LITERATURE REVIEW

Trans fatty acids and their role in the milk of dairy cows

Einar Vargas-Bello-Pérez1,2, and Phil C. Garnsworthy1
1 The University of Nottingham, Sutton Bonington Campus. Loughborough, LE12 5RD, United Kingdom.
2 Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. Casilla 306, Santiago, Chile.

Abstract

E. Vargas-Bello-Pérez, and P.C. Garnsworthy. 2013. Trans fatty acids and their role in the milk of dairy cows. Cien. Inv. Agr. 40(3): 449-473. Lipids obtained from dairy products are an important part of the human diet in many countries. Approximately 75% of the total consumption of fat from ruminant animals comes from bovine milk fat. Trans fatty acids (tFA) are produced during biohydrogenation of mono- and poly-unsaturated FA in the rumen. They are mixtures of positional and geometrical isomers that are incorporated into the milk fat of lactating cows. The most important sources of tFA in the human diet are partially hydrogenated vegetable oils and ruminant milk and meat products. Ruminant-derived lipids often contain 1-8% of total fatty acids as tFA, which are predominantly 18:1 isomers. The most common FA in ruminant fat is vaccenic acid (18:1 trans-11) (VA), accounting for 60-80% of total tFA. Unlike other tFA, VA can be converted to rumenic acid (RA) through the action of stearoyl coenzyme-A desaturase. Today, consumers are becoming aware of the relationship between dietary fat, health maintenance, and disease prevention. These concerns have increased the need to investigate the metabolic fate and bioactivity of dietary FA. By altering the nutrition of cows, farmers can markedly and rapidly modulate the FA composition of milk FA. The largest changes can be obtained either by feeding animals high-quality forage, particularly fresh pasture, or by adding plant or marine oils to the diet. Given that economic factors define future profits for farmers, diet manipulation may be the most practical and appropriate approach to change milk’s FA composition.

Key words: biohydrogenation, dairy cows, milk, ruminal fermentation, trans fatty acids.

Introduction

Lipids obtained from dairy products are an important part of the human diet in many countries. Approximately 75% of the total consumption of fat from ruminant animals comes from bovine milk fat (Livingstone et al., 2012). The composition of milk fatty acids (FA) has numerous effects on milk quality, including its physical properties (e.g., melting point and the hardness of its butter, its crystallization and the fractionation of the milk fat) and nutritional value (e.g., effects on human health). Different FA (short- and medium-chain saturated, branched, mono- and polyunsaturated, cis and trans, and conjugated FA) present in ruminant milk fat are potentially positive or negative factors in the health of consumers (Meremae et al., 2012). Dairy products provide 25-60% of the total saturated fat consumption in Europe, which for
decades has made them a target of concern due to the negative effects of excessive consumption of saturated FAs on human health (Givens and Shingfield, 2006). Some milk FAs, such as butyric acid, oleic acid, polyunsaturated FA (PUFA) and conjugated linoleic acid (CLA) have potential anti-carcinogenic and anti-atherogenic effects. Other FA, such as some saturated (lauric, myristic and palmitic acids) and some trans FA (tFA) have potential negative effects on human health (Cicero et al., 2012; Imamura et al., 2012; Lefevre et al., 2012). A meta-analysis (Elwood et al., 2010) of different studies showed that consumption of dairy products reduces risk factors for ischemic heart disease, stroke and incident diabetes.

Some tFA in the human diet originate from meat or dairy products, but the greater proportion of tFA originates from hydrogenated vegetable or fish oils (FO) (American Heart Association, 2013). A recent review (Brouwer et al., 2010) on the effect of animal and industrially produced tFA on lipoprotein fraction levels in humans suggested that tFA may raise the ratio of plasma LDL to HDL cholesterol regardless of their origin and structure. However, because the profile and quantity of ruminant tFA differs from tFA of industrial origin (e.g., margarines) further research will be needed to confirm the effect of ruminant tFA on the risk of coronary heart disease (CHD) and cancer (Gebauer et al., 2011).

In the early 1990s, the adverse effects of consuming tFA on blood lipid levels (Mensink and Katan, 1990) and CHD (Ascherio et al., 1999) were identified. There is also evidence that tFA are also implicated in increased risk of cardiovascular disease to an extent equal to or greater than the hypercholesterolemic saturated FA (SFA) (Flock et al., 2011; Takeuchi et al., 2012). Despite the fact that the quantity and composition of dietary fat can impact on plasma lipoprotein cholesterol concentrations, the impact of dietary fat on CHD morbidity and mortality has continued to be a controversial topic (Salter, 2013). For example, a recent review (Siri-Tarino et al., 2010) showed that there was insufficient evidence from epidemiologic studies to conclude that dietary SFA are associated with an increased risk of CHD or stroke. There is also evidence that not all SFA (e.g., 18:0) have the same effect on blood lipid profiles and cardiovascular disease risk (Flock and Kris-Etherton, 2013).

In 1994, the UK Department of Health recommended that consumers reduce total fat consumption to 30% of total energy intake, with 10% of energy intake coming from SFA (Wood et al., 2003). At the same time, consumers were also advised to increase their intake of PUFA. In addition to the health benefits of PUFA on human health, CLA isomers have received attention due to their health-promoting effects (Roche et al., 2001; Dilzer and Park, 2012; Livingstone et al., 2012).

According to the Pan American Health Organization (PAHO, 2008), there are no known estimates of tFA intake in Chile; however, it is known that this country is not a major producer of fats as raw materials for human consumption. Approximately 95% of oils (mostly soybean/sunflower oil) are imported from Argentina and a small percentage from Bolivia. In Chile, CHD is the leading cause of death in adults. Epidemiological, clinical and metabolic research has shown strong associations between dietary FA intake and CHD risk factors. SFA and tFA derived from the industrial hydrogenation of oils have been associated with the increased prevalence of CHD. In 2008, Chile adhered to the World Health Organization recommendation to reduce tFA intake to a maximum level of 1% of total energy to reduce CHD prevalence (Torrejon and Uauy, 2011).

By altering the nutrition of cows, farmers can markedly and rapidly modulate the FA composition of milk FA. The largest changes can be obtained either by altering the forage in diets, particularly pastures, or by adding plant or marine lipid supplements to the diet (Loor et al., 2005a, 2005b; Dewhurst et al., 2006; Stamey et al., 2012).
Modifying the milk fat composition through dietary manipulation is not a recent concept (Palmquist and Beaulieu, 1993). In the early 1970s, the concern for reducing saturated fat content in the human diet initiated an era of intense research (Grummer, 1991). In recent years, the interest in reducing fat from dairy products is mainly due to concerns about the hypercholesterolemic effects of medium-chain SFA (Dewhurst and Lee, 2004). Given that economic factors define future profits for farmers, dietary manipulation of the dietary fat content may be the most practical and appropriate approach to change the FA composition of milk.

Lipids from ruminant milk have been estimated to contain over 400 different FA, and this number is mainly due to events that take place within the rumen (Jensen, 2002). Diet has major effects on ruminal bacteria populations and processes within the rumen. For this reason, diet has an important role in determining the fat content and FA composition of milk (Bauman and Griinari, 2003).

