Chapter 1
Manipulation of Dietary and Physiological Factors on Composition and Physicochemical Characteristics of Milk Fat

Lars Wiking, Mette K. Larsen, and Martin R. Weisbjerg

1 Introduction

Fatty acids (FA) in milk origin partly from FA in the feed and partly from de novo synthesis in the mammary gland, commonly with half from feed and half from de novo synthesis. De novo synthesized FA are generally short or medium chain length, maximum C16, and are saturated. Milk FA originating from feed will mirror the feed ration FA according to chain length; however, due to rumen microbial biohydrogenation, the feed FA supplied to the mammary gland will be much more saturated and contain *trans* and conjugated linoleic acid (CLA) intermediates from the rumen biohydrogenation or later desaturation in the mammary glands. The proportion of milk FA from the two sources can deviate considerably from the half-half, depending mainly on FA supply via the feed but also on nutrient supply for the de novo synthesis, and on FA supply/demand from mobilisation/deposition.

Knowledge on how the FA composition of milk is affected by feeding and by physiological factors creates possibilities for manipulation of milk fat composition. The purpose of such manipulations could be nutritional or technological. Nutritionally some FA are regarded as beneficial to human health, whereas other FA are regarded as detrimental, and increasing the former and/or decreasing the latter would create a healthier milk fat. Technologically, more saturated and longer chain FA give a firmer texture of dairy products such as butter and cheese, whereas more

L. Wiking (✉)
Department of Food Science, Aarhus University, Aarhus, Denmark
e-mail: Lars.Wiking@food.au.dk

M. K. Larsen
Department of Food Science, Aarhus University, Aarhus, Denmark

Arla Foods Amba, Videbaek, Denmark

M. R. Weisbjerg
Department of Animal Science, Aarhus University, Tjele, Denmark

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unsaturated FA and shorter chain FA give a softer texture. Higher content of unsaturated FA, in particular, polyunsaturated FA, makes the milk fat more prone to oxidation.

Intensive milk production relies on specialized dairy cow breeds. Holstein is globally by far the most dominating breed, and Jersey is the second most popular breed (http://www.survey-icar.org/cow_survey/). Jersey cows are lower yielding than the Holsteins. However, the Jersey milk has a higher content of fat and protein than the Holsteins. The milk production varies throughout lactation. A normal calving interval is one year, resulting in lactation profiles for milk yield and composition as shown in Fig. 1.1 for the first 30 weeks of lactation. Dairy management and production systems vary depending on climatic conditions, local traditions and development in technology and trade. Traditionally, milk production has been based on grazing of natural grasslands, where calving and milk production were seasonal

