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Abbreviation list

AMS - automatic milking system
DIM - days in milk
DMI - dry matter intake
FA – fatty acid
FFA – free fatty acid
IMF - increased milking frequency
LCFA - long chain fatty acids
LPL - lipoprotein lipase
MCFA - medium chain fatty acids
MF - milking frequency
MFG - milk fat globule
MFGM - milk fat globule membrane
MUFA - mono unsaturated fatty acids
MY - milk yield
PUFA - poly unsaturated fatty acids
SCFA - short chain fatty acids
SFA - saturated fatty acids
UFA - unsaturated fatty acids

Abstract

Milk fat is an important feature in many different milk products and other foodstuffs and it is often crucial for the dairy plants that the milk fat is stable for different manufacturing processes. Lipolysis is the enzymatic degradation of fat and is the one of the causes for an elevated amount of free fatty acids (FFA) in milk. Further, the change in fatty acid (FA) composition in milk can affect the stability of the product and also the manufacturing process. Both internal and external factors, at farm level or at the dairy plants can affect both FA composition and content of FFA. Milking frequency (MF=number of milkings per cow and day) and the composition of feed are two examples of factors generally performed at farm level.

The objective of the present study was to evaluate how FA composition of milk and amount of FFA are influenced by two different ingredients supplemented to concentrate. The added ingredients were palm oil and rapeseed oil. The effects of the two fat supplements were evaluated individually but also during a higher MF to detect if a change in MF can have an effect on milk fat when a specific fat supplement is added in the diet. In total 30 dairy cows, both primiparous (n=16) and multiparous (n=14) of the breeds Swedish Holstein (n=14) and Swedish Red (n=16) were divided into three groups assigned different concentrate in diet; no fat supplement, palm oil supplement and rapeseed oil supplement. The experiment was divided into a nine days adaption period and then five weeks of experimental feeding. Dry matter intake (DMI) and daily milk yield (MY) were registered throughout the experimental period, both during adaption period and experimental feeding. Milk samples were collected for two days, during morning and evening milking at three occasions during the experiment; the last two days during the adaption period, the 4th and 5th week during experimental feeding. The last sampling was performed during the treatment with an increased milking frequency (IMF) where milk samples were taken four times per day. Milk samples were analyzed for milk composition (fat, protein and lactose), milk FA profile, amount of FFA and milk fat globule size (MFG).

Results from present experiment show, as expected, that a higher MF resulted in a higher MY and elevated concentration of FFA in milk. Unexpected was that an IMF did not have a significant effect on size of MFG. However it was observed a tendency for size of MFG that is worth mentioning. Some individual FA were affected by MF where the content of C4:0 was increased and C12:0 and C18:3 (n-6) were decreased when the cows were milked four times per day instead of two times per day. Further it was demonstrated that an increased MF together with a change in diet will not affect the milk fat composition and FA profile in milk. The same results was seen for content of FFA and size of MFG. This present study has also confirmed previous findings that the FA compositions in feed will not be the same as the outcome of the composition in milk. Milk components yields were not affected by diet but cows fed palm oil diet (P) had lower content of fat and protein compared to control diet (C). Diet did not have an impact on content of FFA and size of MFG. Both diets with supplemented fat had lower concentrations of shorter FA (≤ C14) and SFA while content of mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) were higher compared to C diet. Further, as expected cows fed rapeseed oil diet (R) had the highest yield of MUFA and PUFA in milk, where the individual FA; C18:1 (n-9), C18:2 (cis9 trans11, CLA) and C18:3 (n-6) were found in higher concentrations. It was also concluded that a supplement of

rapeseed oil did not influence the milk in a negative way, which has been discussed in former studies. Rapeseed oil as a fat supplement in concentrate could therefore be a good alternative to palm oil in the future.

Sammanfattning

Mjölkfett är en viktig komponent i många olika mjölkprodukter men även i andra livsmedel och det är ofta avgörande för mejeriindustrin att mjölkfettet är stabilt för olika tillverkningsprocesser. Lipolys är den enzymatiska nedbrytningen av fett och är en av orsakerna till en förhöjd mängd av fria fettsyror (FFA) i mjölk. Vidare kan även förändring i fettsyrasammansättning i mjölk påverka stabiliteten och tillverkningsprocessen. Många faktorer, både interna och externa faktorer på gårdsnivå eller på mejerier kan påverka både fettsyrasammansättning och mängd av fria fettsyror. Två av dessa faktorer på gårdsnivå är mjölkningsfrekvens (=antal mjölkningar per ko under en dag) och sammansättningen av fodret.

Syftet med denna studie var att utvärdera hur fettsyrasammansättningen av mjölk och mängden fria fettsyror påverkas av två olika ingredienser kompletterade till kraftfodret, antingen tillsatt palmolja eller tillsatt rapsolja. Effekterna av de två fettillsatserna utvärderades individuellt men även i samband med en högre mjölkningsfrekvens, för att utröna om en ändrad mjölkningsfrekvens kan påverka mjölkfettet när en specifik fettillsats adderas i fodret. Totalt inkluderades 30 mjölkkor, i första laktation (n = 16) eller äldre (n = 14) av raserna Svensk Holstein (n = 14) och Svensk Röd (n = 16), vilka delades upp i tre grupper med olika kraftfoder i foderstaten; inget tillsatt fett, tillsatt palmolja och tillsatt rapsolja. Experimentet delades upp i en nio dagars anpassningsperiod och sen fem veckor med försöksutfodring. Intag av antal kg ts foder och daglig mjölkproduktion registrerades under hela försöksperioden, både under anpassningsperioden samt under försöksutfodring. Mjölkprover samlades in under två dagar, vid morgon- och kvällsmjölkning, tre gånger under experimentet; de två sista dagarna under anpassningsperioden, den fjärde samt femte veckan under försöksutfodringen. Sista provtagningen skedde under en ökad mjölkningsfrekvens där mjölkprover togs fyra gånger per dag. Mjölkproverna analyserades för mjölksammansättning (fett, protein och laktos), fettsyraprofil i mjölken, mängden av fria fettsyror och storleken på mjölkfettkulorna.

Som väntat visar resultat från detta experiment att en högre mjölkningsfrekvens resulterar i en högre mjölkmängd och en förhöjd koncentration av fria fettsyror i mjölken. Oväntat var att en ökad mjölkningsfrekvens inte hade någon signifikant effekt på storleken av mjölkfettkulor. Däremot observerades en tendens till större fettkulor vilket är värt att nämna. Vissa enskilda fettsyror påverkades av mjölkningsfrekvens, där halten av C4:0 ökade och C12:0 och C18:3 (n-6) minskade när korna mjölkades fyra gånger per dag istället för två gånger per dag. Vidare visade resultaten att en ökad mjölkningsfrekvens tillsammans med en förändring av diet inte påverkar mjölkfettsammansättning och fettsyraprofil i mjölk. Samma resultat var synligt för innehållet av fria fettsyror och mjölkfettkulornas storlek. Den här studien har också bekräftat föregående rön om att fettsyrakompositionen i fodret inte kommer att vara samma som utfallet av kompositionen i mjölken. Avkastningen av mjölkkomponenter påverkades inte av diet, men kor som utfodrats med palmolja i kraftfodret (P) hade lägre innehåll av fett och protein jämfört med de

som utfodrats med kontrollkraftfodret (C). Diet påverkade inte innehållet av fria fettsyror samt mjölkfettkulornas storlek. Båda kraftfodren kompletterade med fett hade lägre halter av kortare fettsyror (≤ C14) och mättade fettsyror medan halten av enkelomättade fettsyror och fleromättade fettsyror var högre jämfört med C diet. Vidare hade kor som utfodrats med rapsolja i kraftfodret (R) den högsta avkastningen av enkelomättade fettsyror och fleromättade fettsyror i mjölken, där de individuella fettsyrorna C18: 1 (n-9), C18: 2 (cis9 trans11, CLA) och C18: 3 (n-6) påträffades i högre koncentrationer. Det slogs också fast att ett tillskott av rapsolja inte påverkade mjölken på ett negativt sätt, vilket har diskuterats i tidigare studier. Rapsolja som tillsatt fett i koncentrat kan därför vara ett bra alternativ till palmolja i framtiden.

Introduction

Dairy producers and manufacturers of dairy products want to improve the marketable features of milk and milk products. Earlier breeding goals included parameters such as an increased milk and fat yield but nowadays these are complemented with protein yield (Lindmark-Mårtensson, 2012). This has during recent years created a transition from payment of milk yield (MY) to milk quality (de Koning & Rodenburg, 2004) where milk products with high quality and nutritional value are requested from consumers and manufacturers. The quality of milk must be ensured throughout the production chain to obtain a final foodstuff with a good quality. Different pathways and treatments of the milk at the farm can affect the stability of the milk and induce susceptibility for off-flavours in the milk. Such treatments can be temperature fluctuations, air leakage or agitation when milk is transported via pipes from milking station to the bulk tanks (Cartier & Chilliard, 1990; Slaghius *et al*., 2004; Wiking, 2005). Off-flavours can be a result from the degradation of triglycerides to free fatty acids (FFA), a process that is called lipolysis. These FFA can easily be oxidized or enter other chemical reactions that can give rise to rancidity or other off-flavours in milk (Wiking, 2005). By altering the FA profile of cow's milk it is possible to keep the milk stable until manufacturing of dairy products (O'Donnell, 1993).

The use of automatic milking systems (AMS) is increasing worldwide and in Sweden in September 2013 there were 764 dairy farms (21% of total connected to the Swedish cow control system) using this system (Nils-Erik Larsson, 2014, personal communication). The AMS increases the MY per cow (Wiking *et al*., 2003, 2006; Pettersson *et al*., 2011) but have also been shown to increase the risk for lipolysis (expressed as a high level of FFA), and to some extent responsible for a decreased milk quality (Klei *et al*., 1997; de Koning & Rodenburg, 2004). These effects are claimed to partly be consequences of the harsh treatments of milk, an increased milking frequency (IMF) and irregular milking intervals, which is obtained with AMS (Svennersten-Sjaunja & Pettersson, 2007).

