

## Grazing versus indoor feeding: effects on milk quality

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### Abstract

Changing societal drivers and consumer demands request systems that provide desired products through improved, sustainable production processes.

In this paper dairy product supply chains were analysed, with emphasis on milk quality in relation to feed. Milk fatty acids were analysed in milk produced in different regions, various seasons, and with different feeding systems and also in forages and concentrates. A rapid screening method for conjugated linoleic acid (CLA) in milk fat was developed. Milk from cows on fresh green forage, especially grazed grass, had a much higher unsaturated:saturated fat proportion with more poly-unsaturated FA (beneficial for heart diseases) and more conjugated linoleic acid (CLA isomer rumenic acid, C18:2 c9,t11, possible anti-cancer effects) than milk from silage-fed cows. The FA composition of milk has recently become less favorable than before, e.g., in the 1960s, due to different feeding practices and nobody is aware because it was never monitored, but essential FA and CLA levels have dropped substantially. With low-fat dairy products, human intake of these is declining even further, as ruminant products are the main source of CLA intake.

Farmers from some dairy cooperatives in The Netherlands that produce milk from grazed grass now receive a premium on top of their milk price, so compared with farmers that keep their cows indoors year-round, these primary producers benefit from the higher market value at the end of the production chain.

Keywords: forage, silage, feeding system, seasonal change, milk fatty acids

### Introduction

Changing societal drivers (landscape values, animal welfare) and consumer demands (tasty healthy products) request systems that provide desired products through improved, sustainable production processes.

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). Dairy products contribute 15-20% of the intake of total fat, 25-33% of saturated fat, and about 15% of dietary cholesterol in the US population (Havel, 1997). Milk fat contains approximately 70% saturated, 25% monounsaturated, and 5% polyunsaturated fatty acids (Grummer, 1991), but this can be modified by changing the animal diet. It is thought that lauric acid (C12:0), and especially myristic (C14:0) and palmitic acid (C16:0) raise total and LDL cholesterol, whereas stearic acid (C18:0) is neutral relative to the monounsaturated fatty acid (MUFA) oleic acid (C18:1) (Grundy and Vega, 1998). Cardiovascular risk might be reduced by lowering the intake of undesirable saturated FA or by making alterations in the quality of the fat consumed. Manipulation of the fatty acid composition of milk fat is receiving attention because dietary saturated fatty acids, especially myristic and palmitic acid, can induce hypercholesterolemia in humans (Denke and Grundy, 1992). 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% saturated, 10% polyunsaturated, and 39% monounsaturated fatty acids. When men and women consumed fat-modified dairy products, their plasma total cholesterol was reduced 4.5% compared with intake of conventional dairy products (Noakes *et al.*, 1996).

Ruminant products contain some polyunsaturated fatty acids (PUFA), including omega-3 FA (Dewhurst *et al.*, 2003), with associated health effects for the consumer. The main omega-3 FA in milk is  $\alpha$ -linolenic acid (C18:3 n-3). Another category of

PUFA are conjugated linoleic acids (CLA); the main isomer in milk being rumenic acid (C18:2 *cis*-9, *trans*-11). MUFA in milk consist mainly of C18:1 *cis*-9 (oleic acid) and also C18:1 *trans*-11 (trans vaccenic acid, TVA). Rumenic and vaccenic acid are both *trans*-11 FA produced by rumen micro-organisms and are unique for ruminant fat. They could also be termed omega-7 *trans* fatty acids (Ellen and Elgersma, 2004), to distinguish them from *trans* FA in general, which have a negative health effect. Rumenic acid has been associated with anticarcinogenic properties in rats (Corl *et al.*, 2003; Ip *et al.*, 1999) and possibly in humans (Aro *et al.*, 2000; Belury, 2002). Also other potential beneficial effects of CLA for human health were mentioned, but more work is needed to elucidate the safety and efficacy of isomers and required doses (Belury, 2002). Ingested TVA can be converted into rumenic acid in monogastrics, including humans (Salminen *et al.*, 1998; Santora *et al.*, 2000). Turpeinen *et al.* (2002) estimated that on average in humans 19% of dietary vaccenic acid is endogenously converted into rumenic acid. Banni *et al.* (2001) demonstrated that feeding vaccenic acid to rats (2% in the diet) not only increased tissue levels of rumenic acid, but also had a protective effect against the development of mammary tumours. This implies that, when calculating the dietary intake of rumenic acid, it is reasonable to add 20% of the vaccenic acid present in the diet. CLA contents in products, calculated on the fat fraction, are comparable with those of the milk(fat) of the raw milk from which these products are obtained (Lavillonière *et al.*, 1998; Dhiman *et al.*, 1999b).

