grazing pasture had higher CLA content in milk than did cattle fed pasture plus cereal grain. Feeding full fat extruded cottonseed increased the CLA content in milk (Dhiman *et al.* 1999a; Kelly *et al.* 1998a). Pasture grasses are rich in linolenic acid (C18:3). The oil in soybeans, cottonseeds and sunflower oil are rich sources in linoleic acid (C18:2). An important question is whether the CLA found in milk from dairy cows grazing grass or fed cottonseed is related to dietary intake of linoleic and linolenic acid or not?

Though most animal models in research suggests that intakes needed to induce significant health responses may be as much as 1 gram/day, estimated daily intakes range from 100 mg/d in the EEUU studies to 300-400 mg/d in Europe (Palmquist, 2010). These differences are attributed to greater food intake from ruminant origin in Europe, perhaps mainly cheese. Availability of CLA in the diet from ruminants on pasture-based milk production systems was reviewed by Van Wijlen and Colombani (2010). Because milk fat contains TVA three times as much as CLA, it is significant that humans, as well as dairy cows, are capable of desaturating TVA to synthesize RA endogenously. About 20% of dietary TVA was converted to RA in a human study (Turpeinen *et al.* 2002). Parodi (2006) used this value to conclude that the amount of CLA available to human tissues is 1.4 times that consumed in ruminant products.

**Factors influencing on milk fatty acids composition**
A mass of factors affect the CLA content in milk from dairy cows. As a result, a large variation occurs among the milk samples collected from individual animals fed the same diet and raised under similar conditions. Such factors are divided into three main categories (Khanal and Olson, 2004): (a) diet related, (b) animal related and (c) processing. The two first factors will be mentioned above with more detail.

**Dietary factors**
The dietary related factors that affect milk FA profile of raw bovine milk can be summarized in three major subcategories: 1) pasture feeding 2) lipid feed supplements and 3) seasonal and regional variations in milk FA according to feeding system selected.

**1) Pasture feeding.** Changes in milk FA profile and the presence of conjugated dienes in milk fat was first noticed by Booth *et al.* (1935) when cows were turned out to pasture during the summer. The total conjugated dienes in milk fat change from 0.4 to 0.8% during the winter when cows were kept indoors, while increased to amount of 1.3 to 2.5% during the summer when cows were grazed on pasture (Kuzdal-Savoie and Kuzdal, 1961). The increase in milk fat CLA contents were well-established later in a series of experiments (Jahreis *et al.* 1997; Stanton *et al.* 1997; Kelly *et al.* 1998b; Lawless *et al.* 1998; Elgersma *et al.* 2003b) and continue to be the research mainstay for enhancing CLA content in cows' milk (Dhiman *et al.* 1999a; Schroeder *et al.* 2003; Stockdale *et al.* 2003; Ward *et al.* 2003; Kay *et al.* 2004). Nevertheless, the FA composition of pastures is dependent upon species, variety and growing conditions and, therefore, proper grazing management strategies implemented at farm level might play a major role on sward FA profile and subsequently on milk FA profile (Shaffi *et al.* 1992; Dewhurst *et al.* 2003a). Dairy cows grazing lush green pastures (Dewhurst *et al.* 2003a; Elgersma *et al.* 2003a; Khanal and Olson, 2004) at high daily herbage allowance (DHA) (Elgersma *et al.* 2004b) produce milk fat with the highest levels of CLA. As DHA can be managed easily by dairy farmers, it might be a feasible way to obtain higher concentrations of FA in herbage but considerations have to be made to these assumptions in terms of grazing management due to graze swards at high DHA produce deterioration on sward quality (Curran *et al.* 2010; Roca-Fernández *et al.* 2011b; Roca-Fernández *et al.* 2012a). The potential of plant breeding and management to increase the beneficial FA content of grass and clover species was reviewed by Dewhurst *et al.* (2003a). As well, the timing of cutting or grazing (i.e. the regrowth stage at which herbage is harvested), can influence grass quality (Elgersma *et al.* 2006).

The issues discussed in this section regarding the benefits of fresh herbage, and in particular of grazing fresh pastures, could perhaps reverse the currently increasing trend for cows to be kept indoors year round. However, effects on milk production, landscape values, animal welfare and public opinion will also play a role in this trend. Generally, pasture feeding increases milk fat content of CLA compared to feeding either a total mixed ration (TMR) with similar lipid content or conserved forages (González-Rodríguez *et al.* 2009). Moreover, milk fat CLA content increases with enhancing proportions of pasture in the diet (Stanton *et al.* 1997; Kelly *et al.* 1998a; Dhiman *et al.* 1999b; Roca-Fernández *et al.* 2012b). The CLA-enriching effect of pasture has been attributed to the effects on biohydrogenation and the provision of α-linolenic acid as a lipid substrate for the formation of TVA in the rumen and its subsequent desaturation to *cis-9, trans-11* CLA in the mammary gland (Bauman *et al.* 2003). The lipids in pasture forages consist mainly of glycolipids and phospholipids, which are only 2% of the dietary dry matter (DM) (Van