Together with the effect of AMS that often increases the MF the research puts a lot of focus on how a change in feed and diet can affect the milk fat composition. The changes in feed and diet are often centered to different fat supplements used in concentrates. Fat supplements in feed are used continuously on dairy farms, where one of the reasons is to meet the energy requirement for the high yielding cows. Addition of fat is also desirable to use in order to obtain an increased level of fat in milk and a FA profile that is less

susceptible for lipolysis (Doreau & Chilliard, 1997). Although, due to the biohydrogenation in the rumen, where unsaturated fatty acids (UFA) are transferred to saturated, the research is contradictory regarding this change in FA composition. Some researchers have observed that the composition in milk will not be remarkably changed (Steele & Moore, 1968; Goodridge *et al*., 2001; Weisbjerg *et al*., 2008) and that addition of saturated fat in the diet will not alter rumen function (Mosley *et al*., 2007). Others state the opposite, that an addition of UFA and long chain fatty acid (LCFA) can affect rumen fermentation and bacterial growth in a negative way and thus also influence the milk with undesirable FA and lower contents of desirable FA (Chalupa *et al*., 1984; Doreau & Chilliard, 1997; MacGibbon & Taylor, 2006).

Rapeseed and palm oil are two commonly used fat supplements; rapeseed oil is rich in linoleic and linolenic acid (Scarth & McVetty, 1999) and palm oil is rich in palmitic acid (Wiking *et al*., 2003). Choice of diet, in this case fat supplement, is depending on the economic balance between feed price and milk yield for the farmer (Wiking *et al*., 2003). Palm oil is a fat supplement originating from the plant oil production and is a cost effective ingredient to include in diet (Mosley *et al*., 2007). However it causes comprehensive deforestation and creates major environmental problems. It can be more beneficial for the farmer as well as the environment to use locally produced feedstuff, such as rapeseed, for dairy cows. But many studies have been conducted showing that UFA, such as linoleic and linolenic acid represented in rapeseed, can have a negative impact on the rumen fermentation that will later affect milk fat composition (Maynard & Loosli, 1969; Chalupa *et al*., 1984; MacGibbon & Taylor, 2006). Another experiment, carried out and performed by Robertsson (2013) tested the effect of methyl esters of stearic acid and palmitic acid as fat supplements in feed. The study showed how the methyl esters affected the level of FFA and FA composition in milk and it was demonstrated that there is little or no difference between the different impacts of the methyl esters.

This paper consist of a literature review, describing milk fat in detail and factors affecting it, and an experiment where addition of rapeseed oil and palm oil in diet have been tested; how these supplements affected milk fat composition and the content of FFA in milk. The study also includes how an IMF can affect the milk fat, especially milk fat composition since earlier studies already show that a higher content of FFA are obtained with an IMF (Klei *et al*., 1997; Wiking *et al*., 2006; for review see Svennersten-Sjaunja & Pettersson, 2007). A combination of these factors, fat supplement and MF, will also be assayed and together with previous studies facilitate the understanding how an increase in the use of automatic milking system can affect the quality of milk in the future.

Objective and hypothesis

The aim of the present study is to determine and create a greater understanding of the factors that can affect the FA composition and the risk of poor stability in milk. The objective was to evaluate two different fat supplements, palm oil and rapeseed oil, individually but also during an IMF to detect if a change in MF can have an effect on milk fat when a specific fat supplement is added in the diet.

The hypothesis is that:

- Frequent milking can have different effect on milk fat depending on supplemented fat added to diet.
- Rapeseed oil will not affect the FA profile and amount of FFA in milk fat significantly.
- A higher MF will lead to an elevated amount of FFA.

Literature review

AMS are becoming more common in dairy production today and with this system the farmer can obtain many advantages. Two of the most significant benefits with this kind of system are less required labour and an increased MY, due to an IMF. However, AMS also has some negative consequences, such as a higher content of FFA in milk and increased risk of off-flavours in milk (Wiking *et al*., 2003). These risks can affect the quality of milk and the manufacturing of milk products at the dairy plants. A demand from the dairy plants and other processing industries is that the raw milk should not have any off-flavours which create a quality requirement on the dairy farms. Further a higher milk production requires diets with high energy values that can diminish the risk for a negative energy balance for the cows. Many experiments have been made the last decades where a change in feed and feeding strategies has been evaluated. By changing this part on the farm it may be possible to decrease the content of FFA in milk and still keep the higher MY that is obtained with a AMS. Supplements of fat in diets are one of these strategies, where the advantage is that fiber intake can be maintained and still meet the energy requirement (Coppock & Wilks, 1991).

The mechanical processes that occur at the farm and in dairy plants can affect the stability and quality of the milk, where one of the effects observed is the elevated amount of FFA. However, Wiking *et al*. (2003) point out that the most of the accumulation of FFA take place before the milk enters the dairy plants, as a result of pumping, transport and cooling of the milk. This literature review will only discuss the mechanisms on farm level and focus will be on how feed and MF can affect these unwanted reactions in milk. By changing the composition of the diet an increased amount of FFA can be prevented and therefore create more stable and long-lasting milk and other dairy products. Milk that is less susceptible to lipolysis have generally a low level of FFA and can more or less pass through mechanical treatments in the milking systems unaffected (e.g. pumping and cooling). A change in milk composition due to different compositions in diet can also contribute to a descending milk stability, which will be tested and discussed in this study. Moreover, there will be an introduction to fat and fat synthesis.

Milk fat

Fat is a major component in milk and has a high energy value (McDonald *et al*., 2002). The concentration and composition of fat varies between and within mammals because of the complex fetal development, requiring milk with diverse amount of energy, and milk synthesis (Bauman & Currie, 1980). The complexity gives a high number of various milk fats, and already in 1975 Patton & Jensen listed 437 different FA. With different possible positions of the triacyl-*sn*-glycerols in triglycerides they can even exist in up to 3000 combinations (Patton & Jensen, 1975). The FA composition of a lipid determines its specific chemical and physical properties.

Cow milk contains approximately 3.5-4% of fat but the concentration can differ between and within breeds and individuals (Fox & McSweeney, 1998). More than 95% of the fat in milk consists of triglycerides. The remaining five percent are divided on phospholipids and unesterified sterols, mono- and diglycerides and unesterified FA (Dils, 1986). Compared to other fats, there is a major fraction of triglycerides that exists with concentrations at or above 1.0% of total

amount of FA. With this low concentration among many triglycerides it is only necessary to consider a part of all FA of the triglyceride FA (Table 1). The composition in triglycerides can be affected by diet or other factors such as stage of lactation or breed (Davies *et al*., 1983) and this structure is crucial for how the physical properties of milk fat will be when being processed and exposed to lipolysis (Jensen *et al*., 1991).

Milk fatty acids

Milk FA composition can vary due to both individual and environmental factors. There are also two major metabolic pathways in rumen that can alter the characteristics of the FA in the diet; hydrolysis of consumed esterified FA and hydrogenation of UFA (Grummer, 1991). MacGibbon & Taylor (2006) mention a regular seasonal variation in milk fat composition in most countries where the composition of FA in feed changes depending on season. During spring and summer the amount of palmitic acid (C16:0) in milk generally decreases compared to winter time. Ruminant milk consists of a significant proportion of saturated fatty acid (SFA), approximately 70 to 75% of the total FA (Grummer, 1987; Fox & McSweeney, 1998; MacGibbon & Taylor, 2006) where palmitic acid contributes to the largest part (McDonald *et al*., 2002). The UFA are divided in mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) and are present in milk with a concentration of approximately 25% and 5% respectively. Oleic acid (C18:1) is accounting for the highest content of UFA represented in milk (Grummer, 1987; Lindmark Mårtensson, 2012). The main PUFA in milk are linoleic (C18:2) and linolenic (C18:3) acid. The UFA in ruminants' milk are present in low concentrations compared with milk fat from monogastric animals. This is due to microbial biohydrogenation of dietary FA in the rumen (Davies *et al*., 1983; Dils, 1986), producing SFA instead. This low level of UFA in milk is claimed to be nutritionally undesirable in humans (Fox & McSweeney, 1998). Further *trans*-isomers can be created when incomplete hydrogenation in the rumen occur (Doreau & Chilliard, 1997). Higher amounts of *trans*-FA can inhibit the milk fat synthesis (Palmquist *et al*., 1993) and generally have a higher melting point contributing to a more undesirable nutritional characteristics in milk (Fox & McSweeney, 1998). However, *trans* FA are represented only in small quantities (~5%) in bovine milk (Fox & McSweeney, 1998) and are therefore not thorough discussed in current paper. Ruminant milk and milk products are the major dietary source of the unsaturated conjugated linoleic acid (CLA) that is found in many different isomers, both *cis* and *trans* (Wahle *et al*., 2004). Rumenic acid (C18:2 cis-9, trans-11) is one of these isomers and constitute 75-90% of total CLA (Wahle *et al*., 2004; Lindmark Mårtensson, 2012).