Trans fatty acids are produced during biohydrogenation of PUFA and isomerisation of monounsaturated FA (MUFA) in the rumen. They are mixtures of positional and geometric isomers (Piperova et al., 2004) that are incorporated into the milk fat (Loor and Herbein, 2001; Piperova et al., 2002, 2004). There has been intensive research on tFA, mainly on the negative effects of specific isomers of 18:1 trans that can be found in either dairy products or chemically produced partially hydrogenated vegetable oils (PHVO) (Shingfield et al., 2010). There is also interest in the role of trans isomers of 18:1 in mediating the milk fat depression (MFD) associated with certain dairy diets (Chilliard et al., 2000). MFD has a significant impact on dairy economics, causing important losses to dairy producers. It has been suggested that if milk processors allow dairy producers to reduce the content of milk fat then we can recommend that they produce a “naturally skim milk,” which might be obtained either from cows that genetically produce less fat in their milk (Garrick and Snell, 2005) or by milk fat-depressing diets. However, the European Union for example, defines milk as having a minimum fat content of 3.5% (Council Regulation (EC), 2007). Similarly, dairy companies that separate cream to produce other dairy products will not be interested in naturally skim milk.

More research is needed to understand the exact mechanisms by which tFA cause MFD. Additionally, the influence of tFA on milk composition is of interest due to their biological effects and potential role in human diseases. This review will highlight the literature related to tFA in dairy cow production with the goal of providing nutritionists with information on the role of dietary FA on the milk fat of dairy cows that might help in the development of nutritional strategies to enhance the nutritional value of milk in ruminants.

Lipid metabolism in the rumen

Unlike monogastrics, in which lipid digestion and absorption take place primarily in the stomach and small intestine, in ruminants, the rumen plays an important role. Dietary lipids also affect ruminal fermentation, and ruminal fermentation in turn affects the digestion and utilization of other nutrients. Hence, dietary lipids can have profound effects on ruminant nutrition and animal performance. Grummer (1991) and Mansbridge and Blake (1997) studied ruminal lipids metabolism focusing on manipulation of the physicochemical events in the rumen with two purposes: 1) To control the effect of FA on ruminal bacterial growth so that supplementary lipids do not adversely affect ruminal fermentation and nutrient digestion; and 2) To regulate ruminal biohydrogenation to alter the profiles of FA reaching the small intestine and those secreted in milk.

There are three major factors that influence the transfer of dietary fat to the milk (Wu et al., 1991; Palmquist and Beaulieu, 1993): 1) ruminal
biohydrogenation; 2) absorption (digestibility); and 3) deposition in adipose tissue.

For decades farmers have fed lipid supplements to modify and improve dairy performance, energy metabolism (Lock and Shingfield, 2004) and milk FA composition (Givens and Shingfield, 2006). Both the source and presentation form of lipids influence their effects. Changing the proportion of one category of FA often has consequences for other FA. Thus, feeding a diet that increases PUFA and/or CLA and decreases milk SFA results in a higher 18:1 trans concentration (Lock et al., 2005).

Most common lipid supplements comprise predominantly FA (90-95%) with a chain length greater than 14 carbons and typically contain greater than 75% C18 FA. The degree of unsaturation varies considerably (Grummer, 1991). Ingested lipids are subjected to two important microbial transformations in the rumen: lipolysis and biohydrogenation.

**Lipolysis**

Dietary lipids, if not protected, are rapidly and almost completely hydrolyzed to glycerol and free fatty acids (FFA) by lipases from different strains of bacteria. This process is referred to as lipolysis. Protozoa are not involved to any great extent in hydrolysis, except for phospholipids (Doreau and Ferlay, 1994). According to Bauchart et al. (1990), the extent of ruminal hydrolysis of lipids is high for most unprotected lipids (85-95%). *Anaerovibrio lipolytica*, which is best known for its lipase activity, produces a cell-bound esterase and a lipase (Harfoot, 1978). These lipases hydrolyze acylglycerols completely to FFA and glycerol with little accumulation of mono- or di-glycerides (Jenkins, 1993). Glycerol is fermented to volatile or short chain fatty acids, mainly propionate and butyrate (Doreau and Ferlay, 1994), which are absorbed through the ruminal wall, and may eventually be used as precursors for de novo synthesis of short- and medium-chain FA (SCFA and MCFA, respectively) in the mammary gland (Mansbridge and Blake, 1997). Released FA are subjected to subsequent biohydrogenation and/or used for the synthesis of microbial lipids. The dietary short-chain SFA released by microbial lipolysis are also absorbed through the ruminal wall into the blood stream (Doreau and Ferlay, 1994). Fatty acids are also released from plant galactolipids and phospholipids; hydrolysis of those esterified lipids is attributed to a variety of galactosidases and phospholipases produced by ruminal microbes (Jenkins, 1993).

**Biohydrogenation**

Unsaturated FA (UFA) released in the rumen are subjected to rapid and extensive biohydrogenation of their double bonds by ruminal microbes to saturated FA. This process may have evolved to protect microbes from the toxic effects of UFA because UFA are more toxic to ruminal microbes than SFA (Palmquist and Jenkins, 1980). Biohydrogenation occurs only on FFA with a free carboxyl group adsorbed on feed particles or microbial cells (Harfoot, 1978). The first step in biohydrogenation is an isomerization reaction that converts the cis-12 double bond to a trans-11 isomer, catalyzed by isomerase. Isomerase is not functional unless the FA has a free carboxyl group. This requirement establishes lipolysis as a prerequisite for biohydrogenation (Jenkins, 1993). The extent to which UFA are biohydrogenated depends on several factors, such as the forage to concentrate ratio (Kucuk et al., 2001), the type of protective treatment of the dietary fat (Whitlock et al., 2002), and the amount of dietary lipid (Shingfield et al., 2013). Biohydrogenation is performed mainly by rumen bacteria, although rumen fungi also play a role (Nam and Garnsworthy, 2007).

Lipolysis and biohydrogenation by rumen microbes can be decreased by treating lipids chemically (e.g., formaldehyde treatment or calcium salts) or
physically (e.g., heat) to resist microbial lipolysis and saturation in the rumen. Intact oilseeds also provide a degree of protection from biohydrogenation by microbial enzymes (Petit, 2001).

It has been reported (Doreau and Ferlay, 1994; Loor et al., 2005c) that the process of biohydrogenation of PUFA in the rumen can be decreased with high-concentration diets causing low conversion of 18:1 trans isomers to 18:0. This phenomenon may be provoked by shifts in bacterial populations (Loor et al., 2005c, 2005d) and decreases in ruminal pH (Kalscheur et al., 1997; Loor et al., 2005). Loor et al. (2004, 2005d) proposed other factors (e.g., the amount dietary starch, the degradation rate, and the buffering capacity) that contribute to the accumulation of biohydrogenation intermediates in the rumen with high-concentration diets. A shift in biohydrogenation pathways due to diet can have a significant impact if the shift enhances ruminal production of 18:1 trans-10 and 18:2 trans-10, cis-12, which are associated with MFD (Loor et al., 2005c, 2005d).