Milk produced by farmers therefore plays a key role. As milk FA composition is mainly related to the FA composition of the feed of the animals (Khanal and Olson, 2004), effects of forage, feed and feeding systems will be discussed here.

### Lipids in animals

In contrast to short- and medium-chain FA, ruminants cannot endogenously synthesise these long-chain C18 FA that are desired in meat and milk. Therefore, long-chain FA have to be ingested by the ruminant with the feed.

Linoleic acid (C18:2 *cis*-9, *cis*-12) and  $\alpha$ -linolenic acid (C18:3 *cis*-9, *cis*-12, *cis*-15) are C18 substrates for rumen biohydrogenation (Fig. 1). In herbage, C18:3 predominates, whereas maize contains mainly C18:2. However, the FA content of maize is much lower than of grass. The FA profile of lipids in oils seeds used in concentrates is highly variable. In groundnuts, rapeseed and sesame seed C18:1 is the major FA, cottonseed, soybeans and sunflower seeds are high in C18:2 and linseed is high in C18:3. Currently used cheap concentrate feed ingredients such as coconut meal and palm kernels, however, contain mainly saturated fats (C14:0 and C12:0, respectively). Animal diets could also be supplemented with fish oil, containing predominately FA of 20 or 22 carbons as the major FA, but this might negatively affect milk taste and harm the image of dairy farming and dairy products while plant sources, especially forage, would represent the most natural and environmentally sustainable source.

There are several possible ways to reach the objective of increasing the concentration of desired FA in ruminant products, either to increase the amount (intake) or the concentration of substrate in the feed, to reduce the extent of biohydrogenation in the rumen, or to enhance the activity or amount of the  $\Delta^9$  – desaturase enzyme that converts TVA into rumenic acid in the udder (Figure 1).

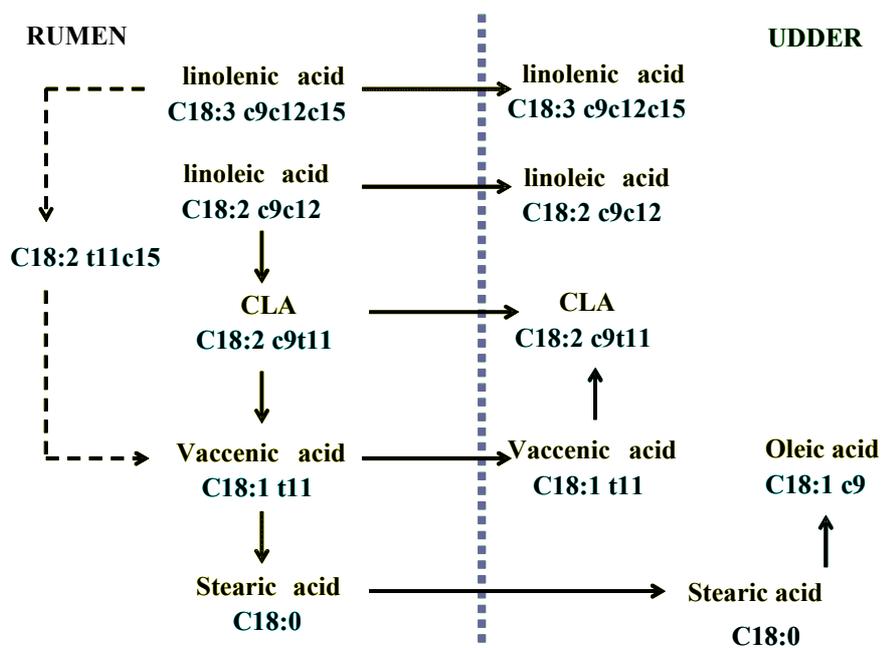


Figure 1. Simplified scheme of biohydrogenation and desaturation pathways of C18 fatty acids in rumen and udder of dairy cow.