Table 1. Major fatty acids present in cow milk adapted from Lindmark Mårtensson (2012)

Item	Fatty acid name	Annual mean 2009 in g/100 g of total fatty acids[1)]
Total FA in group		
≤ C14		22.1
SFA		67.3
MUFA		23.5
PUFA		2.4
Individual FA		
C4:0	Butyric	2.3
C6:0	Caproic	1.7
C8:0	Caprylic	1.1
C10:0	Capric	2.6
C12:0	Lauric	3.4
C14:0	Myristic	11.0
C15:0	Pentadecanoic	1.0
C16:0	Palmitic	32.9
C16:1 (cis9)	Palmitoleic	2.2
C17:0	Margaric	0.6
C18:0	Stearic	10.7
C18:1 (n-9)	Oleic	21.3
C18:2 (n-6)	Linoleic	1.6
C18:2 (CLA)	Rumenic	0.3
C18:3 (n-6)	Linolenic	0.5

1) The annual means are adapted from a registration from different dairy plants in Sweden and collected 2 times during 2009. Each fatty acid is given in weight % of total fatty acids

Milk fat synthesis

Milk FA can be derived either from *de novo* synthesis locally in the mammary gland or from the circulating blood or plasma lipids originated from diet. Lactating ruminants use acetate, β-hydroxybuturate and lactate, derived from rumen fermentation of carbohydrates, for *de novo* synthesis of FA in the mammary gland (Dils, 1983; Walstra, *et al*., 1984). In ruminants' milk, these FA are short chain fatty acids (SCFA: C4:0-C12:0) and medium chain fatty acids (MCFA: C12-C14 and some C16). Further these components contribute to the formation of milk FA with different chain lengths. The long chain fatty acids (LCFA: >C16 and some C16 originate from the uptake of circulating preformed components from the diet or from adipose tissue (Bauman & Davis, 1974; Bauman & Griinari, 2001) and are called plasma or blood lipids.

Preformed fatty acids

Dietary fat degradation occurs in successive steps in the rumen where hydrolysis represents the first mechanism, resulting in non-esterified FA (MacGibbon & Taylor, 2006). These FA are isomerized and hydrogenated by

bacterial enzymes present in the rumen (Doreau *et al*., 2011). The mixture of FA of dietary origin, primarily components derived from degraded LCFA, cannot be absorbed by the rumen wall, like other fats, and are therefore transported to the small intestine. The intestinal mucosa absorbs the FA and some triglycerides while phospholipids and the rest of the triglycerides are incorporated into lipid carriers, chylomicrons and very-low density lipids (VLDL) (Gustafsson, 1991; Sjaastad *et al*., 2010). These lipid carriers are transported via the portal vein to the mammary gland and other peripheral tissues. Before entering the mammary gland the blood enzyme lipoprotein lipase (LPL) hydrolyzes the triglyceride molecules in the capillary wall and the formed FFA, monoglycerides and some glycerol are transported across the base of mammary cell membrane to be re-transposed to triglycerides in the cell (Fox & McSweeney, 1998; Sjaastad *et al*., 2010). At the same time lipolysis can occur primarily in adipose tissue which enables FA to be available for the mammary gland (Bauman & Davis, 1974; Sjaastad *et al*., 2010). The UFA, such as linoleic and linolenic acid, are hydrogenated in the rumen before absorption and transport to the tissues.

De novo synthesis

If FA in milk are not derived from the feed, they are originated from *de novo* milk fat synthesis in the mammary gland. Approximately 50% of the FA are produced here; primarily SCFA and MCFA (Gustafsson, 1999). The *de novo* milk FA synthesis is driven by the precursors acetate from rumen fermentation and β-hydroxybutyrate formed from buturic acid in the rumen wall (Fox & McSweeney, 1998). Ruminants use acetate or β-hydroxybutyrate in the formation of FA where Acetyl-CoA is the activated form and the principal building block of FA. The enzyme FA synthase is a key enzyme participating in the catalytic reaction and elongating process of FA, generating short- and medium chain FA. Before elongation Acetyl-CoA is carboxylated to malonyl-CoA (MacGibbon & Taylor, 2006). Inhibition of the activity of these enzymes can be carried out by LCFA, especially UFA (Sejrsen *et al*., 2007). The short and medium chain FA synthesized *de novo* are neither desaturated nor elongated (Davies *et al*., 1983; Dils, 1983), thus remained saturated.

Both preformed FA and those synthesized by the mammary gland are esterified by glycerol in the alveolar cells. Formation of FA in the alveolar secretory cells in the mammary gland occurs in the endoplasmic reticulum in the cytoplasm. Triglycerides can be synthesized and added directly onto the surface of the growing fat droplet (Mather & Keenan, 1983; Fox & McSweeney, 1998). These newly formed fat droplets fuse together with other droplets forming even bigger globules. The morphology and function of the secretory tissue in the mammary gland are well described by Davis & Bauman (1974) and Mather & Keenan (1983).

Milk fat globule

The milk fat is, like other fats, present in globules with a surrounding membrane consisting mainly of phospholipids, proteins, cholesterols and enzymes. According to the main structural elements of milk the number of milk fat globules (MFG) is 10^{10} globules/ml of milk (Walstra *et al*., 1999). Inside the globule triglycerides, cholesterol esters and other esters are found. When fat in milk is secreted and released from the apical surface of the mammary secretory

cells it is enclosed with this protective membrane (Jenness, 1974). The milk fat globule membrane (MFGM) acts as a barrier to the aqueous environment outside and prevents the globule from fusing together with aqueous phase and other globules in the alveolar lumen. The membrane also protects the FA inside the globule from lipolysis and oxidation (Wiking *et al*., 2004). Most of the MFG are between 1 μm and 10 μm in diameter with an average size of 4 μm (Jenness, 1974; Davies *et al*., 1983; Jensen, 2002). The size of the MFG can affect the level of lipolysis in milk. The size is also crucial for the stability and technological properties of the milk where a smaller size is proved to be more stable and less susceptible to both induced and spontaneous lipolysis (Wiking *et al*., 2003 & 2004). In the previously mentioned study it was stated that size of the MFG is increased by FA, mostly LCFA, originating from the diet but not from *de novo* FA synthesis. This is because these FA are transported from the blood stream directly from diet without being digested in the rumen (Fox & McSweeney, 1998). Wiking *et al*. (2004) found correlations between average size of MFG and the FA C16:0, C16:1, C18:0 and C18:1 meanwhile no correlations could be seen between average size of MFG and shorter FA (C4:0-C14:0) or PUFA (C18:2 and C18:3). Moreover, daily fat production is correlated to the average diameter of MFG which indicate that the membrane compounds are limited when cows produce milk with a high amount of fat (Wiking *et al*., 2004; Weisbjerg *et al*., 2008).

It is important to mention that the size of the fat globules may vary depending on the analytical method of measurement used. This can provide unreliability results, especially when using old measurement methods (Walstra *et al*., 1969). Various parameters can be used to express the mean size of the fat globules. The surface of the fat globule can partially be obtained by a volume surface-weighted mean diameter, d3,2 or volume moment-weighted mean diameter, d4,3 (Fox & McSweeney, 1998).

Lipolysis and FFA in milk

Lipolysis is a consequence from the enzymatic hydrolysis of triglycerides which contributes to the elevation of FFA and an increased risk for rancidity off-flavours in milk. Production of FFA is partly due to the fat globules susceptibility to lipases which are most active in a temperature of 33-37° C and at a pH of 8.5 (Wiking, 2005; Ray *et al*., 2013), where normal pH in milk is 6.7 (Murphy *et al*., 1979). LPL is the enzyme which is mainly responsible for lipolytic reactions in milk and originates from the blood or from microorganisms in the milk (Everitt, 1991; for review see Wiking, 2005). All types of milk contains a high amount of LPL, nevertheless lipolysis and off-flavours is limited since the MFG is protected by the MFGM (Wiking, 2005; Ray *et al*., 2013). Other factors in milk can also prevent lipolysis, such as the pH, ionic strength and that lipase is bound to the micelle surface of casein (Fox & McSweeney, 1998). When lipolysis occurs, LPL separates FA and glycerol in the triglyceride molecules and FFA are then released in the milk (Figure 1). It is the FA present in the position *sn*-1 and *sn*-3 that are subjected to a more frequently occurring lipolysis and formation of FFA. The explanation for this is that these outer positions of the triglyceride molecule are the positions where LPL is first active (Fox & McSweeney, 1998; Quattara *et al*., 2004). The FA most frequently located on these positions are C4:0, C6:0, C18:0 and C18:1 (Walstra *et al*., 1984; Jensen, 2002; Quattara *et al*., 2004).