**Biohydrogenation intermediates**

Small amounts of tFA arise in the rumen as intermediates in the hydrogenation (saturation) of dietary UFA by the hydrogen produced during ruminal fermentation. As a consequence, the fat in butter, cheese, milk, beef, and mutton contains 2-8% tFA by weight (Enig et al., 1990; Katan and Zock, 1995). *Trans* FA are formed in much higher proportions during the industrial hydrogenation of vegetable or fish oils (Katan and Zock, 1995). The major tFA intermediates produced from ruminal metabolism of linoleic acid are 18:1 trans-11 and RA. However, the dynamics of ruminal biohydrogenation pathways allow production of a wide range of positional and geometrical FA isomers as well as modified FA such as hydroxyl and keto derivates (Jenkins et al., 2008; Harvatine et al., 2009). These compounds are absorbed and incorporated into milk fat at varying concentrations. However, production of high concentrations of these FA can be induced only under experimental conditions involving atypical diets (Lock and Bauman, 2004; Shingfield and Griinari, 2007; Harvatine et al., 2009).

**Effect of dietary lipids on rumen fermentation**

The effect of dietary lipids on ruminal fermentation varies according to the type and amount of lipid included in the diet. For example, diets with vegetable oils (high levels of unsaturated FA) cause greater variability in pH due to modifications in the rumen environment and are more exposed to hydrolysis by rumen bacteria than diets containing calcium salts and oilseeds (Fiorentini et al., 2013). Another factor that affects rumen fermentation when supplying lipids is their defaunation effect or reductions in the protozoan and fungal populations due to the intake of UFA (Doreau and Chilliard, 1997). The reduction or elimination of protozoa provokes a decrease in the concentration of ruminal NH$_3$-N, which is due to a reduction in the proteolytic activity of the protozoan population (Doreau and Ferlay, 1995). Lipids added to ruminant diets can disrupt ruminal fermentation, causing reduced digestibility of other nutrients, especially non-lipid energy sources (Jenkins, 1993). It has been shown that ruminal digestion of structural carbohydrates can be reduced 50% or more by less than 10% added fat (Knight et al., 1978). This is accompanied by decreased production of methane, hydrogen and volatile FA, which are products of ruminal fermentation, and a decreased ratio of acetate to propionate (Ikwuegbu and Sutton, 1982). This negative effect of added fat on the fermentation of structural carbohydrates can be alleviated to some extent by hindgut fermentation, but increased fiber excretion in feces often still occurs (Palmquist and Jenkins 1980). Several mechanisms have been proposed to explain how dietary lipids interfere with ruminal fermentation. The generally accepted theories are that close physical attachment of microbial matter to feed particles is necessary for cellular
Compared with fiber and protein, the ruminal digestibility of non-structural carbohydrates is less affected by dietary fat. Several studies have observed normal starch digestion in the rumen of cattle that were fed additional fat even though the dry matter or fiber digestibility was depressed (Jenkins, 1993). Similarly, high-fat diets often depress feed and energy intakes. When cows are fed large amounts of fats that cannot be metabolized, feedback satiety signals may be generated to prevent further intake of food (Choi and Palmquist, 1996).

The detrimental effects of supplemental lipids on ruminal fermentation are affected by several factors, including the degree of unsaturation and the amount and type of fat. Studies have demonstrated that UFA inhibit fermentation more than SFA (Jenkins, 1993; Doreau and Chilliard, 1997). The mechanism behind this phenomenon is not clear.

Glucose deficiency appears to explain the milk protein depression observed with dietary fat supplementation. To maintain adequate protein supplies when fats are included in a diet, the dietary protein content should be increased, and consideration should be given to increasing the supply of fermentable carbohydrates. Even when the protein supply is sufficient, the glucose status may be marginal with high-fat diets due to insulin resistance and glucose requirements for the absorption of FA (Garnsworthy, 1997). Despite the fact that nutritional and endocrine factors such as amino acid availability in the bovine mammary gland and insulin resistance affect protein synthesis, recently, it has been demonstrated that endocrine factors such as the lactogenic hormones hydrocortisone, insulin, and prolactin regulate protein translation (Burgos et al., 2010).

Compared with single-fat sources, blended-fat sources may have less or even no adverse effects on ruminal fermentation and utilization of nutrients. Commercial blends of animal fat and vegetable oil sometimes have little effect on fermentation, and more closely resemble ruminally inert fats, despite their relatively high degree of unsaturation (Jenkins, 1993). Inclusion of FO in the diet of ruminants enhances concentrations of 20:5n-3 and 22:6n-3 in milk (Loor et al., 2005a, 2005b; Angulo et al., 2012) and muscle (Najafi et al., 2012). Additionally, diet
supplementation with FO often induces MFD even at low levels (<2% diet DM; Whitlock et al., 2002). FO is known to inhibit the complete biohydrogenation of C18 PUFA to 18:0 resulting in accumulation of 18:1 \textit{trans}-10 and 18:1 \textit{trans}-11 (Shingfield et al., 2003, Lee et al., 2005, Loor et al., 2005a, 2005b). VA is consequently available for endogenous conversion to RA via the action of stearoyl coenzyme-A desaturase in the mammary gland (Griinari et al., 2000; Palmquist et al., 2005) and explains the effect of FO in enhancing RA concentrations in milk (Shingfield et al., 2003). Enhancement of CLA in ruminant products (meat and dairy) through dietary manipulation is an important contributor towards the recommended CLA intake of 3 g/day (Roche et al., 2001) as part of a complete diet approach (Lee et al., 2008).

\textit{Intestinal flow of trans fatty acids}

Alterations of pathways of biohydrogenation due to diet are imperative because increased ruminal production of 18:1 \textit{trans}-10 and CLA \textit{cis}-12, \textit{trans}-10 have been associated with MFD (Bauman and Griinari, 2003). It is also important because a reduction in VA production in the rumen may decrease endogenous synthesis of RA in tissues (Loor et al., 2005a). Loor et al. (2004, 2005c) reported intestinal flows of 18:1 \textit{trans} isomers and CLA isomers in dairy cows with different forage to concentrate ratios. They found that the nature of supplemental PUFA fed to cows, regardless of the concentrate to forage ratio, should lead to specific increases in the flows of certain CLA, non-conjugated 18:2, 18:1 \textit{cis}, and 18:1 \textit{trans} isomers into the small intestine.

The forage to concentrate ratio of the diet is important for the FA concentration that will be detected in the duodenum. For example, non-supplemented lipid diets rich in forage will increase the concentration of 18:1 tFA in the duodenum (Doreau et al., 2012; Table 1). Increasing the proportion of concentrate raises the content of 18:1 \textit{trans}-10 and, if associated with a high intake of linoleic acid (sunflower or soya oils), causes a deflection of the biohydrogenation of the linoleic acid pathway of \textit{trans}-11 to \textit{trans}-10 (Loor et al., 2005).