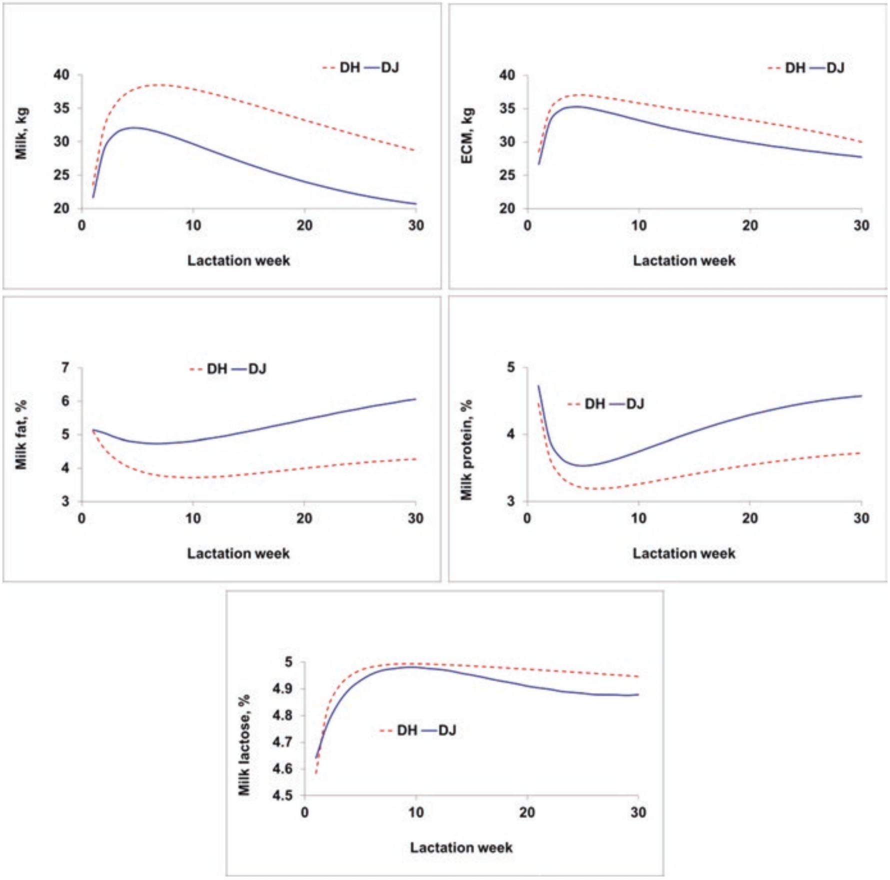

Fig. 1.1 Profiles for daily milk yield, 3.14 MJ/kg energy corrected milk (ECM) and milk concentration of fat and protein during the first 30 weeks of lactation for similar fed Danish Holstein and Danish Jersey (modified from Weisbjerg et al., 2013)

following the abundance of grass. Grazing is still an important part of dairy cow feeding, although with increased production intensity, the extent of grazing declines and is partly or fully replaced with all-year barn feeding with conserved forage and concentrates, typically fed as a total mixed ration (TMR).

2 Manipulation of Dietary and Physiological Factors on Milk Fat Composition

2.1 Forages

Green forages like grass and legumes are a major part of dairy cows' diets, and in some countries, e.g. New Zealand, milk production highly relies on grazing grass clover pastures. In North America, lucerne is the major green supplement to yellow forages like whole crop maize silage forage (Thoma et al., 2013). In Western Europe, the situation is more variable and range from grass and grass-clover as the sole forage and major part of total feed to high maize silage rations, and finally to rations with a high proportion of concentrate (De Vries, Debruyne, & Aarts, 2013; Kristensen et al., 2015).

Green forages are significant sources of FA, mainly linolenic acid (Larsen, Kidmose, Kristensen, Beaumont, & Mortensen, 2013). The yellow whole crop forages like maize silage supply linoleic acid which is the main fatty acid in maize silage as well as in whole cereal crops with developed kernels (Larsen, Hymøller, Brask-Pedersen, & Weisbjerg, 2012).

2.2 Energy and Protein Concentrates

Concentrates used for dairy cows are mainly cereal grains, legume seeds and by-products from the cereal, bioethanol, sugar and oilseed industry. Oilseed meals have been solvent extracted and are characterised by a low residual fat content, whereas oilseed cakes have been pressed and are higher in residual fat content. The fatty acid composition varies a lot among oilseeds, i.e. palm kernel and coconut being rich in C12 and C14, rapeseed being rich in C18:1, sunflower, soybean and cottonseed rich in C18:2 and linseed in C18:3 (NorFor, 2017). Furthermore, fat supplements such as palm fatty acid distillate coming from the oilseed industry are used as cattle feed (Weisbjerg, Wiking, Kristensen, & Lund, 2008). FA composition of some feedstuffs are presented in Table 1.1.

Table 1.1 Fat concentration and FA composition in some feedstuffs. FA composition in by-products like cakes, meals, distillers grains etc. will generally reflect composition in original seed/grain (NorFor, 2017)

	Crude fat	FA	<C12	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	Other FA
	g/kg DM	g FA/kg crude fat	g/100 g FA								
Palmkernel cake	79	800	9.7	46.9	14.1	8.8	1.3	18.5	0.7	0.0	0.0
Rape seed	461	900	0	0	0	4.4	1.4	54.3	19.5	11.4	9
Cotton seed	210	900	0	0	1.4	23.4	1.1	22.9	47.8	0	3.4
Soya beans	222	900	0	0.2	0.1	9.8	2.4	28.9	50.7	6.5	1.4
Sun flower seed	480	900	0	0	0	5.6	2.2	25.1	66.2	0	0.9
Linseed	390	900	0	0	0	5.5	4.3	21.1	13.3	55.7	0.1
Wheat grain	25	700	0	0	0.1	17.8	0.8	15.2	56.4	5.9	3.8
Maize grain	46	900	0	0	0	11.4	1.9	25.3	60.7	0.7	0
Grass	39	540	0	0	0	20.2	2.7	2.7	14	56.9	3.5
White clover	36	480	0	0.2	0.4	17.2	2.9	5	16	55.4	2.9
Red clover	32	480	0	0.3	0.5	18.8	3.7	8	23.2	42.9	2.6

Table 1.2 Nutrients eaten and nutrients absorbed by ruminants (Volden, 2011; Weisbjerg et al., 1992)

Nutrients in feed	Nutrients absorbed
Carbohydrates	VFA (acetic, propionic and butyric acid), microbial matter, glucose
Protein	Microbial protein rumen escape feed protein
Fat	Hydrogenated fatty acids from feed, microbial fatty acids