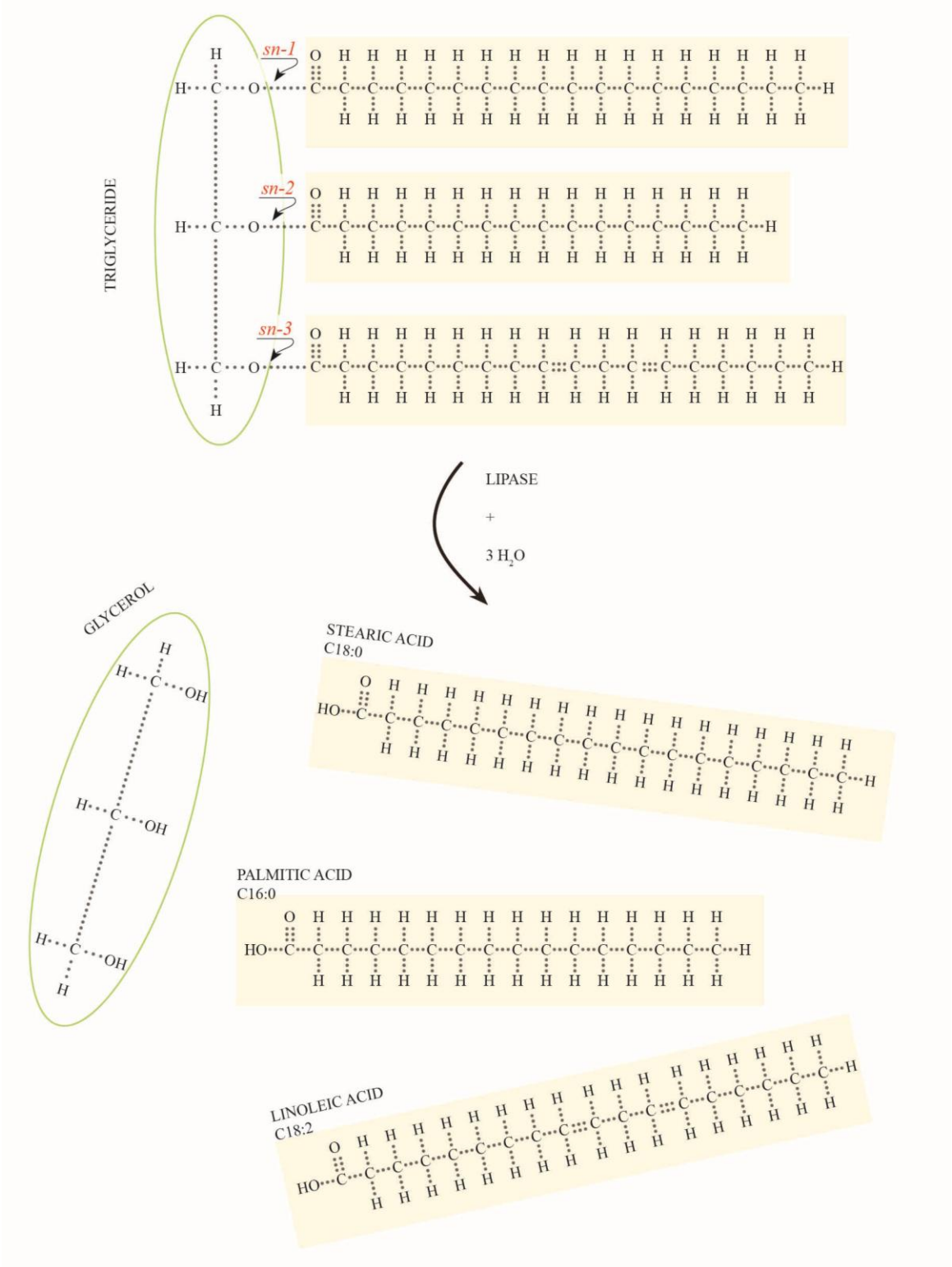

Figure 1. Lipolytic degradation of a triglyceride molecule where the enzyme lipase and water split the triglyceride into glycerol and 3 free fatty acids, adapted from Sjaastad *et al*., 2010.

Table 2. Threshold values for rancid off-flavour in milk from different measurement methods in various studies.

Threshold[1)]	Reference	Method used
2.01	Frankel & Tarassuk (1955)	Extraction- titration method
2.74	Kinter & Day (1965)	Sensory test panel
1.0	Tuckey & Stadhouders (1967)	BDI method with cultured milk sample
1.5-2.0	Tuckey & Stadhouders (1967)	BDI method with uncultured milk sample
1.85-2.05	Pillay *et al*. (1980)	BDI method
~1.0	Fox & McSweeney (1998)	
3.16-3.51	Santos *et al*. (2003)	Sensory test panel

[1)] Threshold values measured in mEq/100 g fat

The content of FFA in milk is often described as acid degree value (ADV) and measured in fat as mmol or mEq of FFA/100 g of fat. In milk freshly drawn from the cow the amount of FFA is usually low but during storage of the milk content of FFA can quickly be increased, making it important to use threshold values for off-flavours and quality assurance. Thresholds levels of FFA from various researchers are presented in Table 2. Fox & McSweeney (1998) specify the threshold level to be around 1 but other values have been mentioned in other studies, ranging from 1.5 in the experiment by Tuckey & Stadhouders (1967) to 3.51 in the experiment by Santos *et al*. (2003) where a sensory test panel felt the rancid flavour at this specific level. This discrepancy may be due to the difficulty to find the relationship between rancid off-flavour and the level of FFA in milk. The difference between threshold values can further be explained to the variation in composition of FA in FFA. Therefore a value of some FFA exceeding this threshold in milk is undesirable due to a higher risk for rancidity meanwhile other FFA will not give the milk a rancid flavour (Deeth & Fitz-Gerald, 1983; Fox & McSweeney, 1998). Different methods measuring the ADV can also be an explanation to this variation among studies. Wiking *et al*. (2005) point out that, although this relationship is hard to define, milk can still get a rancid off-flavour with an elevated content of FFA.

Other descriptions of FFA and risk for a decreased quality is mentioned in the literature. One of the descriptions is the FFA level that is created from a spontaneous lipolysis. According to Deeth & Fitz-Gerald (1983) and Cartier & Chilliard (1990) this level of FFA is defined as stored and cooled raw milk without any other effect of treatment, except the spontaneous reaction itself. During cool storage of milk the content of FFA can increase, with the maximum formation of FFA at a temperature of ~15 °C (Tarassuk & Henderson, 1942; Deeth & Fitz-Gerald, 1977). This is in agreement with a study performed by Wiking (2005) where a rapid elevated FFA content at a temperature of 20 °C was observed. It was also demonstrated that the formation of FFA is highest during the first 24 hours. In general, milk is stored in temperatures below LPL's optimal temperature (33-37° C), which contributes to a lower risk for lipolysis. Moreover this enzyme can be inhibited by its own rest products, which are obtained when the enzyme acts on the milk fat (Walstra & Jennes, 1984). It is usually the LCFA that inhibit lipolysis because of their ability to bind to the active site of the LPL (Bengtsson & Olivecrona, 1980; for review see Spörndly *et al*., 1999). The frequency of lipolysis occurring depends

partly on stage in lactation, where the milk from cows in late lactation is more susceptible for lipolysis, much due to larger MFG. A larger globule has a weaker MFGM due to a limited amount of membrane material (Wiking *et al*., 2003; Wiking, 2005; Weisbjerg *et al*., 2008). In contrast to previous findings Thomas *et al*., (1954) were not able to reveal any direct evidences that cows in late lactation would deliver milk with a higher content of FFA than cows in early lactation.

Lipolysis can either be derived from a spontaneous or an induced reaction. The reason for a spontaneous reaction is not fully understood but according to some scientists it does not need an activation treatment. Instead it can occur due to heritability of the specific trait; cows in negative energy balance or stage of lactation (Everitt, 1991; Palmquist *et al*., 1993). Further Ray *et* al. (2013) describe in a review that lipolysis can be related to a balance between activating and inhibiting factors in the milk. Fats with a high amount of SFA are more susceptible to lipolysis (Everitt, 1991). Other factors contributing to a higher risk for lipolysis can be diseases contributing to a poor udder health. Mastitis can make the MFGM weaker and reduce the amount of casein which can bind some of the lipase (Everitt, 1991). Induced lipolysis is mainly based on a damaged MFGM that can be originated from different treatments, referred to activation treatments, in a dairy production such as homogenization, temperature change, air leakage or agitation (Cartier & Chilliard, 1990; Everitt, 1991; Slaghius *et al*., 2004). The MFGM and milk fat stability can also be affected by the diet, MY, pregnancy status and MF. Lipolysis can be accelerated by several factors, but the rate depends on the milk's susceptibility for mechanical stress and lipolysis which differs between individual cows (Wiking *et al*., 2003).

Milk quality – off flavours

Since milk fat is present in many different forms and concentrations, each with a unique chemical and physical property, it can be used for many products with different quality at the dairy plants. A demand from the dairy plants and other processing industries is that the raw milk should not have any off-flavours which create a quality requirement on the dairy farms. The treatments and pathways that the milk pass through are often affecting the fatty acid (FA) composition in milk which in turn can affect the flavour and consistency of the milk and other dairy products, both in a favorable and a non-favorable way. In some products, after being processed, the FA can cause a tasteful characteristic, such as the strong flavor in blue cheese or Parmesan (Badings, 1984; Fox & McSweeney, 1998). However, these FA can also, after being released from the milk fat globule, be degraded to FFA by the enzyme Lipoprotein lipase (LPL), a process that is called lipolysis. These FFA can easily be oxidized or enter other chemical reactions that can give rise to off-flavours in milk (Wiking, 2005). By altering the FA profile of cow's milk it is possible to keep the milk stable until manufacturing of dairy products (O'Donnell, 1993). Some researchers claim that a stable milk should not include FA present on the outer positions on the triglyceride, such as C4:0, C6:0, C18:0 and C18:1, where LPL is most active (Walstra *et al*.,, 1984; Fox & McSweeney, 1998; Quattara *et al*., 2004). FA including double bonds that easily can be oxidized can also contribute to this unstability (Fox & McSweeney, 1998). It is also feasible to influence the milk in dissimilar ways and affect the functionality of fat

depending on the end product that is desired (Grummer, 1991; Scarth & McVetty, 1999; Mosley *et al*., 2007).

Off-flavours in milk can ascend from several reasons; some reactions in milk fat are more common such as lipolysis and oxidation. Rancidity is one of many off-flavours and can be divided into oxidative rancidity and hydrolytic rancidity, the later having its origin from the hydrolytic degradation of milk fats (lipolysis), contributing to a production of FFA (Deeth & Fitz-Gerald, 1983). An increase in FFA can cause deterioration of milk sensory properties, taste and odor but also technological properties in milk which generally is undesirable in butter and milk but can be an important characteristic in some cheeses (Fox & McSweeney, 1998). Ruminant milk fat has a high content of SCFA compared to milk from other mammals (Fox & McSweeney, 1998; MacGibbon & Taylor, 2006). It has been observed that it is particularly the SCFA (C4-C12) causing the off-flavours, when FFA are released from triglycerides by the enzyme lipase. Even low levels of short chain FFA can impair taste and processing quality of the milk, since the threshold values for these FFAs are particularly low (Fox & McSweeney, 1998; Wiking, 2005; Quattara *et al*., 2011). If the composition of the milk fat changes to longer and unsaturated FA the taste and quality of the milk can be impaired (Wiking *et al*., 2003).