Table 1. Duodenal proportions (g/100 g of duodenal 18:1 isomers) of 18:1 \textit{cis} and \textit{trans} isomers.

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Non dietary lipid supplementation</th>
<th>Dietary lipid supplementation$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis-9</td>
<td>19.6 ± 10.8</td>
<td>21.2 ±17.6</td>
</tr>
<tr>
<td>Cis-11</td>
<td>4.8 ± 3.1</td>
<td>6.1 ± 3.7</td>
</tr>
<tr>
<td>Cis-12</td>
<td>1.5 ± 0.6</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>Cis-13</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Cis-15</td>
<td>3.7 ± 3.3</td>
<td>3.2 ± 2.3</td>
</tr>
<tr>
<td>Trans-4</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Trans-5</td>
<td>0.7 ± 0.5</td>
<td>0.7 ± 0.6</td>
</tr>
<tr>
<td>Trans-6+7+8</td>
<td>2.5 ± 0.9</td>
<td>2.3 ± 1.7</td>
</tr>
<tr>
<td>Trans-9</td>
<td>1.8 ± 0.5</td>
<td>2.1 ± 1.4</td>
</tr>
<tr>
<td>Trans-10</td>
<td>5.0 ±3.2</td>
<td>16.2 ± 18.4</td>
</tr>
<tr>
<td>Trans-11</td>
<td>30.8 ±12.3</td>
<td>20.4 ± 16.7</td>
</tr>
<tr>
<td>Trans-12</td>
<td>3.9 ± 1.1</td>
<td>2.9 ± 1.7</td>
</tr>
<tr>
<td>Trans-13+14</td>
<td>11.1 ± 3.4</td>
<td>10.5 ± 4.9</td>
</tr>
<tr>
<td>Trans-15</td>
<td>4.9 ± 2.5</td>
<td>4.5 ± 2.3</td>
</tr>
<tr>
<td>Trans-16</td>
<td>6.0 ± 2.6</td>
<td>3.1 ± 2.1</td>
</tr>
<tr>
<td>Sum of trans</td>
<td>63.9 ± 16.1</td>
<td>71.7 ± 17.4</td>
</tr>
</tbody>
</table>

$^1$Data on dietary lipid supplementation was taken from a meta-analysis reported by Glasser et al. (2008) in which the lipid sources were as follows: animal fat, hydrogenated fats, fish oil, linseed, soybean, sunflower, cottonseed, rapeseed, palm, other vegetal lipids, and an animal-vegetal blend.
A key factor that may help to understand how tFA are absorbed in the small intestine is the study of the expression of fatty acid-binding protein (FABP). In a recent report, Hayashi et al. (2013) studied the metabolism and intracellular transport of lipids in cattle. They found that FABP, which has great affinity for LCFA, has high protein expression levels in the duodenum and jejunum relative to other gastrointestinal regions in both calves and cows. In addition, they reported that both liver- and intestinal-type FABP are located in the cytosol of jejunal epithelial cells (where they were found in the villi rather than in the crypts).

Milk fat composition

Lipids in milk normally comprise 60-70% SFA (e.g., myristic, palmitic and stearic acids) (Dewhurst and Lee, 2004), 25-35% MUFA (e.g., oleic acid) and up to 5% PUFA (e.g., linoleic, CLA, α-linoleic acids) (Corl et al., 2003; Lock and Shingfield, 2004) (Tables 2 and 3). Normally, milk contains low levels of LCPUFA such as eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) (Gulliver et al., 2012). Milk fat contains significantly higher concentrations of SCFA and MCFA and relatively lower concentrations of UFA compared with other dietary sources of vegetable and animal fat (Kennelly, 1996). Milk fat has been criticized because it contains a less desirable balance of FA than many vegetable or fish oils. Two principal MCFA in butterfat, myristic acid and palmitic acid, have been identified as major dietary factors responsible for raising plasma low-density lipoprotein cholesterol levels (Denke and Grundy, 1992; Nestel et al., 1994). In particular, myristic acid, of which dairy products are a major source, is reputedly more potent than palmitic acid in raising cholesterol (Zock et al., 1994; Bradbury et al., 2010). However, a review from Parodi (2009) suggested that not all SFA represent a high risk for hypercholesterolemia. Those FA are 4:0, 6:0, 8:0, 10:0 and 18:0, which are present in milk but are not incorporated into chylomycrons and have no serum cholesterol-raising effect (Bloom et al., 1951).

Table 2. Lipids in milk.

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>% of total lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipid</td>
<td>1.11</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.46</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>95.80</td>
</tr>
<tr>
<td>1, 2 Diacylglycerol</td>
<td>2.25</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.28</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>0.08</td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>0.02</td>
</tr>
<tr>
<td>Fat (g dl⁻¹)</td>
<td>3.25</td>
</tr>
</tbody>
</table>

Modified from Jensen (2002).

Table 3. Fat composition of milk in dairy cows.

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>Per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole milk</td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3.9</td>
</tr>
<tr>
<td>SFA</td>
<td>2.5</td>
</tr>
<tr>
<td>MUFA</td>
<td>1.0</td>
</tr>
<tr>
<td>PUFA</td>
<td>0.1</td>
</tr>
<tr>
<td>tFA</td>
<td>0.1</td>
</tr>
<tr>
<td>Semi-skimmed milk</td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.7</td>
</tr>
<tr>
<td>SFA</td>
<td>1.1</td>
</tr>
<tr>
<td>MUFA</td>
<td>0.4</td>
</tr>
<tr>
<td>PUFA</td>
<td>Trace</td>
</tr>
<tr>
<td>tFA</td>
<td>0.1</td>
</tr>
<tr>
<td>Skimmed milk</td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.3</td>
</tr>
<tr>
<td>SFA</td>
<td>0.1</td>
</tr>
<tr>
<td>MUFA</td>
<td>0.1</td>
</tr>
<tr>
<td>PUFA</td>
<td>Trace</td>
</tr>
<tr>
<td>tFA</td>
<td>Trace</td>
</tr>
</tbody>
</table>

SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; tFA= trans fatty acids. From The Dairy Council (2013). Data were taken from the 2002 summary edition of The Composition of Foods Standards Agency (2002).

Trans fatty acids in milk

The most important sources of tFA for humans are partially hydrogenated vegetable oil, ruminant
milk and meat products (Brouwer et al., 2010). According to Hulshof et al. (1999) in many European countries approximately 50% of tFA come from dairy fat. Ruminant-derived lipids often contain 1-8% of total fatty acids as tFA (Craig-Schmidt, 1998), which are predominantly 18:1 trans isomers (Emken, 1995). The most common 18:1 trans isomer in ruminant fat is VA (18:1 trans-11; Table 4), accounting for 60-80% of total tFA (Emken, 1995; Craig-Schmidt, 1998; Salter et al., 2006). Unlike tFA from PHVO, ruminant tFA (VA) can be converted to CLA cis-9, trans-11 (RA) through the action of stearoyl coenzyme-A desaturase (Salter et al., 2006).