2.3 *Response to Energy and Nutrient Supply*

Due to the rumen metabolism, nutrients absorbed by ruminants such as dairy cows deviate considerably from the nutrients eaten (Table 1.2), in contrast to nutrition of monogastric animals, where the nutrients from the feed are transferred more directly to the animal. The microbial community in the rumen (bacteria, protozoa and fungi) will ferment both fibrous and non-fibrous carbohydrates to be used for their growth, and they will degrade protein to supply nitrogen and to a lesser degree energy for their growth. Short-chain volatile FA mainly acetic, propionic and

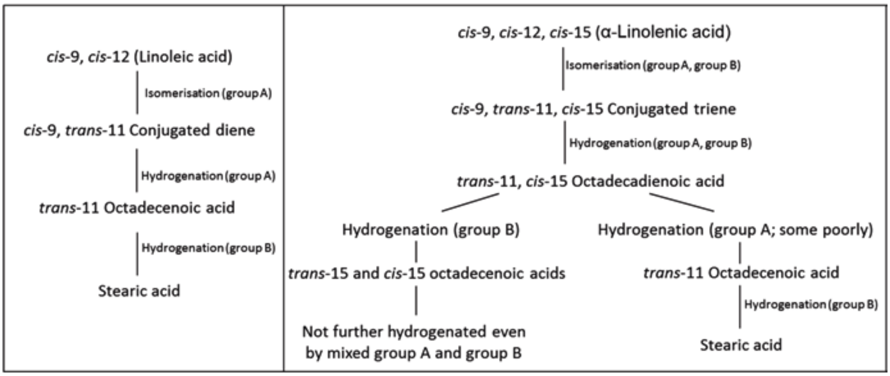

Fig. 1.2 Rumen metabolism of linoleic and linolenic acid. Group A and B refer to two classes of biohydrogenating bacteria (adapted from Harfoot & Hazlewood, 1997)

butyric are produced from the carbohydrates during their fermentation and the short-chain FA are absorbed over the rumen wall as the major energy source for the cow. FA are not fermented in the rumen, but glycerides are hydrolysed, the glycerol part being fermented, and unsaturated FA are extensively (70–90% efficiency) hydrogenated (Weisbjerg, Børsting, & Hvelplund, 1992). Hydrogenation results in a large number of intermediates, which can be seen in Fig. 1.2 (Harfoot & Hazlewood, 1997).

2.4 De Novo FA Synthesis

The substrate for the de novo FA synthesis in the mammary gland is mainly acetate and β-hydroxybutyric acid (Harvatine, Boisclair, & Bauman, 2009). β-hydroxybutyric acid originate from butyrate (Table 1.2) which is metabolised to β-hydroxybutyric acid during absorption over the rumen wall. The FA synthesis is catalyzed by acetyl-CoA carboxylase and fatty acid synthase (Wakil, Stoops, & Joshi, 1983) and result in FA with chain length from 4 to 16 (Harvatine et al., 2009).

De novo synthesized FA are saturated, and FA of feed origin transported to the mammary glands are also mainly saturated due to rumen biohydrogenation. Saturated FA can be desaturased in the mammary gland catalyzed by stearoyl-CoA desaturase. Stearoyl-CoA desaturase is located in the endoplasmic reticulum with C18 and C16 FA as primary substrates (Palmquist, 2006). This enzyme adds a *cis9* double bond in FA and C18:0 is the main substrate. This enzymatic activity is higher in Holstein cows compared to Jersey, and the differences between animals are mainly genetic related (Poulsen et al., 2012).

3 Feed Effects on Milk Composition

3.1 Milk Fat Content

Milk gross composition can be controlled by feeding to some extent. Increased energy intake obtained by increased energy concentration in the ration, e.g. by increased concentrate offer, increased concentrate/forage ratio in mixed rations or increased forage digestibility can reduce milk fat concentration and consistently increase milk protein concentration (Alstrup et al., 2014; Hymøller, Alstrup, Larsen, Lund, & Weisbjerg, 2014). Increased fat in feed consistently decreases milk protein concentration (Weisbjerg et al., 2008), whereas the effect on milk fat concentration is more variable. Often small increases in fat supplementations to rations with low fat concentrations will increase milk fat production, and thereby severely decrease protein/fat ratio in the milk, whereas higher inclusions to >4–5% FA in ration DM might reduce milk fat concentration (Østergaard, Danfær, Daugaard, Hindhede, & Thysen, 1981). However, the use of saturated fat with chain length ≥ C16 seems to increase milk fat also at high concentrations in the ration (Weisbjerg et al., 2008). The effect of specific carbohydrates can mainly be ascribed to their effect on energy intake and rumen fermentation pattern, where non-dietary fibre (NDF) reduce the energy intake and increase the acetate proportion of rumen VFA, thereby increasing milk fat and reducing milk protein (Alstrup et al., 2014). Starch will increase the energy intake and the propionate ratio in the rumen, subsequently increasing milk protein and reducing milk fat (Stensig, Weisbjerg, & Hvelplund, 1998). Sugar may increase butyrate levels in the rumen, resulting in higher milk fat concentration and possibly increased milk protein (Stensig et al., 1998).