The other reaction developing rancidity is oxidation of fats at the double bond (Maynard & Loosli, 1969). It is an auto catalyzed chain reaction where a free radical is formed when oxygen reacts with double bonds in the FA and then decomposed into smaller substances (Everitt, 1991; Fox & McSweeney, 1998). According to a food safety program from Washington State Department of Agriculture (WSDA) an oxidative rancidity is cause from contamination of milk with small amounts of copper or iron (WSDA, 2010). It is principally more common with fat oxidation in UFA, although oxidation may exist within SFA (Fox & McSweeney, 1998). Occurrence of oxidation in milk can vary between and within cows and some feed additives can also increase the risk for oxidation. It has been shown that linseed cake gives labile milk while soy- and rapeseed products make the milk more resistant for oxidation (Everitt, 1991).

Impact of management on FA in milk

Many great things can be achieved with a change in the FA composition in feed to dairy cows. Two of the most discussed topics today are the favorable health effects with milk (Sejrsen *et al*., 2007; Doreau *et al*., 2011) and a more stable milk for manufacturing of milk products (Wiking *et al*., 2003 & 2004). Since FA from the diet are directly transposed into milk and often presented in more than half of the FA in milk it can be possible to also change the milk fat composition through diet. But due to some modifications of FA in the rumen and partly in the mammary gland this can only be obtained to a limited extent (Sejrsen *et al*., 2007). Since the genetic correlation between milk fat yield and MY and other components in milk is quite high the selection for an increased milk fat yield is difficult to obtain, without changing the other milk components (Bauman & Griinari, 2001). Sejrsen *et al*. (2007) concluded that it is possible to change the composition of milk but impossible to create improvement of one component without affecting the others. Instead it is common to change the feed and feeding routines and thereby a change in the FA composition may be achieved (Bauman & Griinari, 2001). There are also other factors affecting the

FA composition and the amount of FFA in milk; stage of lactation, MF and milking interval and also quality of the milking equipment, these factors are discussed below.

Fat supplementation

Production of milk requires a high metabolic activity and added fat in feed is therefore a good energy source that can facilitate high production. A good nutrient reserve, particularly in adipose tissue, is also desirable, to ensure that the mammary gland receives the components for milk fat synthesis (Bauman & Currie, 1980). Fat as a supplement is frequently incorporated in concentrates in the diet for ruminants. Often this feeding strategy is used because of the desire to meet the energy requirements by the cow but nowadays the reason is also to increase the fat content in milk (Doreau & Chilliard, 1997). In plant fats there are generally a higher concentration of UFA than in animal products (Maynard & Loosli, 1969). Because of the low amount of essential FA and PUFA, such as linoleic (C18:3) and linolenic acid (C18:2), in cow's milk many trials with supplemented vegetable oils and fats have been made during the past decades (Goodridge *et al*., 2001). This is predominantly due to the purpose of nutritional supply in milk for humans (Davies *et al*., 1983). For some researchers and farmers this is contrariwise with other information where it has been proved that an elevated amount of UFA in milk can increase the risk for oxidation and thereby rancidity (Fox & McSweeney, 1998; Wiking *et al*., 2003). A diet enriched with too much fat, especially LCFA, would usually result in a higher production of propionate and a lower production of acetate and butyrate, which are the precursors to milk fat (Chilliard *et al*., 1991; Mosley *et al*., 2007; Weisbjerg *et al*., 2008).

As mentioned earlier, a supplement of a specific FA does not necessarily mean that the content of this particular FA will increase in the milk, which is due to biohydrogenation in the rumen (Walstra *et al*., 1984; Goodridge *et al*., 2001). Steel & Moore (1968) showed that a dietary supplement of linoleic acid had little effect on the same component in milk, where other researchers contradict these statements (Palmquist *et al*., 1993; Wiking *et al*., 2003 & 2004; Weisbjerg *et al*., 2008). Wiking *et al*., 2003 showed that a diet composed of a high content of roasted whole soybean, rich in UFA, increased the amount of C18:0, C18:1, C18:2 and C18:3 in milk. To counteract the biohydrogenation that occur in the rumen, protected fats and oils that are not susceptible for ruminal degradation have been developed (Davies *et al*., 1983; Jensen *et al*., 1991; Goodridge *et al*., 2001). If the supplemented fats are protected from rumen degradation and biohydrogenation the FA will pass rumen microbial fermentation unchanged and be transported postruminally for digestion, absorption and then incorporated into milk fat (Grummer, 1991). The FA composition in milk can then be affected and may be change to a more desirable composition and it could also be possible to avoid negative characteristics in milk fat (Goodridge *et al*., 2001). These protected fats can be saponified, hydrogenated or covered with proteins or formaldehyde (Doreau *et al*., 1997). Jensen *et al*. (1991) stated that a diet with protected oils rich in C18:2 can change the milk fat composition significantly, resulting in milk with a higher content of LCFA and PUFA and a lower content of SCFA and SFA.

Other feeding routines can be applied at the farm to change the FA composition in milk but some changes are not always desirable. Sejrsen *et al*. (2007) mention that the type of forage used can affect the ratio of saturated and unsaturated FA in milk but also increase the amount of *trans* FA, that can be the result from an incomplete biohydrogenation. Previous research highlight that an elevated level of undesirable *trans* FA can be derived especially when feeding the cows fat supplemented diets in combination with diets containing high amounts of starch (Sjersen *et al*., 2007).

It is common to use saturated fat, such as palm oil, rich in palmitic acid, as a supplement in diets for dairy cows. Wiking *et al*. (2003) tested a feeding strategy where Holstein cows were fed diets with different FA compositions; one high in saturated fat (50% palmitic acid), another high in unsaturated fat and the last one high in FA used in *de novo* synthesis. The milk analysis demonstrated that feeding a high amount of saturated fat can generate a higher fat content and a larger diameter of the MFG in milk compared to the two other diets. This is also proved by Weisbjerg *et al*. (2008) who found a positive correlation between average diameter of MFG and daily fat yield when addition of FA in diet was performed. Doreau & Chilliard (1997) clarify in their review that different fat sources can have a dissimilar impact on the milk production and composition, due to different effects on rumen fermentation. The previous research explain that MCFA and UFA can reduce cellolytic activity in rumen which impairs the degradation of carbohydrates, thus the amount of acetate which is an important compound in milk fat synthesis (Doreau & Chilliard., 1997). A supplement of plant oils rich in LCFA, containing 16 and 18 carbon atoms, can reduce the activity of *de novo* synthesis (Chalupa *et al*., 1986; Fox & McSweeney, 1998; Wiking *et al*., 2004; MacGibbon & Tyler, 2006; Dai *et al*., 2011). Wiking *et al*. (2005) registered a low average fat content in milk fat when feeding cows a diet with a high level of roasted soybeans that are rich in C18:2. This indicates that a supplement with a high concentration of PUFA, such as rapeseed and soybean, will inhibit the formation of precursors for milk fat in the rumen, and further the *de novo* synthesis and has been referred to milk fat depression in cows (Bauman & Griinari, 2001 and Peterson *et al*., 2003). Rapeseed oil is rich in UFA; 61% oleic acid (C18:1), 21% linoleic acid (C18:2) and 11% linolenic acid (C18:3) (Scarth & McVetty, 1999) and contains a very low level of SFA (Scarth & McVetty, 1999; Jensen, 2002). This high level of UFA may reduce the fat content in milk. According to Ray *et al*. (2013) the risk of rancidity will decrease when feeding rapeseed oil to underfed cows.

Milking frequency and milking interval

Various studies show that a higher MF will result in an increased milk yield in cows (Erdman & Varner, 1995; Klei *et al*., 1997; Stelwagen, 2001; Soberon *et al*., 2011), while the content of fat and protein can decrease (Erdman & Varner, 1995; Wiking *et al*., 2006). Wiking *et al*. (2006) discussed that the length of a study can influence the results, explaining that short-time studies testing an IMF show no differences in fat yield or fat percentage while a long-term study (Klei *et al*., 1997) may show a total increase of 4.7% fat in milk throughout the entire lactation. With a higher number of milkings per day the FFA content and size of MFG will increase (Wiking *et al*., 2006). This is demonstrated in several studies where the MF has been increased from 2 to 3 milkings per day (Klei *et al*., 1997), from 2 to 4 milkings per day (for review see Svennersten-Sjaunja & Pettersson, 2007) and from 2 to 4 on half udder level (Wiking *et al*., 2006).

During an altered MF the FA composition may be changed (Sapru *et al*., 1997). Wiking *et al*. (2006) found that the proportion of PUFA was smaller in milk from a udder half milked four times compared with the other udder half milked two times. A higher MF can give an elevated activity in the mammary secretory cells and therefore a stimulated production of SCFA. SCFA are more susceptible for lipolysis, due to that they are often located on the outer positions on the triglyceride molecule where LPL is first active (Quattara *et al*., 2004). This sensibility for lipolysis can create a high amount of FFA in milk. Wiking *et al*. (2006) demonstrated in a study that an increase of FFA from 1.14 mEq/100 g of fat to 1.49 mEq/100 g of fat was obtained when milking twice or four times a day respectively.

Voluntary visits to the milking robots are obtained with AMS, and this together with a higher MF results in a change in length of the milking intervals (Hogeveen *et al*., 2001). These changes can in some cases lead to uneven and irregular intervals which may further affect the milk fat stability and elevated levels of FFA (for review see Svennersten-Sjaunja, 2002). Slaghius *et al*. (2004) revealed that shorter intervals (4 h and 8 h) gave an increased amount of FFA compared to a longer interval (12 h). It is important to keep regular milking intervals, especially during experiments when milk analyses are performed, to receive as good results as possible. The number of visits to the AMS can differ between cows and between farms, which may be due to a non occurring voluntary milking behaviour, a pasture-based production (Jacobs & Siegford, 2012) or a large herd size (Artmann, 2001). An average of 2.17 to 2.9 milkings per day has been reported (Klungel et al., 2000; Pettersson *et al*., 2011; Castro *et al*., 2012). Those farms having a lower MF but still high amounts of FFA implies that it can also be factors other than MF that affect the amount of FFA in milk.