Due to public health concerns for chronic diseases in the Americas, the Pan American Health Organization/World Health Organization (PAHO/WHO) designed a Regional Strategy and Plan of Action for an Integrated Approach to the Prevention and Control of Chronic Diseases (2006–2015), which included diet, physical activity, and health (PAHO Resolution CD47.R9).

In 2007, the PAHO assembled a Task Force called the “Trans Fat Free Americas” (TFFA), and its members were asked to evaluate the impact of tFA on human nutrition and health and to discuss practical ways to eliminate tFA from the food supply, including regulatory actions and recommendations for healthier alternative fats. The Task Force was composed of experts from different Latin American and Caribbean countries (Argentina, Brazil, Costa Rica, Chile, Guatemala, Jamaica, Mexico, Peru, Puerto Rico, Uruguay, and Venezuela), the United States (the Centers for Disease Control and Prevention, Harvard University, National Institutes of Health, and Department of Agriculture), and Canada (Health Canada and Public Health Agency of Canada). The TFFA estimated that consumption of tFA is approximately 2-3% (4.5-7.2 g/d) of total calories consumed in the United States, 3% (7.2 g/d) in Argentina, and 2% (4.5 g/d) in Chile. According to estimates by Task Force investigators from the School of Public Health at Harvard University, the health impact of removing industrially produced tFA from the food supply in the Americas (excluding the United States and Canada) may reduce tFA consumption to 4.5 g/d (approx. 2% of daily energy intake), which would represent 30,000-130,000 CHD events prevented.

In Chile, soya bean or sunflower oil are the main sources of hydrogenated fats; approximately 30% of industrial hydrogenation is for margarine manufacture, and the remaining 70% of hydrogenated fats are obtained from salmon oil, salmon and soya bean oil blends, or rendered animal fat imported from Argentina. In addition, an indication of the

<table>
<thead>
<tr>
<th>Forage</th>
<th>Lipid supplement</th>
<th>trans-4</th>
<th>trans-5</th>
<th>trans-6-8</th>
<th>trans-9</th>
<th>trans-10</th>
<th>trans-11</th>
<th>trans-12</th>
<th>trans-13+14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryegrass silage</td>
<td>-</td>
<td>0.02</td>
<td>0.02</td>
<td>0.20</td>
<td>0.17</td>
<td>0.20</td>
<td>0.99</td>
<td>0.20</td>
<td>0.44</td>
</tr>
<tr>
<td>Ryegrass pasture</td>
<td>-</td>
<td>0.03</td>
<td>0.03</td>
<td>0.34</td>
<td>0.30</td>
<td>0.51</td>
<td>4.73</td>
<td>0.41</td>
<td>0.85</td>
</tr>
<tr>
<td>Red clover silage</td>
<td>-</td>
<td>0.03</td>
<td>0.03</td>
<td>0.27</td>
<td>0.30</td>
<td>0.31</td>
<td>1.00</td>
<td>0.27</td>
<td>0.56</td>
</tr>
<tr>
<td>Grass silage</td>
<td>-</td>
<td>0.03</td>
<td>0.03</td>
<td>0.27</td>
<td>0.30</td>
<td>0.31</td>
<td>1.04</td>
<td>0.29</td>
<td>0.64</td>
</tr>
<tr>
<td>Maize and grass silages</td>
<td>Sunflower oil</td>
<td>0.06</td>
<td>0.05</td>
<td>0.95</td>
<td>0.46</td>
<td>7.22</td>
<td>1.44</td>
<td>0.86</td>
<td>0.43</td>
</tr>
<tr>
<td>Maize and grass silages</td>
<td>Rapeseed oil</td>
<td>0.05</td>
<td>0.08</td>
<td>0.56</td>
<td>0.50</td>
<td>0.62</td>
<td>2.20</td>
<td>0.68</td>
<td>1.60</td>
</tr>
<tr>
<td>Maize silage</td>
<td>Fish oil</td>
<td>0.04</td>
<td>0.05</td>
<td>0.44</td>
<td>0.48</td>
<td>1.76</td>
<td>9.17</td>
<td>0.70</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Modified from Shingfield et al. (2013).
tFA content on nutrition labels has been mandatory since November 2006, and the content has to be less than 0.5 g of tFA per serving for the “trans free” label.

Conjugated linoleic acid

CLA is produced during the biohydrogenation of PUFA in the rumen (O’Donnell-Megaro et al., 2012). CLA is an intermediate in the rumen hydrogenation of linoleic acid, whereas VA (18:1 trans-11) is a common intermediate in the biohydrogenation of linoleic and cis- and trans-linoleic acids (Chilliard et al., 2000). Grinari et al. (2000) demonstrated that endogenous synthesis of RA from VA represents the primary source of CLA in milk fat (Shen et al., 2007). A recent study (Lee and Jenkins, 2011) suggested that ruminal microbes are capable of the formation of several CLA (at least 8 isomers) and 18:3 isomers from linolenic acid, indicating that the production of CLA isomers in the ruminal contents varies depending on the predominate microbial species and the diet offered to the animal.

Dairy products and meat from ruminants represent the only significant natural sources of CLA (Lawson et al., 2001; Burdge et al., 2005). CLA has been suggested to have numerous beneficial physiological effects such as changes in body composition (Gaullier et al., 2004; Chichlowski et al., 2005) and lower insulin resistance, which are associated with reduced cardiovascular heart disease (Aminot-Gilchrist and Anderson, 2004). Other health effects, including anti-carcinogenic, anti-atherogenic, anti-obesity, and anti-diabetic effects and immune system enhancement, have been investigated in different animal models, and to a lesser extent in humans (Belury, 2002).

An increase in milk CLA, particularly RA, is associated with an increase in other tFA, particularly VA (18:1 trans-11), which acts as a precursor of RA via the enzymatic action of stearoyl coenzyme-A desaturase, and to a lesser extent 18:1 trans-10 (Lock and Garnsworthy, 2002; Lock and Shingfield, 2004). This was also reported by Chilliard et al. (2000) and Hurtaud and Peyraud (2007), who found a linear relationship between the content of CLA in milk and 18:1 trans isomers.

Dietary starch and fiber levels affect biohydrogenation of FA by rumen microbiota and hence, the equilibrium of CLA isomers passing to the duodenum (Offer et al., 2001). Supplementation of dairy cow diets with plant oils (soya bean, sunflower, linseed, rapeseed and maize) results in significant increases in milk fat CLA concentrations. Moreover, plant oils high in linoleic acid give the greatest response in cattle (Chilliard et al., 2007). Feeding pasture to dairy cows has also been shown to increase the milk fat content of CLA (Lerch et al., 2012). However, other studies have shown that milk CLA increases with diets low in 18:2, such as diets including FO supplements; because of these observations, it has been suggested that CLA can be synthesized by tissues (i.e., adipose tissue) (Chilliard et al., 2000).

Lock and Garnsworthy (2002) demonstrated that dietary lipid manipulation could alter the RA content in milk fat when linoleic or linolenic acids were fed to cows. They found similar increases in milk RA content when either dietary linoleic or linolenic acids were increased, and higher concentrations were observed when a blend of oils (linseed, rapeseed, soya, sunflower oils and linseed and sunflower oils) were increased together. They also found that there was considerably more RA (approximately 80% more) in supplemented milk than milk produced in the rumen in agreement with the concept of endogenous synthesis of RA in the mammary gland via the enzymatic action of stearoyl coenzyme-A desaturase.