Most linoleic and linolenic acid are biohydrogenated in the rumen. A varying proportion of PUFA, ranging from less than 2 to over 20% is recovered as TVA, an important product of the biohydrogenation of both linoleic and linolenic acid.

Lipolysis and biohydrogenation have been extensively studied *in vitro*, notably with soybean oil and to a lesser extent with linseed oil. The FA composition of linseed oil more or less resembles that of fresh grass, in that lipids in fresh grass also contain a high proportion of linolenic acid.

In the experiments with linseed oil of Chow *et al.* (2004), an accumulation of the intermediates C18:2 *trans*-11, *cis*-15 and TVA were observed. Because of its similarity with linseed oil, the C18:2 *trans*-11, *cis*-15 is most likely also an important intermediate in the rumen of grass-fed cows. It is likely that this is reflected in the FA profile of milk produced by grass-fed dairy cows. Both C18:2 (from maize and other grains) and C18:3 can be converted to TVA in the rumen and might lead to CLA in the udder by action of the  $\Delta^9$  – desaturase enzyme, but only C18:3 gives rise to the intermediate C18:2 *trans*-11, *cis*-15 in the rumen (Fig. 1). Comparing winter diets with diets on fresh grass revealed that in grass-fed cows the C18:2 *trans*-11, *cis*-15, when expressed as proportion of the sum of C18:2 + C18:3, ranged between 0.30 and 0.46, whereas on winter diets this was only between 0.029 and 0.042 (B. Vlaeminck, pers. communication). Changing from a grass and maize silage based winter diet to fresh grass elevated the level of this intermediate.

### Lipids in plants

Dairy cows' diets are usually composed of a mix of fresh forages (mainly leaf blades), conserved forages (herbage leaf blades and also stems in spring; seeds and stems in case of forage maize) and concentrates (seeds), all of which contain lipids of vegetable origin. Diet composition differs greatly among seasons and regions.

Some information is available in the literature about the lipid concentration and composition of forages and factors influencing them. The lipid fraction in leaves of herbs and grasses ranges from 30 – 100 g kg<sup>-1</sup> DM, much of which is contributed by lipids in the chloroplasts (Bauchart *et al.*, 1984). Sources of variation in lipid concentration are plant species, growth stage, temperature and light intensity (Hawke, 1973). There are five major fatty acids in grasses and approximately 95% consists of C18:3, C18:2 and C16:0 (Hawke, 1973). Fresh grass contains a high proportion (0.50-0.75) of its total fatty acid content in the form of  $\alpha$ -linolenic acid, C18:3. Levels of  $\alpha$ -linolenic acid vary with plant and environmental factors such as stage of maturity, genetic differences (Elgersma *et al.*, 2003a, b), season and light intensity (Dewhurst and King, 1998).

Quantifying the concentrations and composition of fatty acids in grasses in response to environmental factors could help to design management strategies to increase precursors for beneficial FA in products from ruminants. However, there is a lack of standardized procedures for sampling, storing, extracting and analysing lipids in leafy material. Drying, freeze-drying or thawing prior to extraction may affect the results and hampers comparison of data among the various laboratories. A ring test was set up in the autumn of 2005 for analyzing fresh and ensiled grass in different laboratories, and results are awaited.

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 big influences 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, during senescence, after detachment (grazing or cutting) and during storage.

In detached plants, immediately after cutting and perhaps during the early stages of ingestion and ensiling, the metabolism of plant cells can continue. Also, there is activity of the enzymes of dead tissue. The processes of respiration and proteolysis are best known, however also lipolysis occurs.

In ruminants grazing fresh pastures, the first stages of lipolysis could be mediated by plant lipases (Lee *et al.*, 2003). These enzymes are widely 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.

Dewhurst and King (1998) studied the effect of ensiling on the fatty acids in the material. Wilting prior to ensiling reduced the content of total fatty acids by almost 30%, with for linolenic acid even 40% loss. These authors suggest that the ensiling process itself has little influence, provided compaction and sealing of the silos is good. This may not always be the case in big bale silages. Adding silage additives (formic acid, formalin) resulted in much smaller losses, which was also found for formic acid by Doreau and Poncet (2000). Hay making reduced total FA by over 50%, with loss of linolenic acid to an even higher percentage (Doreau and Poncet, 2000). Similar observations were seen in haylage (70% DM) by Elgersma *et al.* (2003c).