Stage of lactation, lactation number and breed

Milk from cows producing small amounts of milk per milking is more susceptible for an elevated amount of FFA and because of that is important for the farmer to ensure that the expected MY in AMS is not too small (Rasmussen *et al*., 2006). It has been shown that milk from cows in late lactation has a higher level of FFA than milk from early lactation (Klei *et. al.*, 1997; Sapru *et. al.*, 1997). Thomas *et al*. (1954) could however not see such differences. The lactation number during the first 100 DIM can affect the level of FFA and Klei *et al*. (1997) stated that cows in second lactation showed a higher amount of FFA compared to primiparous cows. Different breeds can also result in different concentrations of FFA where Holstein cows tend to give milk with higher levels of FFA than Jersey cows when milked two times a day with a milking interval of 12 hours (Karijord *et al*., 1982).

Milking equipment

Several studies have been made regarding milking equipment impact on the stability of milk. Wiking *et al*. (2003) concluded that the pumping temperature of the milk played a major role in the stability of milk where the experiment showed that warmer temperature made the milk unstable. It was also revealed that by cooling milk to a temperature of 5^{o} C the majority of the lipids kept crystallized and made the milk fat globule more stable. Other possible technical risk factors on milk quality can be air inlet in the teat cups, bubbling and a too

long post run time of the milk pump (de Koning *et al*., 2004). Storage time of the milk may have an effect on the content of FFA. Wiking *et al*. (2006) saw that when sampling and analyzing raw milk directly no significant differences could be observed between 2 times milking compared to 4 times milking. When the milk had been stored for 24 hours the difference between the two milking frequencies was significant.

Materials and methods

The study was conducted as a continuous treatment design carried out in the Swedish Livestock Research Center (SLRC) at Lövsta, at The Swedish University of Agricultural Sciences in Uppsala. The experiment was approved by the Local Ethics Committee, Uppsala County and conducted during seven weeks from December 2013 to January 2014.

Animals, housing, milking and diet

The cows in the study were of 16 Swedish Red (n=16) and 14 Swedish Holstein (n=14) breed and were housed in a loose housing system. They were fed silage and water *ad libitum* and concentrate distributed in feeding stations, according to an ordinary ration, based on an individual MY, used at Lövsta. In the study the cows had a nine days adaption period in order to get them used to the new milking routines and to adapt them to the same diet. During the last two days of the adaptation period, milk samples were collected, representing a pre-treatment sample. After the adaption period the feeding treatment started where cows were divided into three groups fed with concentrate supplemented with different fats. The feeding treatment lasted for five weeks. During these five weeks a change in MF was also performed. In total 30 cows were used in the study and all of them were in mid lactation (142±46 DIM) at the start (Table 3).

Table 3. Cow status in diet groups at the beginning of experiment

Diet/CowID	Breed	Milk yield[1)]	Lactation nr	DIM[2)]	SCC[3)]
Control diet					
24	SRB	30.88	1	82	17 000
982	SLB	29.47	2	173	25 000
1005	SLB	31.64	2	185	21 000
1557	SRB	40.49	3	75	14 000
1565	SRB	35.56	3	105	45 000
1611	SRB	31.24	2	68	102 000
1624	SRB	42.94	2	66	11 000
5406	SLB	30.91	1	195	62 000
6534	SLB	32.47	1	191	25 000
6544	SLB	34.26	1	158	26 000
Palm oil diet					
9	SLB	20.14	1	113	44 000
13	SLB	27.24	1	164	227 000
35	SRB	33.48	1	68	140 000
976	SLB	32.08	2	160	43 000
1542	SRB	37.79	3	141	113 000
1583	SRB	25.70	2	194	289 000
1628	SRB	30.31	2	123	52 000
1665	SRB	35.88	1	157	34 000
1668	SLB	35.10	1	153	21 000
6535	SLB	28.90	1	210	20 000
Rapeseed oil diet					
19	SRB	31.9	1	101	62 000
25	SRB	34.47	1	65	45 000
1604	SRB	32.37	2	189	124 000
1612	SRB	36.23	2	184	15 000
1621	SRB	35.60	2	115	36 000
1672	SRB	29.00	1	139	20 000
5408	SLB	28.54	1	159	73 000
5410	SLB	39.41	1	141	16 000
6512	SLB	31.80	2	172	125 000
6536	SLB	34.65	1	211	41 000

[1)] Milk yield is given in kg/day and based on the average milk yield from all milkings at first sampling occasion during adaption period
[2)] Days in milk (DIM) at start of experiment
[3)] Number of somatic cells (SCC) is given in cells/ml of milk and based on the average number of cells from all milkings at first sampling occasion during adaption period

They had an average daily MY of 32.6±5.5 kg. Both primiparous (n=16) and multiparous (n=14) cows were included. The cows were split into three balanced groups with ten individuals in each group, including primiparous and multiparous and cows of the two breeds, Swedish Red and Swedish Holstein. Milking was performed in a DeLaval AMR^{TM} (Automatic Milking Rotary) during the whole experiment. During the adaptation period and the first four weeks the cows were milked two times daily with a 12 h milking interval, at 05 and 17. During the 5th week of the experiment the MF was increased and the cows were instead milked four times daily, with 6 h milking intervals, at 05, 11, 17, 23. For a detailed experiment schedule see Table 4.

Since the effect of dietary fats on milk fat content can vary depending on lactation stage only individuals that were in mid-lactation when the experiment started were chosen. This in order to avoid differences in milk fat contents among the cows and to have cows producing larger amount of milk that is less susceptible for an elevated amount of FFA. Average DIM were at start of the experiment 130±55 for C, 148±45 for P and 148±41 for R, respectively.

The different concentrates were manufactured by Teknosan (Spannex Group, Stockholm, Sweden). Experimental diets were concentrates with individually rations adjusted to their nutrient requirement. The average concentrate ration was 12.7±2.7) and the ration ranged between 6.0 and 17.7 kg per day depending on MY, and was kept stable throughout the experiment. The three treatment groups were fed different concentrates; one with no fat added (C), one with palm oil fat ingredient (P) and one with rapeseed oil fat ingredient (R). Concentrate ingredients and chemical composition of the concentrate mixtures are given in Table 5 and Table 6, respectively.

Table 4. Experimental design showing adaption period, days with milk samplings (Monday and Tuesday), start and end of experimental feeding and the period with an increased milking frequency

December			January		
Date	**Day**		**Date**	**Day**	
9	Mo	9 days adaptation period, 2x milking	1	We	Start of 3rd wk, 2x milking
10	Tu		2	Th	
11	We		3	Fr	
12	Th		4	Sa	
13	Fr		5	Su	
14	Sa		6	Mo	
15	Su		7	Tu	
16	Mo	Milk sampling	8	We	Start of 4th wk, 2x milking
17	Tu		9	Th	
18	We	Start of 1st wk of experimental feeding, 2x milking	10	Fr	
19	Th		11	Sa	
20	Fr		12	Su	
21	Sa		13	Mo	Milk sampling
22	Su		14	Tu	
23	Mo		15	We	Start of 5th wk, 4x milking
24	Tu		16	Th	
25	We	Start of 2nd wk, 2x milking	17	Fr	
26	Th		18	Sa	
27	Fr		19	Su	
28	Sa		20	Mo	Milk sampling
29	Su		21	Tu	
30	Mo				End of experimental feeding
31	Tu				

Table 5. Ingredient composition of experimental diets, as reported by concentrate manufacturer, Teknosan

	Diet		
Item	**C[2)]**	**P**	**R**
Ingredients[1)]			
Vegetable oil MPB		4.0	
Rapeseed oil			4.0
Wheat middlings	12.0	12.0	12.0
Expro	23.0	23.91	23.77
Palm kernel expeller	8.0	8.0	8.19
Pelleted beet	7.74	6.0	6.0
Beet molasses	2.50	2.0	2.0
Barley	12.0	14.0	12.0
Wheat	13.0	8.26	10.22
Oat	19.0	19.0	19.0
Limestone, salts, vitamins & trace elements	2.76	2.82	2.82

[1)] Ingredients are given in kg/100kg
[2)] C = control diet, P = palm oil diet and R = rapeseed oil diet

Table 6. Chemical compositions of experimental diets, as reported by concentrate manufacturer, Teknosan

		Diet		
Item		**C**[1)]	**P**	**R**
Chemical composition	**Unit**			
ME	MJ/kg	11.30	12.11	12.04
Crude protein	%	16.29	16.0	16.03
Crude fat	%	3.84	7.75	7.73
Crude fiber	%	9.72	9.61	9.58
Ash	%	6.96	6.84	6.83
NDF	%	25.84	25.56	25.50
Starch	%	23.73	21.91	22.07
Calcium	g/kg	7.50	7.50	7.50
Phosphorous	g/kg	5.25	5.26	5.25
Potassium	g/kg	8.40	8.01	7.99
Copper	mg/kg	6.90	6.90	6.90
Magnesium	g/kg	4.0	4.0	4.0
Sodium	g/kg	4.70	4.70	4.70
Selenium	mg/kg	0.40	0.40	0.40
AAT	%	10.64	10.41	10.40
PBV	%	1.14	1.31	1.32
Lysine	g/kg	7.53	7.53	7.51
Metionine	g/kg	2.94	2.92	2.92
Vitamin A	Int. unit	6 000	6 000	6 000
Vitamin D	Int. unit	2 000	2 000	2 000
Vitamin E	mg/kg	40	40	40

[1)] C = control diet, P = palm oil diet and R = rapeseed oil diet

Sampling and analysis

Individual feed intake and MY were registered on daily basis throughout the whole experiment. Samples from feed, both concentrate and silage, were collected once a week from start till the end of the experiment. Chemical composition of the concentrates was analyzed for ash, crude protein, NDF and EG-fat (Table 7). FA composition of the diets was also analyzed and is presented in Table 8.