3.2 Fatty Acids

Roughly, FA with a chain length up to C16 originate from de novo synthesis, which to some extent is genetically controlled, whereas the longer chained FA to a large extent is determined by the content and composition in the feed. A very large number of studies have reported how different feeding regimes affect milk fatty acid composition, and several attempts have been made to perform meta-analyses to predict FA composition in milk fat based on feed composition. Such models seem valid as long as they are used in feed conditions similar to those that are included in the meta-analysis. In Table 1.3, typical values for FA distribution of the main FA in milk are shown.

The content of C4–C14 in milk fat is mainly dependent on the de novo synthesis of these FA; although some feed items contain minor amounts. Feeding fat supplements to dairy cows decreases de novo synthesis and use of fat sources containing more than 80% C16 has decreased the content of C4–C14 in milk fat by 22–43 g/kg FA (Piantoni, Lock, & Allen, 2013; Weisbjerg et al., 2013), corresponding to about

Table 1.3 Variation in composition of major fatty acids (FA) in milk as affected by different treatments

Treatment	Proportion of fresh grass	Hay or grazing	Oilseed supplements	Fat supplements
Reference	Couvreur et al. (2006)	Coppa et al. (2011)	Larsen et al. (2012)	Weisbjerg et al. (2013)
Fatty acids (g/kg FA)				
C4–C14	262–284	211–264	200–244	212–260
C16:0	241–310	230–326	217–337	242–397
C18:0	103–112	75–116	101–180	74–152
C18:1 *cis*9	194–211	174–223	162–266	147–256
C18:1 *trans*	24–59	18–75	11–31	12–30
C18:2 n6	13–16	8–14	14–18	15–20
C18:3 n3	2–7	6–10	5–8	4–8
CLA *cis*9 *trans*11	5–17	6–24	4–10	3–9
VLCFA[a]	1.5–2.8	3.2–4.4		

[a] Very long chained (>C18) polyunsaturated FA

half of the observed increase of C16 in milk fat. Feeding oil or oilseed supplements affected milk content of C4–C14 differently in different studies. Thus, 200–300 g oils or 1–1.5 kg oilseeds resulted in no reductions in the content of C4–C14 in milk (McNamee, Fearon, & Pearce, 2002), reductions corresponding to half of the reductions in C16 content (Larsen et al., 2012; Weisbjerg et al., 2013), or reductions similar to the reductions in C16 content (Fearon, Mayne, Beattie, & Bruce, 2004). The distribution within C4–C14 is feed dependent and when the de novo synthesis is reduced due to increasing fat supplements, C12–C14 FA are decreasing more rapidly than C4–C10 FA in the produced milk (Weisbjerg et al., 2008).

Palmitic acid, C16:0, is the dominant FA of milk and originates from feed as well as from the mammary de novo synthesis. The distribution between these two sources of C16 varies and in milk from high yielding dairy cows, the output of C16 in milk has been reported as 2.6–5.6 times the input (Larsen et al., 2012), where the lower values were obtained at high levels of fat supplementation from feed. Thus, to reduce the C16 content in milk fat it is important to reduce the amount in feed but controlling the de novo synthesis may be even more important. An obvious source of C16 in feed is fractionated palm fat, which has been used extensively to increase milk production, and supplementation of a fat source containing more than 80% C16 by 2–3% of dry matter intake (DMI) has increased C16 in milk fat by 50–90 g/kg FA compared to no fat supplement (Piantoni et al., 2013; Weisbjerg et al., 2013). The de novo synthesis is reduced when serum concentrations of C18 FA are increased e.g. by feeding cows oils or oilseeds. A decrease in C16 content of 50–100 g/kg FA is reported after feeding 1–1.5 kg per cow per day oilseeds of various types (Larsen et al., 2012; McNamee et al., 2002; Weisbjerg et al., 2013). Such feeding rich in C18 FA also increase the content of C18 FA in milk fat.