The concentration of CLA in milk can be increased in pastured cows when their feed is supplemented with oils (Mendoza et al., 2011). Other studies (Lawless et al., 1998) have concentrated on supplementing the feed of grazing cows with full-fat soya beans or full-fat rapeseed...
to increase milk CLA concentrations. Alternatively, FO has been used as an effective means to increase the concentration of CLA in milk (AbuGhazaleh and Holmes, 2007). However, the transfer of dietary (n-3) LCPUFA to milk (Offer et al., 2001) is inefficient mainly due to the following factors: biohydrogenation of these dietary lipids in the rumen (Figure 1); their selective incorporation into certain plasma lipoprotein fractions (e.g., HDL) which are ineffective at delivering FA into the mammary gland; and the role of the fatty acyl substrate of the mammary gland enzymes associated with FA esterification (Offer et al., 2001).

**Factors affecting milk fat composition**

Ruminant milk FA composition is linked to intrinsic (animal species, breed, genotype, physiological stage) and extrinsic (environment and nutrition) factors (Palmquist and Beaulieu, 1993; Chilliard and Ferlay, 2004). In addition, other key factors include the chemical composition of the basal diet and the forage concentration and dietary supplementation with vegetable oils (type and quantity) or marine oils (fish and algae oils). Recently, it has been shown that dietary components can alter the expression of genes related to FA mammary lipogenesis (Bauman et al., 2011).

In a given animal species, the effects linked to breed or genotypes are significant but restricted, and they can only be altered over longer terms. The effect of lactation stage on milk fat content and FA composition is associated with body fat mobilization in early lactation (Chilliard et al., 1991, 2007), but the early stage only lasts a few weeks each year. Garnsworthy et al. (2006) investigated the variation in citrate with stage of lactation and *de novo* FA synthesis without any confounding dietary effects. They found that cows in late lactation produced milk with a FA profile similar to cows in mid lactation. The differences in yield of FA between mid and late lactation were due to differences in milk fat yield, suggesting that the stage of lactation does not affect the relative incorporation of FA from *de novo* synthesis vs. preformed sources when diet composition is constant.

**Forage source**

It has been reported (Elgersma et al., 2006) that fresh grass dry matter contains 1-3% FA, with
the highest values during spring and autumn, and approximately 75% of these FA are 18:3n-3. Compared with mixed winter diets, pasture often increases milk fat concentrations of 18:0, 18:1, 18:3 and CLA and decreases the concentrations of 10:0-16:0 (Chilliard et al., 2007). Likewise, when forage is dried to limit wilting losses, its 18:3n-3 concentration is higher, which supports the production of milk with similar or higher 18:3n-3 concentrations than pasture and higher VA and RA concentrations than grass silage (Chilliard et al., 2007).

**Dietary fat in dairy cow diets**

Supplementation with lipids has been used to increase the energy concentration of the diet and to avoid the negative effects associated with dry matter intake, digestibility, and milk production and composition sometimes experienced with cereal grains (Garnsworthy, 1997). Oil supplementation in dairy diets is used not only to increase energy density but can also be used to reduce methane emissions (Amaro et al., 2012). Dietary supplementation with lipid sources rich in 12:0 and 14:0 FA (Machmuller, 2006) and high in PUFA such as linseed oil (Martin et al., 2008), sunflower oil (McGinn et al., 2004) and mixtures of sunflower and fish oil (Woodward et al., 2006) can reduce methane production.

Many sources of supplemental fat have been fed to beef and dairy cattle under experimental conditions. These include blends of animal and vegetable fat (Ohajuruka et al., 1991), tallow (Rabiee et al., 2012), yellow grease (Kargar et al., 2012), fishmeal (Yazdani, 2011), pima cottonseed (Broderick et al., 2013), soya beans (Abdi et al., 2013), rapeseeds (Damgaard et al., 2013), canola seeds (Mohammed et al., 2011), peanut byproducts (meal, skin, hulls, silages and hay; Hill et al., 2002), safflower seeds (Alizadeh et al., 2012), sunflower seeds (Mohammed et al., 2011), flaked fat (Elliott et al., 1994), prilled fat (Harvatine and Allen, 2006), hydrogenated fat (Kargar et al., 2012), calcium soaps of fat (Karcagi et al., 2010), medium-chain triglycerides (Sun et al., 2013), and free FA (Funston, 2004). The FA profile of these fat sources varied widely. Coppock and Wilks (1991) reported the FA profiles of many of the commonly used fats. The major FA in most feed lipids is linoleic acid, whereas linolenic acid predominates in most forage lipids (Staples et al., 1998). Tallow (40.9%) and yellow grease (46.8%) contain different oleic acid contents (Coppock and Wilks, 1991). Granular fats, such as calcium soaps of palm oil (Tomkins and Drackley, 2010), and prilled fats (Harvatine and Allen, 2006), contain mainly saturated palmitic and stearic acids (Staples et al., 1998).

Rumen microorganisms cannot utilize large quantities of fat, although limited quantities of FA can be incorporated into microorganisms during cell synthesis. More important than the quantity of FA in the diet is their form because long-chain free UFA have a detergent effect on bacterial cell walls. Under normal circumstances, the ester linkages of triglycerides are rapidly hydrolyzed by bacterial lipases in the rumen. Once released from the ester combination, unsaturated fatty acids

The fat content of ordinary dairy diets for ruminants is less than 50 g/kg of DM, and digestive problems can occur by increasing the total fat content of the diet to more than 100 g/kg of DM (Garnsworthy, 1997). However, this does not necessarily apply to small ruminants because MFD in sheep (Reynolds et al., 2006) and goat (Gagliostro et al., 2006) is unusual. The tolerance to high concentrations of dietary fat (>5% of DM) in small ruminants may be explained by the fact that their ruminal biohydrogenation pathways appear to be more constant than dairy cows, reflecting differences between species that are related to several factors such as eating behavior, rumination, rumen digestion kinetics and transit rates and resulting in less exposure of the mammary gland to tFA isomers that may depress milk fat synthesis (e.g., 18:1 trans-10; Shingfield et al., 2010).
are subsequently hydrogenated to reduce their toxicity (Garnsworthy, 1997). Fiber particles in the rumen can be physically coated by fat, making them inaccessible to microbial attack. The magnitude of these effects depends on the level, source and type of fat, dietary carbohydrate source and feed intake. Similarly, another problem is that long-chain FFA can form soaps with calcium and magnesium in the rumen. This process detoxifies the FA, but it can also reduce the availability of minerals (Garnsworthy, 1997).