Some work has been carried out to investigate the effect of nitrogen fertilization on FA concentration in the grass. Boufaïed *et al.* (2003) applied 120 vs 0 kg N ha<sup>-1</sup> on *Phleum pratense*, resulting in significant increases of C16:0 (18%), C18:2 (12%) and C18:3 (40%) in the herbage and an overall increase of 26% in the concentration of FA.

Elgersma *et al.* (2005) hypothesized that the protein concentration in the herbage, the leaf blade proportion of the canopy and regrowth period of the sward might affect the concentration of fat and the proportions of FA in the herbage. Regrowth period affected the total FA concentration, and significantly lower concentrations of C18:3 and C16:1 were found after a longer period of regrowth. N application resulted in higher concentrations of all FA. The FA composition was not affected by N application, but a longer regrowth period significantly decreased the proportion of C18:3 and increased those of C18:2 and C16:0. A strong positive overall linear relation was found between the concentrations of total FA and C18:3 with the N concentration in the herbage. These results were confirmed by further experiments (Witkowska *et al.*, 2006).

#### **Effect of feeding system on milk FA composition**

Cows on pasture (Dhiman *et al.*, 1999a; Elgersma *et al.*, 2004a) grazing lush green grass (Dewhurst *et al.*, 2003; Elgersma *et al.*, 2003d; Khanal and Olsen, 2004) at a high herbage allowance (Elgersma *et al.*, 2004b) produce milk with the highest concentrations of PUFA.

Experiments showed a quick response of the CLA content in milk to changing cows from indoor feeding to pasturing (Kelly *et al.*, 1998) and *vice versa* (Elgersma *et al.*, 2004a). The maximum effect is reached within about 5 days (Chilliard *et al.*, 2001).

Grazing cows at a high herbage allowance can select and ingest upper layers of the canopy, where young leaf blades predominate. It was therefore hypothesized that the protein concentration in the herbage, the leaf blade proportion of the canopy and regrowth period of the sward might affect the concentration of fat and the proportions of FA in the herbage. A high herbage allowance results in milk with high concentrations of CLA, probably because it enables selection of leafy plant parts with higher lipid concentrations.

As fertilization and herbage allowance can be managed easily by farmers, it might represent an interesting and feasible way to obtain higher concentrations of fatty acids in herbage. Also decisions on the timing of cutting or grazing, i.e., the regrowth stage at which herbage is harvested, can be a practical means of influencing herbage quality. Scollan *et al.* (2005) stated grassland offers considerable scope to help creating product differentiation in increasingly competitive markets.

Alternatively, also the concentrate composition could be modified (e.g., Ashes *et al.*, 1992). Last but not least, apart from milk FA composition, also the milk production level and the milk fat concentration have to be taken into account.

### Regional and seasonal variation in milk FA composition

Milk fat consists of a range of FA, but for this overview we will focus on proportions of unsaturated fatty acids (UFA), omega-3 FA, and the concentration of CLA, as these are the desired categories of FA.

Seasonal variation in milk FA composition has long been recognized and is caused by difference in feed between summer and winter. Nałęcz-Tarwacka (unpublished results) studied 429 cows of 23 farms in central Poland during two years. The proportion of UFA was 344 g kg<sup>-1</sup> in summer when all cows had green grass, and 308 g kg<sup>-1</sup> in winter when they were fed maize silage, grass silage and brewers' grain. The omega-3 FA declined from 9 to 8 g kg<sup>-1</sup>, and the CLA concentration from 7.2 to 5.4 g kg<sup>-1</sup>.

In another study, Reklewska *et al.* (2003) found CLA concentrations in milk of Black-and-White cows in Poland to be higher in summer (8.4 g kg<sup>-1</sup>) and autumn (8.9 g kg<sup>-1</sup>) than in winter (6.3 g kg<sup>-1</sup>). They also compared CLA in milk from cows on a total mixed ration (TMR) and on pasture, respective values were 6.1 and 11.7 g kg<sup>-1</sup>. A further study compared seasonal changes in cows indoors, on three different feeding systems (Table 1).