C18 FA in milk is derived from feed or from body mobilization, which means that higher contents are obtained when feed is rich in C18 FA or when the cow is in negative energy balance. C18 FA in feed are to a wide extent unsaturated and are extensively hydrogenated by the rumen microorganisms. The content and distribution of a vast range of C18 FA in milk are frequently reported and such results are used to document and explain how rumen processes are affected by feeding. Oleic acid (C18:1 *cis*9) is the second most abundant fatty acid in milk. Almost all C18:1 *cis*9 in milk is formed from C18:0 by mammary stearoyl-CoA desaturase. Some C18:1 *cis*9 can be transferred to milk from feed as rumen hydrogenation is incomplete, and a major feed source of C18:1 *cis*9 is rapeseed products (Larsen et al., 2012).

Linoleic acid, C18:2 n6, is the most abundant polyunsaturated FA in milk. The only source of C18:2 n6 in milk is C18:2 n6 from feed. In diets without fat supplements, grains and maize silage are significant sources, legumes and herbs are typically richer sources than grass species, and the richest sources of C18:2 n6 are oilseed products of soy and sunflower. The recovery from feed to milk depends very much on the degree of biohydrogenation and recoveries of 7–12% have been reported after feeding different levels of oilseeds (Larsen et al., 2012), whereas feeding freshly cut grass or herbs instead of TMR increased the transfer efficiency of n6 FA from 16% to 50–64% (Petersen, Søegaard, & Jensen, 2011).

Linolenic acid, C18:3 n3 is another essential FA present in milk. The primary source of C18:3 n3 in milk is grass-based feed, but linseed and rapeseed may be used as an alternative source. The level of biohydrogenation of C18:3 n3 from feed is usually high and recoveries in milk in the range of less than 10% are normal (Larsen et al., 2012; Petersen et al., 2011). The relative differences in C18:3 n3 concentrations in milk fat can be large although the absolute differences are small. Differences may be caused by differences in C18:3 n3 content in feed; however, in some cases, differences are mainly due to differences in biohydrogenation. Havemose, Weisbjerg, Bredie, and Nielsen (2004) fed cows maize silage or grass silage and found a threefold increase in the C18:3n3 concentration in milk, from 2 to 6 g/kg FA in milk from cows fed grass silage, which is due to the higher content of C18:3 n3 in grass compared to maize. Petersen et al. (2011) increased the n3 FA content from 7 g/kg FA in milk from TMR feed, over 11 g/kg FA% in milk from cows fed pure fresh cut white clover/rye grass mixture, to 21 g/kg in milk from cows fed species-rich fresh cut herbs. At the same time, the transfer efficiency of n3 FA increased from 7% in TMR fed cows over 8% for clover/grass fed cows to 16% for the herb fed cows. Feeding increased amounts of linseed resulting in increased contents of C18:3 n3 in milk, and Jersey cows have lower initial levels compared to Holstein (Larsen et al., 2012). The increased C18:3 n3 level is accompanied by reduced transfer efficiencies from 7% at no linseed supplement to 4% at the highest level of linseed (Larsen et al., 2012).

The main CLA present in milk is CLA *cis*9 *trans*11 (normally 80–90% of CLA) accompanied by smaller amounts of CLA *trans*7 *cis*9 and CLA *trans*10 *cis*12

(Shingfield, Bonnet, & Scollan, 2013). The main source of CLA *cis*9 *trans*11 is C18:1 *trans*11, which is desaturated by mammary desaturase. Thus, the content of CLA *cis*9 *trans*11 is mainly dependent on the C18:1 *trans*11 supply to the udder and the desaturase activity. Content of CLA *cis*9 *trans*11 in milk is increased when high amounts of grazing are used and very high correlations between dry matter intake from pasture and CLA *cis*9 *trans* 11 content in milk have been reported (Couvreur, Hurtaud, Lopez, Delaby, & Peyraud, 2006). Also, fat-rich feed can increase the content of CLA *cis*9 *trans*11 as the fat-rich feed may reduce the final rumen hydrogenation and thus result in a higher amount of C18:1 *trans*11. In an oilseed feeding experiment (Larsen et al., 2012) there was a higher increase in CLA *cis*9 *trans*11 content for Holstein cows at highest level of oilseed feeding compared to other oilseed levels and compared to Jersey cows. However, if large amounts of linoleic acid are fed together with high starch rations, the acidic rumen fermentation can favour production of the CLA isomer C18:2 *trans*10 *cis*12. This CLA isomer is known to cause milk fat depression and will, therefore, be accompanied by a lowering of the milk fat percentage (Bauman, Mather, Wall, & Lock, 2006).