Soest, 1994). Lipids in plants are not static entities, but are continuously subject to turnover, meaning that lipid degradation is a normal process in the living plant and that lipases are normally present. At the short term, this will not have a big influence on the FA composition of the lipid fraction in plants. There are at least three occasions when the lipid fraction in plants or plant parts may significantly be modified, i.e. during senescence, immediately after detachment (grazing or cutting) and during storage after cutting (Elgersma et al. 2006). In detached plants, after cutting and perhaps during the early stages of ingestion and ensiling, the metabolism of plant cells can continue. Moreover, the activity of the enzymes of dead tissue is important. In ruminants grazing fresh pastures, the first stage of lipolysis could be mediated by plant lipases (Lee et al. 2003). These enzymes are present in plants and their regulation might be altered due to the double stress of elevated temperature and anoxia imposed on the plant metabolism of intact plant cells after ensiling or ingestion by ruminants. A potential plant breeding goal to reduce the rate and extent of lipolytic activity in the rumen could be to select forage plants with reduced lipolytic activity.

Dewhurst and King (1998) studied the effect of ensiling on the FA composition in the material. Wilting prior to ensiling reduced the content of total FA by almost 30% (from 24.6 to 17.5 g/kg DM), with a reduction of up to 40% for linolenic acid. These authors suggested that the ensiling process itself has little influence, provided compaction and sealing of the silo is good. This may not always be the case in big bale silages. Adding silage additives (formic acid or formalin) resulted in much smaller losses, which was also found for formic acid by Doreau and Poncet (2000). Hay making reduces the total FA by over 50%, with a greater loss of linolenic acid (Doreau and Poncet, 2000). Similar observations were made for haylage (70% DM) by Elgersma et al. (2003b).

*In vitro* studies with rumen cultures suggest that glycolipids are hydrolyzed and hydrogenated similarly to triglycerides (Dawson et al. 1974, 1977; Singh and Hawke, 1979). Forage maturity also seems to be an important factor affecting milk fat content of CLA. Diets containing forage at the early growth stage resulted in increased milk fat CLA compared to diets that included late-growth or second cutting forage (Chouinard et al. 1998). However, forage lipid content and composition seems only to partly explain observed differences in milk fat content of CLA. Synergistic effects between lipid substrate and other pasture components may alter rumen biohydrogenation. Alterations in feed intake have had variable effects on milk fat content of CLA. Restricting feed intake by 30% resulted in milk fat concentration of CLA being increased in one study (Jiang et al. 1996) and reduced in another (Stanton et al. 1997). Timmen and

Patton (1988) more severely restricted feed intake and observed that the concentration of CLA in milk fat more than doubled. Alterations in feed intake would obviously affect substrate supply and change the rumen environment. Both of these factors would contribute to a change in the ruminal biohydrogenation process. Jahreis et al. (1997) compared conventional farming with high external inputs of fertilizer and concentrates, with or without grazing during summer; with low external N input ecological farming with summer grazing. It was observed that organic farming produced milk with the highest contents of both CLA and TVA in its milk fat, especially during May to September. The authors explained these results by the different feeding strategies between the two systems considered and claimed that fresh forage diet in organic farming was richer in PUFA than silage and concentrate diet fed in conventional farming, also pointed that the grass/legume silages were richer in PUFA compared to maize silages, and indicated that rations higher in fibre cause specific rumen bacterial populations, as measured by a higher content of branched-chain FA in milk fat. The higher concentration of linolenic acid in organic milk is consistent with recent observations of higher concentrations of this FA in milk produced from red or white clover silages compared to milk produced from grass silage (Dewhurst et al. 2003b). It was also found that grazing a cool season pasture with a C-3 carbon pathway containing more than 50% of their total FA as linolenic acid increases milk fat CLA (Dhiman et al. 1999b) more than its warm season counterparts with C-4 carbon pathways containing <40% of total FA as linolenic acid (White et al. 2001). A mature pasture, which has higher proportions of C14:0 and C16:0 and less linolenic acid, is also not likely to produce as much CLA content as does the lush green pasture when grazed by lactating cows (Loor et al. 2002b). Similarly, cows receiving all of their diet from grass produced milk fat with higher CLA contents compared to cows receiving either one-third or two-third of their diet from grass (Dhiman et al. 1999b; Roca-Fernández et al. 2012b ).