Theoretically, supplementation of dairy cow diets with fat could provide some of the following advantages: increased dietary energy concentration because fat contains three times more net energy for lactation than protein- and carbohydrate-rich feeds (Palmquist, 1984); improved energetic efficiency because reduced energy loss in the form of heat, methane, and urine may be expected (Palmquist and Jenkins, 1980) and because dietary FA are incorporated directly into the milk fat by the mammary gland (Wu and Huber, 1994); and reduced risk of rumen acidosis and decreased milk fat percentage induced by feeding high levels of cereal grains in the diet (along with high-PUFA diets) (Palmquist and Conrad, 1978; Palmquist, 1984).

Lipids can be manipulated in several ways to avoid adverse effects in the rumen and to retain availability in the small intestine (Palmquist, 1984). These modified fat products are known as “by-pass fats” or “rumen-inert fats.” Protection against rumen microbial action can be achieved by encapsulation in formaldehyde-treated casein, via the formation of calcium soaps or by selecting fatty acids with high melting points and small particle sizes (Garnsworthy, 1997). The development of commercial products based on calcium soaps of LCFA offers dairy cattle producers a method of increasing the energy concentration of rations without disturbing rumen fermentation (Reis et al., 2012). However, protection against microbial action in the rumen could also neutralize beneficial effects; for example, the use of marine oils in ruminants increases long-chain UFA (i.e., DHA and EPA) but it can also modulate the biohydrogenation process, increasing the flow of 18:1 trans-11 that will be desaturated to CLA in the mammary gland (AbuGhazaleh and Holmes, 2007).

**SFA and oleic acid**

There is a huge potential to alter the concentration of medium-chain SFA (10:0 to 16:0) in the milk of dairy cows. For example, it has been reported that cows fed with hay-based diets can increase these FA (10:0, 12:0, 14:0 and 16:0) to 56% of milk fat, whereas supplementation with linseed oil can decrease them to 29% (Roy et al., 2006). Lipid supplements rich in medium-chain FA can increase these FA; for example, calcium salts of palm oil increases the palmitic acid concentration (Chilliard et al., 2007).

Similarly, it has been shown (Collomb et al., 2004; Loor et al., 2005c, 2005d; Gonthier et al., 2005) that stearic acid in milk can be increased either by dietary 18:0 intake or by supplementation with 18-carbon UFA because these FA are hydrogenated to 18:0 in the rumen (Loor et al., 2004, 2005c, 2005d).

Similarly, the secretion of oleic acid can be increased through its direct gut absorption and mammary gland secretion or, more importantly, through ruminal biohydrogenation followed by mammary desaturation of 18:0. In addition, its concentration can be increased by manipulating the distribution of oleamides, which are amides of oleic acid that resist ruminal biohydrogenation and increase milk oleic acid concentrations when fed to lactating cows (Jenkins et al., 2008).

**Milk fat synthesis**

Fatty acids incorporated into milk triglycerides are derived from two sources, uptake of preformed FA from the peripheral circulation and FA synthesis in mammary secretory cells. Depending
on the cow’s breed, stage of lactation and diet, FA synthesis de novo contributes proportionately 60% on a molar basis or 40% by weight to total the FA secretion in milk (Shingfield et al., 2010).

Mammary epithelial cells synthesize short- and medium-chain FA using acetate and 3-hydroxybutyrate as substrates. Fatty acid synthesis de novo accounts for all 4:0 to 12:0, most of the 14:0 (ca. 95%) and approximately 50% of 16:0 secreted in milk, whereas all 18-carbon and longer chain FA are thought to be derived from circulating plasma lipids (Chilliard et al., 2000). De novo FA synthesis has an absolute requirement for acetyl-CoA, the presence of two key enzymes, acetyl-CoA carboxylase (ACC) and FA synthase and a supply of NADPH reducing equivalents (Barber et al., 1997). Both 3-hydroxybutyrate and acetate contribute equally to the initial four-carbon unit. Acetate is converted to acetyl-CoA in the cytosol and used to extend the chain length of synthesized FA via the malonyl-CoA pathway, whereas 3-hydroxybutyrate is incorporated directly following activation to butyryl-CoA. The conversion of acetate to malonyl-CoA, which is catalyzed by ACC, is considered the rate-limiting step (Bauman and Davis, 1974).

Effect of tFA on milk fat synthesis

High amounts of tFA, either from dietary sources or from incomplete ruminal biohydrogenation, are regarded as inhibitors of milk fat synthesis (Palmquist and Beaulieu, 1993). When dairy cattle are fed high-concentrate diets, MFD occurs due to an increase in the 18:1 trans isomer content (Piperova et al., 2000). Similarly, Wonsil et al. (1994) have proposed that the production of 18:1 trans isomers is the result of incomplete biohydrogenation of dietary PUFA.

Milk fat depression

Trans fatty acids have been implicated in MFD (Table 5). Researchers have postulated a possible role for 18:1 trans isomers in milk fat during MFD, indicating that biohydrogenation is incomplete (Kargar et al., 2012). However, other studies (Kalscheur et al., 1997) have provided data showing that dietary manipulation results in important increases in the milk fat content of 18:1 trans but no corresponding reduction in milk fat yield (Bauman and Griinari, 2003).

Decreases in milk fat synthesis are common in cows fed diets containing high proportions of concentrates, FO and marine algal lipids or ionophores (Bauman and Griinari, 2001 and 2003). Although certain attributes of a diet are important in the establishment of MFD, there is considerable interaction between the composition of the basal diet, the amount and source of dietary lipid supplementation, and the feeding frequency (Shingfield and Griinari, 2007).

A number of theories have been proposed to explain diet-induced MFD. The major theories have attributed the decreases in milk fat synthesis during diet-induced MFD several possible causes: 1) Reductions in the supply of acetate and 3-hydroxybutyrate for de novo FA synthesis in the mammary gland (Palmquist and Jenkins, 1980); 2) Elevated insulin secretion stimulating the preferential partitioning of FA towards adipose tissue at the expense of the mammary gland (Vernon and Finley, 1988); 3) Direct inhibition of mammary lipogenesis by tFA formed during biohydrogenation of dietary UFA in the rumen (Shingfield et al., 2010); and 4) Maintenance of milk fluidity when incorporating high-melting point tFA into the milk fat (Gama et al., 2008).

Of the hypotheses developed to explain diet-induced MFD, the biohydrogenation theory appears to be the most robust and offers a more convincing explanation for MFD over a wider range of diets (Shingfield et al., 2010). However, increases in ruminal outflow of CLA trans-10, cis-12, do not explain the decreases in milk fat synthesis in all cases of diet-induced MFD, with the implication that additional biohydrogenation intermediates and/
or mechanisms must be involved (Shingfield and Griinari, 2007). MFD diets (high concentrate, high starch, PUFA, and occasionally pasture) also alter the ruminal environment (decrease in ruminal pH with high concentrate) and fermentation products (increased propionate production). MFD diets, thus, lead to a simultaneous increase in numerous FA isomers, complicating the elucidation of related mechanisms (Gama et al., 2008; Glasser et al., 2010).