A range of *trans* FA are formed during rumen biohydrogenation and those which are not finally hydrogenated can be *trans*ferred to milk. The C18:1 *trans* FA include all positions from 4 to 16, of which C18:1 *trans* 11 (vaccenic acid) is the most abundant under most practical feeding conditions. However, certain feeding conditions involving high levels of polyunsaturated FA and high levels of concentrate may impair normal rumen function to such an extent that formation of C18:1 *trans* 10 is favoured (Bauman et al., 2006; Shingfield et al., 2013). Also, branched and odd-chain FA are synthesized microbially in the rumen and the content in milk can be used as a diagnostic tool of rumen function (Vlaeminck, Fievez, Cabrita, Fonseca, & Dewhurst, 2006).

The very long chained polyunsaturated FA (>C18, (*VLCFA*)) constitute less than 1% of total FA in normal milk fat, but in the milk fat globule membrane (*MFGM*) they constitute 3–3.5% of total FA. Arachidonic acid (ARA, C20:4n-6) is the dominating VLCFA and constitute around one-third of these FA. The n-3 VLCFA (C20:5n-3 and C22:5n-3) constitute 20–25% of VLCFA in both milk fat and MFGM. Docosahexaenoic acid (DHA, C22:6n-3) is absent in cow milk (Jensen & Nielsen, 1996).

Besides feeding, lactation stage also affects FA composition in milk. During mobilization the concentrations of C16 and C18 FA in milk fat increase, because body fat is used as a fat source (Stoop, Bovenhuis, Heck, & van Arendonk, 2009). The stage of lactation also affects the fatty acid composition, where C16 increases during early and mid-lactation and remains at the same level at mid to late lactation, and there is a corresponding decrease in C18 FA. The amount of unsaturated FA follows the content of C18 FA until late lactation, where there is an increase in unsaturated FA (Stoop et al., 2009).

4 Physicochemical Attributes of Milk Fat Affected by Feed and Management

4.1 Effect of Feed on Milk Fat Globules

It has been known for more than 300 years that fat exists as globules in milk (Leewenhoeck, 1674). The diameter of the milk fat globule (MFG) ranges from 0.1 to 12 μm with an average of around 4.5 μm. The average diameter of the MFG depend on breed of cow. Cows like Jersey and Gurneys produce milk with not only a higher fat content but also larger MFG than cows like Frisians and Holsteins. Figure 1.3 shows the difference in MFG diameter between the three most frequent breeds in Denmark.

The feeding also influences the diameter of MFG. Increasing the dietary fat supplements in cows increase the average diameter of the MFGs especially when the fat supplements is based on most of saturated FA (Weisbjerg et al., 2008; Wiking, Bjorck, & Nielsen, 2003). This seems to be regulated through an increased daily fat yield, which is positively correlated to increased average diameter of the MFGs (Wiking, Stagsted, Björck, & Nielsen, 2004; Wiking, Theil, Nielsen, & Sørensen, 2010). In contrast, fresh grass feeding resulting in lower fat yield is followed by a reduction in MFG size (Couvreur, Hurtaud, Marnet, Faverdin, & Peyraud, 2007). A theory about the mechanisms behind the increase in MFG diameter when the cow produces more fat is that it can be due to a limited amount of milk fat globule membrane (MFGM) material present during milk fat synthesis, so the fat is enveloped in large globules which require less surface material. This is indicated by a decreased activity of the MFGM enzyme, γ-glutamyl transpeptidase, and lower concentration of polar lipids in whole milk with increasing MFG size (Liu, Logan, Cocks, & Rochfort, 2017; Wiking et al., 2004). Oppositely, the level of another membrane component, cholesterol, correlated positively with daily fat yield (Larsen, 2012). Thus, another simple theory could be that a larger milk fat synthesis increases the

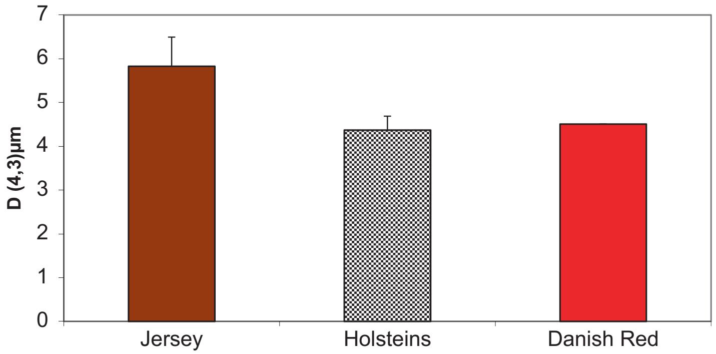

Fig. 1.3 Volume weighted diameter ($D_{4,3}$) of the three Danish cattle breeds

fusion of oil droplets during the transport from the basal to the apical boundaries in the mammary epithelial cells. It is especially the population of larger fat globules (average diameter >8 μm) which increase when the cow produces more fat (Wiking et al., 2004). During the lactation period of cows, the fat yield often decreases which result in smaller average MFG (Altenhofer et al., 2015; Walstra, 1969).