Table 1. CLA concentrations (g kg<sup>-1</sup> of milk fat) of cows on different feeding systems in Poland, depending on the season (Reklewska *et al.*, 2003).

Feeding system	Summer	Winter
Indoor-pasture ecological	10.0	7.1
Indoor-pasture conventional	5.0	6.9
Indoor TMR	6.7	0.4

Remarkably, contrary to the general trend of lower CLA concentrations in winter, cows on the conventional indoor-pasture system had a higher value in winter; the pasture in summer was ad libitum and in winter less pasture was offered.

Also cattle breed could play a role: Żegarska *et al.* (2001) reported milk compositions of summer milk from cows on pasture and found a higher CLA concentration for Polish red (11.9 g kg<sup>-1</sup>) than for Black-and-White (9.4 g kg<sup>-1</sup>).

Individual animal variation should be included as well. Animals on the same diet showed a three-fold difference in milk conjugated linoleic acid (CLA) content (Kelsey *et al.*, 2003).

Elgersma *et al.* (2004a) found a range in CLA contents in milk of six individual cows of 14-36 g kg<sup>-1</sup> of milk fat on grazed grass, and 4.0 – 5.8 g kg<sup>-1</sup> at the end of a 2-week transition period to a 1:1 grass/maize silage diet. In a second experiment, the CLA contents in milk fat of 12 cows ranged from

12.4 to 27.8 g kg<sup>-1</sup> on grazed grass, and from 4.0 to 8.6 g kg<sup>-1</sup> four days after transition to maize silage. In general, CLA levels differed among cows, but patterns in response to diet changes were similar. The ranking of cows for CLA concentration in their milk at the various sampling days was rather similar Elgersma *et al.* (2004a). Also Lock and Garnsworthy (2002) found consistency in the ranking of individual cows for *cis*-9, *trans*-11 C18:2 concentration over time on the same diet or when cows were switched between diets. This would offer scope for selecting high-CLA animals for breeding purposes, provided the trait is at least moderately heritable.

#### **FA analysis - methodology**

The concentration of CLA in fat is usually determined by gas chromatography (GC), which is a costly and time-consuming analysis. Scientific progress is partly hampered by the labour intensive, slow and costly standard analysis of FA using gas chromatography.

A screening method (details are confidential and cannot be provided here) was developed to predict the CLA concentrations in milk fat by using an alternative analysis. The correlation between both methods was very high ( $R^2 = 0.998$ ) across a range of CLA concentrations (2 – 32 g kg<sup>-1</sup> fat) (Elgersma and Wever, 2005). The method is more rapid and could be used for screening purposes.

For lipid analyses in herbage, various methods are used. As mentioned previously, a ring test has been initiated to investigate effects on FA data.

#### **From farm to factory to consumer – marketing and future perspectives**

It was discovered by chance that during the last decades, the FA composition of milk has become less favorable in The Netherlands and nobody is aware because it was never monitored, but essential FA and CLA concentrations have dropped substantially. The total FA production from cows could be higher as a result of higher milk fat concentrations, which would have to be taken into account for a total picture, next to the FA concentrations. However, with low-fat dairy products being marketed, human intake of these FA is declining even further, as ruminant products are the main source of CLA intake. Health effects of CLA need further study in humans, but the beneficial effect of unsaturated fats in general seems commonly accepted. CLA concentrations are correlated with those of unsaturated fatty acids and could perhaps be used as indicators for the FA composition of milk.

In the Netherlands, the findings mentioned in this review could perhaps undermine the currently increasing trend for cows to be kept indoors year round.

Farmers from a small Dutch dairy cooperative that produce milk from grazed grass now receive a premium on top of their milk price. So in this case primary producers benefit from the higher market value at the end of the production chain.

#### **Conclusions**

This review showed that milk quality can be changed by farmers by feeding strategy and there are longer-term options for animal breeding. There are possibilities for a chain-approach from farmer to industry to consumer and small-scale examples are presented, but there are still questions regarding health claims in humans. Also, monitoring milk quality is no routine procedure yet. Research and standard protocols for sampling, storage and lipid analysis in forages are needed. An integrated chain approach is essential for putting science into practice.

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