Some studies have recognized that the quantity of CLA isomers produced in the rumen is insufficient to explain the MFD phenomenon (Maxin et al., 2011). One explanation is that either there are additional FA isomers (unidentified or not detected) that inhibit milk fat secretion and contribute to MFD (Harvatine et al., 2009) or that other nutrients or mechanisms are also involved in MFD (Gama et al., 2008; Shingfield et al., 2010) because MFD-inducing diets usually affect ruminal fermentation and modify supply of different nutrients derived from digestion (VFA; acetic, propionic and butyric; glucose, LCFA, proteins and CLA isomers) of dairy cows. Because the effect of 18:1 trans-10 on milk fat synthesis has not been fully elucidated, CLA isomers such as 18:2 trans-9, cis-11 (Gama et al., 2008) and 18:2 cis-10, trans-12 (Saebø et al., 2005), have to be considered when studying MFD.

Some studies (Rulquin et al., 2007; Maxin et al., 2011) have quantified the effects of several

### Table 5. Milk FA composition in cows fed control or an MFD diet.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Common name</th>
<th>Control</th>
<th>Milk fat depressing diet</th>
<th>SED</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td>Butyric</td>
<td>34.8</td>
<td>16.3</td>
<td>1.61</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>6:0</td>
<td>Caproic</td>
<td>21.4</td>
<td>7.3</td>
<td>0.92</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>8:0</td>
<td>Caprylic</td>
<td>15.6</td>
<td>4.9</td>
<td>1.40</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>10:0</td>
<td>Capric</td>
<td>34.0</td>
<td>12.5</td>
<td>1.51</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>12:0</td>
<td>Lauric</td>
<td>40.5</td>
<td>16.7</td>
<td>1.71</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>14:0</td>
<td>Myristic</td>
<td>112.3</td>
<td>48.5</td>
<td>3.90</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>14:1</td>
<td>Myristoleic</td>
<td>8.6</td>
<td>5.1</td>
<td>0.92</td>
<td>0.023</td>
</tr>
<tr>
<td>16:0</td>
<td>Palmitic</td>
<td>279.8</td>
<td>107.7</td>
<td>9.95</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>16:1</td>
<td>Palmitoleic</td>
<td>18.2</td>
<td>9.5</td>
<td>0.95</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>18:0</td>
<td>Stearic</td>
<td>88.0</td>
<td>44.4</td>
<td>5.42</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>18:1 trans isomers</td>
<td>Elaidic (t9)+ Vaccenic (t11)</td>
<td>16.9</td>
<td>75.6</td>
<td>6.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>18:1 cis isomers</td>
<td>Oleic</td>
<td>170.1</td>
<td>95.7</td>
<td>1.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>18:2 (n-6)</td>
<td>Linoleic</td>
<td>26.5</td>
<td>31.1</td>
<td>1.90</td>
<td>0.126</td>
</tr>
<tr>
<td>18:3 (n-3)</td>
<td>Linolenic</td>
<td>5.1</td>
<td>2.5</td>
<td>0.30</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total PUFA</td>
<td></td>
<td>38.8</td>
<td>41.0</td>
<td>9.40</td>
<td>0.864</td>
</tr>
<tr>
<td>Total SFA</td>
<td></td>
<td>626.4</td>
<td>242.0</td>
<td>22.65</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total de novo FA</td>
<td></td>
<td>223.8</td>
<td>89.9</td>
<td>12.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total LCFA</td>
<td></td>
<td>611.8</td>
<td>373.9</td>
<td>21.00</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

PUFA= polyunsaturated fatty acids; SFA= saturated fatty acids; LCFA= long-chain fatty acids. Modified from Piperova et al. (2000) and Jensen (2002).

1A diet containing 25% forage, 70% concentrate and supplemented with 5% soybean oil. 2Standard error of the difference.

3Probability that treatments are not different.
nutrients derived from digestion on milk fat concentration and milk fat yield to evaluate their biological effect on MFD. The importance of those studies is that the measurements of the responses of the milk fat concentration and milk fat yield to these nutrients can be used to estimate milk fat changes after dietary modifications, provided that the changes in the nutrient supply following dietary modifications can be predicted.

For the last 15 years, the study of MFD has been an important research topic. Recently, Bauman et al. (2011) made some significant points on MFD: Clearly, MFD is a reduction in milk fat by specific FA produced during biohydrogenation. Despite the fact that there may be different CLA isomers involved in MFD, CLA \textit{trans}-10, \textit{cis}-12 is the most studied. In addition, the mammary lipid synthesis capacity is affected by the coordinated effects of lipogenic enzymes when MFD occurs, and more importantly, research into diet-induced MFD has been key to understanding milk fat synthesis mechanisms. Investigations over the past decade into MFD have had an impact on dairy systems, including their management and nutritional strategies.

**Conclusions**

This review has summarized some aspects of milk fat composition, synthesis and depression as well as FA metabolism in dairy cows. This review has also addressed the role of dietary FA in milk fat content and composition in dairy cows. However, there are still a many questions to answer that will help us to understand the regulation of milk fat synthesis that is central to the development of nutritional strategies to enhance the nutritional value of milk in ruminants.

**Acknowledgments**

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**Resumen**

E. Vargas-Bello-Pérez y P.C. Garnsworthy. 2013. Ácidos grasos \textit{trans} y su rol en la leche de vacas lecheras. \textit{Cien. Inv. Agr.} 40(3): 449-473. La grasa de productos lácteos es muy importante en la dieta humana en muchos países. Aproximadamente, el 75% del consumo total de grasa de productos de rumiantes proviene de la grasa láctea. Los ácidos grasos \textit{trans} (AGT) son producidos durante la hidrogenación ruminal de AG poliinsaturados. Son una mezcla de isómeros que son incorporados en la grasa láctea de vacas lactantes. La principal fuente de AGT de la dieta humana proviene de los aceites vegetales parcialmente hidrogenados y de la leche y carne de rumiantes. Usualmente los lípidos de la leche y carne de rumiantes contienen entre 1 a 8% de AGT del total de AG presentes, y en su mayoría compuestos por isómeros 18:1. El AGT más común en la grasa de rumiantes es el vaccénico (18:1 \textit{trans}-11), el cual representa entre un 60 a 80% del total de AGT. A diferencia de los AGT de los aceites vegetales parcialmente hidrogenados, los AGT de rumiantes (ácido vaccénico) pueden ser convertidos en ácido ruménico a través de la enzima estearoil coenzima-A desaturasa. Actualmente, los consumidores están conscientes de la relación entre la grasa de la dieta, la salud y la prevención de enfermedades. Estas preocupaciones han motivado la necesidad de investigar el destino metabólico y la bioactividad de los AG de la dieta. Mediante la manipulación de la nutrición de la vaca lechera, los productores pueden modular y alterar la composición de la grasa láctea. Los cambios más grandes se pueden observar mediante la calidad del forraje de la dieta (pastos) o
mediante la utilización de suplementos vegetales o marinos en la dieta. Dado que la factibilidad económica está definida por las ganancias de los productores, la manipulación de la dieta de la vaca lechera es la alternativa más factible y práctica para modificar la composición de la grasa láctea.

**Palabras clave:** Ácidos grasos trans, fermentación ruminal, hidrogenación, leche, vacas lecheras.

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