**2) Lipid feed supplements.** For decades, dietary lipid supplementation has been used to meet the energy requirements in dairy farming under unfavorable areas, where food supply can be temporarily scarce and high productive lactating ruminants (Palmquist and Jenkins, 1980; Gargouri et al. 2006; Sánz-Sampelayo et al. 2007). In most cases, protected plant oils were supplied with the aim of sustaining high yields of milk or fat (Casals et al. 2006; Gargouri et al. 2006). However, nowadays, particular emphasis is laid on the effect of lipid supplementation on milk FA profile. Plant lipids can be fed either as whole oilseeds, protected oils or free oils, but results indicate that the latter, more accessible to rumen microorganisms, bring about the most notable changes in milk fat composition (Dhiman et al.

2000; Loor *et al.* 2002a; Ward *et al.* 2003; Whitlock *et al.* 2003; Lock *et al.* 2004; Chilliard *et al.* 2007).

Supplementing roasted or extruded oilseeds seems to have a greater effect on milk fat CLA content than raw seeds (Dhiman *et al.* 2000), because of low release of oil from raw seeds in the rumen compared with heat-treated seeds. Low release of oil might lead to complete biohydrogenation of linoleic acid in the rumen and little or no effect on CLA content. Moreover, microorganisms responsible for biohydrogenation of PUFA must have access to the oil, which is very poor with raw seeds. Extruding, micronizing or roasting of soybeans resulted in two or three fold increases in milk fat CLA contents compared with a control diet containing ground soybeans (Chouinard *et al.* 2001). It appears that milk FA was accessible to rumen microbes when the oilseeds were processed and that heat treatment further increased the accessibility. Despite the positive effects of vegetable oil supplementation on milk FA profile (i.e. increased TVA and RA content), it should be pointed out that, in some cases, high concentrations of *trans-10* C18:1 are indeed observed (Roy *et al.* 2006; Gómez-Cortés *et al.* 2008). *Trans-10* C18:1 may have potentially negative effects on consumers' health (Kühlsen *et al.* 2005; Roy *et al.* 2007) and, when plant lipids are added to high-concentrate diets, progressive increases in the milk content of this C18:1 isomer are often accompanied by reductions in *trans-11* C18:1 and, subsequently, in *cis-9, trans-11* C18:2 (Bauman and Griinari, 2001; Roy *et al.* 2006; Shingfield *et al.* 2008).

**3) Seasonal and regional variations.** Seasonal variation in milk FA composition has long been recognized and, in this respect, several authors refer to Riel (1963). Although it has been reported by Mattsson (1949), who found concentrations of conjugated dienoic acids of 6-24 g/kg (with one milk fat with 37 g/kg) as well as lower levels for stall-fed cows. In the Netherlands, Stadhouders and Mulder (1955) reported a three-fold difference in conjugated dienoic acid concentrations in milk of cows fed in stalls *versus* pastured (7 *vs.* 25 g/kg). Jahreis *et al.* (1996) attributed the variations in milk FA profile depending on season and farm management systems. Higher pasture quality in spring (young and growing) than in summer (senescence) has a positive influence on milk FA profile of dairy cows; the physiological stage of forages has significance when making hay or silage (Dewhurst *et al.* 2006).

Different studies carried out in Austria, Germany, France and Switzerland showed that the FA profile of bovine milk from the highlands differed from that of the lowlands (Bugaud *et al.* 2001; Collomb *et al.* 2002; Kraft *et al.* 2003; Collomb *et al.* 2004; Leiber *et al.* 2004) as well as between seasons (Ferlay *et al.* 2008). Generally, in milk fat from pasture-fed cows in the mountains, higher concentrations of

the main *n-3* FA (α-linolenic acid) were found, as well as lower concentrations of SFA. Leiber *et al.* (2004, 2005) hypothesized that the increase in the α-linolenic acid content of alpine summer milk was due to pasture feeding and the absence or low amounts of concentrates. The concentration of CLA was also very high in summer milk fat from the highlands (Collomb *et al.* 2004). These high values are due to pasture effects but differences in the botanical composition of grass in the mountains (Bugaud *et al.* 2001; Collomb *et al.* 2002) could modify the FA profile of milk, as reported for the lowlands in the case of grass supplemented with legumes (Dewhurst *et al.* 2003c; Van Dorland, 2006).

Mountain pastures were characterized by a higher diversity in the botanical composition of grass than in the lowlands. Also, environmental conditions as well as low inputs feeding systems due to pasture-based feeding regimes without or with low amounts of concentrates could increase the lipomobilization by dairy cows. All these effects could explain changes in the milk FA profile. Elgersma *et al.* (2006) reported average concentrations of CLA and RA in milk from different countries during pasture feeding are 2-3 times those during barn feeding. The highest concentrations of RA and CLA were found in Switzerland, which relates to long periods of pasture feeding and to unique meadow grasses and herbs from the mountains and highlands while the lowest concentrations were found in The Netherlands when cows are fed indoors.