Besides affecting the physical size of MFGs, feeding also have some effect on the lipid composition of the milk fat globule membrane (MFGM). As previously described, feeding highly affect the fatty acid composition in the core milk fat. The effects on the FA composition of MFGM are lesser. MFGM phospholipids contain high levels of palmitic and oleic acid, while the short and medium-chain FA are present in very low levels (Mcpherson & Kitchen, 1983). Smith, Bianco, and Dunkley (1977) found that feeding a supplement rich in linoleic acid increased the unsaturation of the phospholipids in the MFGM. However, this unsaturation was less than that of the core lipids. Likewise, feeding a fresh pasture based spring diet results in more unsaturated FA in the MFGM versus a corn silage based diet (Lopez, Briard-BIon, & Ménard, 2014). Palmquist and Schanbacher (1991) observed that by feeding palmitic acid to the cow, it is possible to increase the saturation of the lipids in the membrane.

Recently, several papers have studied the difference in FA composition between small and large MFGs. These indicate that small MFGs contain less short-chained FA (Mesilati-Stahy, Mida, & Argov-Argaman, 2011; Rahmatyar & Wiking, 2012; Timm & Patton, 1988) and some studies also reported more content of polyunsaturated FA in the small MFG (Mesilati-Stahy et al., 2011; Rahmatyar & Wiking, 2012). However, literature is contradictory. For example, Briard, Leconte, Michel, and Michalski (2003) found the opposite that the methods used for size separating MFGs differ among studies, thus different size group are obtained which might explain some of the variation. The purpose of studying such compositional variations are driven by finding unique functionality of MFG subpopulations. Several studies have produced cheeses from milk enriched with either large or small MFG and especially the lipolysis increase in cheeses with increased MFG size (Michalski & Briard, 2004; O'Mahoney, Auty, & McSweeney, 2005). Current focus is on large differences between MFG and the small extracellular vesicles, that contain no triglycerides, e.g the extracellular vesicles are much richer in sphingomyelin (Blans et al., 2017).

4.2 Effect of Cow Feeding on Crystallization and Melting of Milk Fat

Since the FA composition of bovine milk fat is highly affected by feeding, it also changes the crystallization and melting behavior of milk fat (Bertram, Wiking, Nielsen, & Andersen, 2005). The high diversity of FA in milk fat gives a high diversity of triacylglycerides which promote a very broad melting range of milk fat, from −40 to 40 °C. Further, the melting profile is characterized by three melting fractions, a low melting fraction from −25 to 10 °C, a medium fraction from 10 to 19 °C, and the high melting fraction from above 20 °C (Timms, 1984). It is well known that butter produced during summer, when cows are grazing, is softer than butter produced from winter milk. This effect has been correlated to the changes in the FA composition over the season, where the unsaturated-to-saturated FA ratio increases during the summer, which is related to grazing. To balance a more uniform texture of butter throughout the year, manufacturers can give the cream a season-dependent temperature treatment to manipulate fat crystal size and composition, which defines the spreadability and hardness of the butter. Larger effects can be obtained by feeding high amounts of unsaturated oilseed, for example feeding extruded linseeds can lower the level of saturated FA from 71 to 61% in milk, which result in 14% less crystalized fat at 5 °C (Smet et al., 2010).

Recent studies indicate that it is mainly the lowest melting fraction, which is affected by the feeding of the cows (Buldo, Larsen, & Wiking, 2013; Larsen, Andersen, Kaufmann, & Wiking, 2014). This fraction is important for the functionality, e.g. spreadability and hardness, for dairy products stored in the fridge. Buldo et al. (2013) increased the C16:0 in milk through feeding and observed that the melting point of the lowest melting fraction is positively correlated to the concentration of C16:0 and negatively correlated to the concentration of C18:1 *cis*-9 in milk fat. By comparing organic and conventional milk, Larsen et al. (2014) also reported that only the lowest melting fraction varied between dairies and season and this was positively correlated to the proposition of C16 and negatively correlated to C18:1 *cis*-9.