Table 7. Chemical compositions of silage and experimental diets from analyses (DM basis)

		Diet[1)]		
Item	**Silage**	**C**	**P**	**R**
Composition[2)]				
DM	41.2	88.0	87.6	88.3
Ash	9.2	7.5	8.0	7.3
Crude protein	12.3	18.5	19.6	18.2
NDF	55.1	28.8	26.9	25.6
Fat (EG)	---[3)]	4.2	6.1	6.5

[1)] Diets are concentrates supplemented with either no added fat (C), 4% added palm oil fat ingredient (P) or 4% added rapeseed oil ingredient (R)
[2)] Composition is given in g/100g DM
[3)] Not determined

Table 8. FA compositions, both grouped FA and individually FA, of silage, ingredients added in diets and experimental diets

	Ingredient				Diet[1)]		
Item	**Silage**	**Soy bean**	**Palm oil fat**	**Rapeseed oil fat**	**C**	**P**	**R**
Total FA[2)] in group							
FA ≤C14	1.99	1.70	0.34	0.07	8.79	5.02	4.38
SFA	19.96	17.79	20.86	7.16	26.65	23.88	16.93
MUFA	3.74	23.13	64.68	59.96	30.33	45.57	46.19
PUFA	61.55	55.89	12.52	28.65	38.63	27.38	32.22
FA composition[3)]							
C6:0	0.04	0.00	0.00	0.00	0.00	0.00	0.00
C12:0	1.27	1.00	0.00	0.00	5.84	3.43	2.98
C14:0	0.68	0.70	0.34	0.07	2.96	1.58	1.40
C15:0	0.09	0.00	0.00	0.00	0.06	0.00	0.02
C16:0	15.26	13.14	15.62	4.50	15.55	15.77	10.03
C16:1(n-7)	0.83	0.00	0.06	0.12	0.30	0.07	0.21
C17:0	0.03	0.00	0.00	0.00	0.02	0.00	0.02
C18:0	1.54	2.53	3.21	1.73	1.90	2.35	1.92
C18:1(n-9)	2.91	22.93	63.84	58.69	29.40	44.93	45.08
C18:2(n-6)	15.07	49.87	11.62	19.05	35.17	25.06	26.10
C18:3(n-3)	46.08	5.84	0.26	9.51	3.31	2.02	6.06
C20:0	0.44	0.21	0.50	0.58	0.19	0.23	0.36
C20:1(n-9)	0.00	0.20	0.73	1.05	0.57	0.57	0.83
C22:0	0.61	0.22	1.19	0.28	0.13	0.51	0.21
C22:1(n-9)	0.00	0.00	0.05	0.00	0.03	0.00	0.02
C22:4(n-6)	0.40	0.18	0.63	0.09	0.15	0.30	0.06
C24:1	0.00	0.00	0.00	0.11	0.04	0.00	0.05

[1)] Diets are concentrates supplemented with either no added fat (C), 4% added palm oil fat ingredient (P) or 4% added rapeseed oil ingredient (R)
[2)] Measured fatty acids in group (g/100g)
[3)] Fatty acid in g/100g of total identified fatty acids

MY was registered at every milking during the whole treatment period. The time elapsed since last milking was also recorded at every milking. Milk sampling was performed on three separate occasions during the experiment period. The first sample was collected during the adaptation period, the second sample was collected during the 4th week, before frequent milking was started, and the third sample was collected during the 5th week, during frequent milking. At the two first occasions fresh milk samples were collected during morning and evening milking for two days. At the last occasion during the 5th week, with higher MF, fresh milk samples were taken four times a day for two days. Milk was first collected in proportional milk samplers in the AMR and then transferred to sampling tubes of ~450 mL that was heated to 37^{o} C in a water bath. Milk from one of these tubes, representing one cow, was then

portioned into four smaller test tubes. Milk from one of these tubes, representing one cow, was then portioned into three smaller test tubes and analyzed.

Sample 1 was preserved with bronopol and stored at 5^{o} C for 1-3 days before analysis for milk composition, using mid-infrared spectroscopy (Fourier Transform Instruments, MilkoScanFT120 Foss, Hillerød, Denmark).

Sample 2 was kept at 5^{o} C and stored for 48 hours but was then moved to a freezer holding -20^{o} C and stored until analysis for content of FFA and FA composition at BioCentrum, at The Swedish University of Agricultural Sciences in Uppsala. Before analysis of content of FFA and FA composition the milk was pooled, mixing milk from all milkings during each two sampling and from each cow according to individual MY. Determination of total content of FFA was performed by using a solvent extraction followed by titration, according to a variant of the method constructed by Deeth *et al*. (1975). To receive the FA quantity and profile a gas-liquid chromatography (GLC) separation and quantification technique was used. Prior to GLC a milk extraction was carried out according to the method 1B:1983, International Dairy Federation. After extraction a transmethylation of triglycerides was performed according to the procedure made by Christie (1982).

Sample 3 was the last milk sample and only collected from evening milking the first day and from morning milking the second day. This milk sample was sent to Foulum Research Center, Denmark for MFG analysis. Size of MFG was determined by integrated light scattering as described by Wiking *et al*. (2003), at Foulum, Århus University, Denmark. The average volume-weighted diameter ($d_{4,3}$) was calculated by the instrument software described by Wiking *et al*. (2004).

Statistical analysis

Feed intake, MY, milk composition, FA profile and content of FFA were statistically analyzed as a randomized block design using the MIXED procedure model of SAS for Windows software (Version 9.3; SAS Insitute Inc., Cary, NC). Different models were used depending on the variable measured and sources of variation in the models included different effects depending of the variable measured. Each individual was designated as a random effect in the model used for FA composition, content of FFA and mean of MFG size. In the model used for feed intake, MY and milk composition cow was used as a repeated effect. Statistical differences were considered to exist at $P<0.05$, and a tendency was considered to exist at $P\leq0.05<0.01$. Data is presented as LS-mean for 30 cows with standard errors (SE) unless otherwise stated.

The models for milk parameter yield had the form of:

$$Y_{ijklmn} = \mu + (diet)_i + (MF)_j + (DIM)_k + (breed)_l + (lact.nr)_m\ (MY)_n\ (diet*MF)_{ij} + \varepsilon_{ijklmn}$$

The models for feed intake, content of FFA and MFG mean had the form of:

$$Y_{ijklmn} = \mu + (diet)_i + (MF)_j + (DIM)_k + (breed)_l + \varepsilon_{ijklmn}$$

Yijklmn - is the dependent variable
μ - is the overall mean
(diet)i - is the fixed effect of diets divided on three levels
(MF)j - is the fixed effect of milking frequency divided on two levels
(DIM)k - is the fixed effect of days in milk
(breed)l - is the fixed effect of breed divided on two levels
$(lact.nr)_m$ - is the fixed effect of lactation number divided on three levels
$(MY)_n$ - is the fixed effect of milk yield per day
εijklmn - is the random error

Results

Cows in the experiment adapted well to the management and diets, where they had a normal intake of both concentrate and silage throughout the whole experiment.

Feed intake

Total registered feed intake and the intake of silage and concentrate are presented in Table 9. While intake of concentrate had only small variations, though significant (P=0.0005), among experimental diets, silage intake was significantly (P<0.0001) lower in cows fed P compared to those cows fed C and R diets. Diets had small but significant (P=0.0002) variations on total feed intake. MF had a significant (P=0.0006 effect on total DMI, this probably because of a tendency (P=0.078) towards an effect on silage intake, where an increase of 10.5% was observed over all diets when MF was elevated. An altered intake of concentrates with higher MF could not be found. A combination of diet and higher MF did not reveal any significant effect on total DMI, intake of concentrate or intake of silage.

Table 9. Effect of diet and MF on total DMI, intake of concentrate and intake of silage from cows fed the control diet or diets supplemented with palm oil or rapeseed oil. Numbers are based on LSmeans with SE values

	Diet[1)]						P-value=		
Item	**C**	**SE**	**P**	**SE**	**R**	**SE**	**Diet**	**MF**	**Diet*MF**
Intake[2)]									
Total DMI	23.7	0.36	21.4	0.36	23.5	0.36	0.0002	0.0006	0.273
Concentrate	11.8	0.15	11.1	0.15	10.8	0.15	0.0005	0.179	0.963
Silage	12.4	0.29	10.8	0.29	13.0	0.29	<0.0001	0.078	0.214

[1)] Diets are concentrates supplemented with either no added fat (C), 4% added palm oil fat ingredient (P) or 4% added rapeseed oil ingredient (R)
[2)] Intake is given in kg DM/day

Milk parameters

Effect of diet and MF on MY and milk composition are viewable in Table 10. As expected, parity, DIM and breed had a significant (P<0.05) effect on MY (data not shown). A higher MY was observed for multiparous and Holstein cows. However there was no significant difference detected between first and second lactation cows. An increase in MF from two milkings to four milkings per day gave a significant (P<0.0001) increase in MY from 33.0 ± 0.44 kg/d to 40.5 ± 0.60 kg/d. This result was based on average MY from the two first sampling occasions, where the cows were milked twice daily, and compared with MY from the last sampling occasion where an IMF was performed. The elevated MY during higher MF was observed among all experimental diets.

The milk component yields were not significantly affected by diet. Effect of diet*MF had a tendency (P=0.051) on protein yield, where an increased protein yield was detected with an IMF. Effect of MF on component yields was significant (P<0.0001). Milk from cows in all diet groups had a higher yield of

fat, protein and lactose with a higher MF (Table 10) Milk from cows fed P diet had a significantly (P=0.0001) lower content of fat and protein while lactose content showed no significant (P=0.784) alteration during both levels of MF. As well as for protein yield effect of diet*MF tended (P=0.059) to be significant on protein content, but this time an IMF decreased the protein content. Effect of MF was significant (fat and protein: P<0.0001, lactose: P=0.0002) for milk composition. Content of fat and protein decreased with a higher MF while lactose content was slightly increased.