**Animal factors**

**1) Breed.** It has been found that given the same diet Holstein cows produce higher CLA levels in milk fat than do Jerseys or Normande cows (Lawless *et al.* 1999; White *et al.* 2001). In summer milk from grazing cows, higher CLA concentrations were found in Polish Red (11.9 g/kg) than in Black-and-White cows (9.4 g/kg) (Żegarska *et al.* 2001). Other authors (Soyeurt *et al.* 2007; Stoop *et al.* 2008) have also reported breed effects on milk FA profile. Kelsey *et al.* (2003), however, have reported minor variation in milk fat CLA content between Holsteins *vs.* Jersey cows fed the same diet and highlighted that the breed contributed only about 1% of the total variation on milk FA composition.

**2) Stage of lactation.** The milks from early lactation (30 days) contained less C4:0 to C12:0 than those from middle (120 days) and late (210 days) lactation periods in New Zealand cows (Auldist *et al.* 1998). The difference was independent of seasonal (feed) effect and was attributed to the physiological inability of cows in early lactation to consume enough DM to meet energy requirements. The synthesis of C4:0 to C12:0 in the mammary gland increased during early lactation then decreases and the mobilization of FA from adipose tissue enhanced (Palmquist *et al.* 1993).

Seasonal calving and stage of lactation affect the CLA contents, but the differences were small. Rowney and Christian (1996) evaluated the effects of diet and stage of lactation on milks for cheeses. They found that diet quality had the greatest effect.

**3) Parity.** Kelsey *et al.* (2003) have found that parity contributed about 10% of the variation on milk FA composition in milk fat from dairy cattle. It was similar to the earlier finding (Lal and Narayanan, 1984) where dairy cows with 7 or higher lactation produced 15% more CLA in milk fat compared with cows of either 1 to 3 or 4 to 6 lactations. Similarly, Stanton *et al.* (1997) reported that increased dairy cows' lactation number had a positive effect on milk fat CLA content.

**4) Animal to animal.** Of all the animal related factors, animal-to-animal variation appears to be the most important (Khanal and Olson, 2004).

The variation among individual cows would primarily be related to two factors: (a) rumen production of TVA and CLA, and (b) the activity of $\Delta^9$-desaturase. While the rumen output of CLA contributes only marginally to the overall CLA content in milk, it has been observed that individual cows may vary over 3-fold in the activity $\Delta^9$-desaturase (White *et al.* 2001; Kelsey *et al.* 2003).

Kelly *et al.* (1998a) have reported a larger variation in milk fat CLA content for cows fed sunflower oil than either peanut or linseed oil. Similarly, variations in CLA content among individual cows were higher with diets such as all pasture (Kelly *et al.* 1998b), TMR supplemented with free oil (Kelly *et al.* 1998a), or a diet with higher forage-concentrate ratio (Jiang *et al.* 1996), all of which are conducive to higher CLA concentrations. Cows in confinement fed TMR had smaller variations in milk fat CLA content compared to cows grazing pastures (White *et al.* 2001).

Similarly, variation in milk fat CLA content for the same group of individual cows was higher while grazing on pasture than when receiving either a TMR diet or a diet of conserved forage (*ad libitum* access) supplemented with grain (Khanal, 2004). It was also found that individual cow variation was greater during summer than in winter and in dairies that graze cows during summer than dairies that either did not graze nor had only 1/3 of its cows grazed. It has been observed that concentration of CLA in milk fat was the lowest from February to March and the highest from August to September both in USA (Khanal, 2004) and Canada (Riel, 1963).

**Milk differentiation and added value of dairy products**
Scollan *et al.* (2005) stated that pasture-based milk production systems offer considerable scope to create product differentiation and contribute to produce milk with higher added value in increasingly competitive dairy markets due to enhanced milk quality (such as with higher levels of CLA in milk fat).

## CONCLUSION

The assessment of the important role that dietary factors (pasture feeding, lipid feed supplements, seasonal and regional variations) and animal factors (breed, stage of lactation, parity and animal to animal) plays on milk fatty acids composition of grazing dairy cows should be the basis on which build sustainable pasture-based milk production systems in humid regions. Using proper grassland management strategies is crucial at farm level in order to maximize milk production per cow and per hectare by optimizing sward quality of grazed grass and pasture dry matter intake with a response in milk quality due to higher levels of polyunsaturated fatty acids. Due to pasture-based milk production systems have high dependence on fresh grass for feeding dairy cattle is possible to increase the added value of the final product, the milk, with higher content of conjugated linoleic acid which might help dairy farms to be more profitable and by reducing feeding costs and getting best quality product.

## ACKNOWLEDGEMENT

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