5 Free Fatty Acids in Milk

A serious sensory quality defect related to milk fat is the formation of free fatty acids (FFA) due to hydrolysis of triglycerides. Especially the short-chained FFA (C4–C8) contribute with a distinct rancid flavour. FFA is caused by hydrolysis of the triglycerides by mainly lipoprotein lipase (LPL) in raw milk. It originates from the mammary gland, where it is involved in the uptake of blood lipids for milk synthesis. The enzyme is active in lipid-water interfaces. Its optimum temperature is 33 °C, and pH optimum is about 8.5. It is a relatively heat labile enzyme which is mostly inactivated by a high temperature-short time heat treatment. In milk, LPL is mainly associated with the casein micelles. LPL is first brought into contact with the triglycerides when the MFGM is disrupted and casein will recoat the formed lipid-water interface. In fact, just cooling the raw milk will change the configuration of the MFGM and bring LPL together with caseins in contact with the triglycerides (Dickow, Larsen, Hammershøj, & Wiking, 2011). The enzyme is activated by apolipoprotein CII, which is normally found in the blood and therefore can be transferred to the milk. This activator assists LPL to bind onto the fat globule. In spite of the high amount of LPL in milk, lipolysis is limited since milk fat is protected by the membrane and raw milk is normally stored at temperatures far below the optimum temperature of LPL. Furthermore, the products of the hydrolysis of the triglycerides, the FFA, inhibit the enzyme presumably due to FFA bindings to the LPL. The level of FFA in milk can be affected by milking systems and set-up at the farm, milking frequency and to some extent feeding.

5.1 Effect of Cow Feeding on Milk FFA

Changes in the composition of the cow's feed can cause milk to be more susceptible to lipolysis. Feeding concentrate with a large amount of saturated lipids results in an elevated fat percentage and large-sized fat globules, which are very prone to lipolysis (Wiking et al., 2003) and milk originating from feeding unsaturated fat or from diets stimulating de novo synthesis caused a reduced fat percentage and this milk fat is more stable during pumping. Underfeeding of the cow can also lead to increased FFA concentration in the milk (Thomson, Van der Poel, Woolford, & Auldist, 2005).

5.2 Milking Frequency

A higher milk yield can be obtained by increasing the milking frequency, i.e. from twice to three times per day. However, this also increases the content of FFA in milk (Klei et al., 1997; Wiking, Nielsen, Båvius, Edvardsson, & Svennersten-Sjaunja, 2006). The mechanism behind this accumulation of FFA is rather unknown. It has been observed that the increase in FFA is not found immediately after milking, but first after storage, indicating that a weaker membrane is formed (Wiking et al., 2006). Moreover, MFG size increases when the cows are milked more often (Wiking et al., 2006). Milk fat content increases during milking (Nielsen, Larsen, Bjerring, & Ingvardtsen, 2005), so at higher milking frequencies, the residual high-fat milk from the previous milking contributes to a higher proportion of the cisternal milk, this may explain the higher milk fat content at short intervals between milkings (Nielsen et al., 2005) which might also have some effect on FFA.

5.3 Milking Systems

The contribution from specific milking systems on FFA formation has always drawn attention. The introduction of pipeline systems resulted in increased FFA content in milk due to high air intake from the claw or the pumps. In addition, the milk is often lifted in the pipes above cow level, which increases the pressure on the MFG and thereby increases the degree of damages (Fleming, 1979). The contact between a MFG and an air bubble results in rupture of the MFG. Herring bone parlors and rotary parlors, normally provide a better quality regarding FFA since air intake and milk flows are better controlled.

The introduction of automatic milking system (AMS) in the late 1990s brought the attention on FFA again. The increased milking frequency and the continuous pumping of warm milk into the tank are factors contributing to elevated FFA levels in these systems. The average milking frequency in AMS is between 2.4 and 2.6 a day. This implies that a large share of the cows attends the milking robot three or four times per day.

6 Conclusion

This chapter has described the tools and mechanisms to manipulate composition and some physiochemical attributes of the milk fat. Focus is on the effect of the feed ration. Especially, the effect of the feed ration on the milk FA composition have been intensively studied. In general, the de novo FA, C4–16, in milk increase by starch-based concentrates and decrease by feeding fat supplements. The long-chain FA, >C16, can to some extent be manipulated by changed concentration and composition of FA in the diet. For instance, a large relative increase in C18:3 n3 in milk can be obtained by feeding linseeds rich in C18:3 n3, or by feeding fresh grass or herbs. The hydrogenation in the rumen, however, limits some of the effects from polyunsaturated FA in feedstuff. The fat composition and amount in feed also affects physical attributes like the size of the MFG and the fat crystallization/melting behaviors. Besides feeding, FA composition and MFG size are highly affected by cow breed and modestly affected by lactation state. Finally, milking management that include choice of milking systems and milking frequency, also affect the quality of the milk fat, i.e. formation of FFA.

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