Table 10. Effect of diet and MF on milk yield, milk components yield and milk composition of milk from cows fed the control diet or diets supplemented with palm oil or rapeseed oil. Values are presented as LSmeans with SE

		Diet[1)]						P-value		
Item	**MF[2)]**	**C**	**SE**	**P**	**SE**	**R**	**SE**	**Diet**	**MF**	**Diet*MF**
Milk yield[3)]										
	2x	32.98	0.59	32.72	0.64	33.26	0.72	0.843	<0.0001	0.974
	4x	40.61	0.91	40.16	0.94	40.63	1.00			
Milk components yield[4)]										
Fat yield	2x	1.28	0.050	1.23	0.053	1.34	0.059	0.152	<0.0001	0.218
	4x	1.56	0.056	1.43	0.058	1.54	0.061			
Protein yield	2x	1.06	0.031	1.02	0.033	1.11	0.036	0.269	<0.0001	0.051
	4x	1.40	0.034	1.36	0.035	1.39	0.038			
Lactose yield	2x	1.44	0.056	1.45	0.060	1.53	0.067	0.761	<0.0001	0.139
	4x	1.91	0.059	1.94	0.062	1.94	0.068			
Milk composition[5)]										
Fat	2x	4.51	0.075	4.32	0.074	4.55	0.081	0.0001	<0.0001	0.115
	4x	3.70	0.067	3.44	0.069	3.52	0.071			
Protein	2x	3.63	0.030	3.54	0.029	3.66	0.032	<0.0001	<0.0001	0.059
	4x	3.34	0.027	3.20	0.027	3.27	0.028			
Lactose	2x	4.66	0.021	4.65	0.021	4.65	0.023	0.784	0.0002	0.996
	4x	4.76	0.019	4.75	0.020	4.75	0.020			

[1)] Diets are concentrates supplemented with either no added fat (C), 4% added palm oil fat ingredient (P) or 4% added rapeseed oil ingredient (R)
[2)] Level of milking frequency (2x=two times per day, 4x=four times per day)
[3)] Milk yield is given in kg/day
[4)] Component yields are given in kg/day
[5)] Composition is given in % per milking

FFA and MFG size

Effect of diet with different levels of MF on amount of FFA and size of MFG are viewable in Table 11. Diet and diet*MF did not have a significant effect on FFA and MGF size. Content of FFA was clearly increasing with an elevated MF. MF tended (P=0.096) to have an effect on size of MFG where a vaguely larger MFG was observed with a higher MF.

Table 11. Effect of diet and MF on concentration of FFA and size of MFG in milk (pooled milk samples) from cows fed control diet or diets supplemented with palm oil or rapeseed oil. Numbers are based on LSmeans with SE values

Item	MF[2]	Diet[1]						P-value		
		C	SE	P	SE	R	SE	Diet	MF	Diet*MF
FFA[3]										
µ Eq./ml	2x	0.118	0.013	0.126	0.012	0.107	0.014	0.413	<0.0001	0.465
	4x	0.273	0.017	0.256	0.016	0.254	0.018			
mEq./100 g fat	2x	2.85	0.409	3.45	0.407	2.77	0.410	0.379	<0.0001	0.151
	4x	6.09	0.409	5.10	0.407	5.00	0.410			
MFG size[4]										
	2x	4.08	0.088	3.85	0.089	3.87	0.110	0.165	0.096	0.526
	4x	4.16	0.093	4.10	0.078	4.07	0.085			

[1] Diets are concentrates supplemented with either no added fat (C), 4% added palm oil fat ingredient (P) or 4% added rapeseed oil ingredient (R)
[2] Level of milking frequency (2x=two times per day, 4x=four times per day)
[3] Free fatty acids are given in mEq./ml
[4] Milk fat globule size is given in µ volume weighted mean

Fatty acid composition in milk

Results of diet on FA composition in milk are presented in Table 12. Effect of diet was significant (P<0.01) for all groups with total FA; ≤C14:0, SFA, MUFA and PUFA. Milk from cows fed with C diet showed, as expected, a higher content of FA ≤C14:0 and SFA while contents of MUFA and PUFA were lower compared to P and R diets. As expected cows fed R diet had the highest yield of MUFA and PUFA in milk. The individual FA; C18:1 (n-9), C18:2 (cis9 trans11, CLA) and C18:3 (n-6) were found in significantly (p<0.01) higher concentrations in milk from cows fed diet R compared with milk from cows fed C or P diet.

A significant (p=0.047) effect of MF was observed in PUFA where a higher MF lowered the concentration of PUFA in milk among all diets. The other groups of total FA was not significant affected by MF. Some individual FA were significantly affected by MF. A higher MF showed to increase the concentration of C4:0 (P=0.007) and C18:0 (P=0.048). Further an elevated MF demonstrated that the concentration of C12:0 and C18:3 (n-6) was significantly (P=0.0081 and P=0.006 respectively) decreased in milk from cows fed every different diet. There was no significant interaction between diet and MF for any of the studied FA.

Table 12. Fatty acid composition in milk from cows fed control diet or diets supplemented with palm oil or rapeseed oil. Effect of MF is also shown. Numbers are based on LSmeans with SE values.

		Diet[1)]						P-value=		
Item	**MF[2)]**	**C**	**SE**	**P**	**SE**	**R**	**SE**	**Diet**	**MF**	**Diet*MF**
Total FA[3)] in group										
FA (≤C14)	2x	31.21	0.586	28.52	0.587	26.69	0.588	<0.0001	0.351	0.690
	4x	30.65	0.587	27.62	0.586	26.79	0.590			
SFA	2x	75.65	0.883	71.02	0.884	66.59	0.886	<0.0001	0.576	0.484
	4x	76.16	0.883	70.31	0.883	68.00	0.889			
MUFA	2x	24.73	0.759	29.42	0.759	32.68	0.761	<0.0001	0.688	0.712
	4x	24.57	0.759	29.74	0.759	31.76	0.764			
PUFA	2x	2.59	0.099	2.73	0.099	2.90	0.099	0.0094	0.047	0.960
	4x	2.40	0.099	2.60	0.099	2.72	0.100			
Individual FA[4)]										
C4:0	2x	3.82	0.130	3.73	0.125	3.70	0.134	0.712	0.007	0.951
	4x	4.08	0.129	4.00	0.124	4.03	0.134			
C6:0	2x	2.54	0.070	2.33	0.070	2.20	0.070	0.0002	0.097	0.909
	4x	2.62	0.070	2.41	0.070	2.33	0.070			
C8:0	2x	1.59	0.044	1.42	0.044	1.30	0.044	<0.0001	0.266	0.792
	4x	1.54	0.044	1.35	0.044	1.29	0.044			
C10:0	2x	3.83	0.126	3.27	0.126	2.96	0.126	<0.0001	0.215	0.794
	4x	3.66	0.126	3.09	0.126	2.93	0.126			
C12:0	2x	4.72	0.135	4.17	0.135	3.69	0.136	<0.0001	0.0081	0.760
	4x	4.38	0.135	3.78	0.135	3.50	0.136			
C14:0	2x	14.14	0.307	13.04	0.307	12.34	0.308	<0.0001	0.471	0.796
	4x	13.96	0.307	12.65	0.307	12.36	0.309			
C15:0	2x	1.03	0.042	0.87	0.038	0.84	0.044	<0.0001	0.983	0.964
	4x	1.02	0.041	0.88	0.037	0.83	0.044			
C16:0	2x	33.11	0.685	29.63	0.632	25.53	0.722	<0.0001	0.858	0.477
	4x	33.57	0.678	28.88	0.626	26.10	0.715			
C16:1 cis9	2x	1.09	0.108	1.18	0.108	1.01	0.108	0.373	0.760	0.857
	4x	1.13	0.108	1.10	0.108	0.98	0.109			
C17:0 (+some C16:1 cis13)	2x	0.51	0.020	0.43	0.019	0.40	0.022	<0.0001	0.687	0.876
	4x	0.50	0.020	0.43	0.019	0.40	0.021			
C18:0	2x	9.59	0.449	11.11	0.428	12.88	0.465	<0.0001	0.048	0.885
	4x	10.07	0.446	11.99	0.426	13.58	0.463			
C18:1 (n-9)	2x	19.09	0.639	23.40	0.640	25.58	0.641	<0.0001	0.501	0.834
	4x	18.87	0.640	23.34	0.639	24.79	0.643			
C18:2 (n-6)	2x	1.55	0.083	1.49	0.083	1.44	0.083	0.773	0.139	0.864
	4x	1.39	0.083	1.40	0.083	1.38	0.083			

C18:2 cis9trans11 (CLA)	2x	0.44	0.035	0.55	0.036	0.68	0.036	<0.0001	0.073	0.972
	4x	0.38	0.036	0.50	0.035	0.63	0.036			
C18:3 (n-6)	2x	0.083	0.011	0.119	0.010	0.131	0.012	0.002	0.006	0.249
	4x	0.072	0.011	0.102	0.010	0.089	0.012			

[1)] Diets are concentrates supplemented with either no added fat (C), 4 % added palm oil fat ingredient (P) or 4 % added rapeseed oil ingredient (R)
[2)] Level of milking frequency (2x=two times per day, 4x=four times per day)
[3)] Measured fatty acids in group (g/100g)
[4)] Fatty acids are given in g/100g identified FA

Discussion

This study has revealed important results that an IMF together with a change in diet will not affect the milk fat composition and FA profile in milk. The hypothesis that frequent milking can have different effect on milk fat depending on supplemented fat added to diet is therefore not supported. This combination of MF and diet did not affect the content of FFA and size of MFG either. It is also demonstrated, together with earlier findings, that rapeseed oil supplement will not influence the FA profile in milk more negatively than a supplement with added palm oil. Further, this present study has confirmed previous findings that the FA composition in feed will not be the same as the composition in milk. Approximately 50-60% of the FA in supplemented fat are transferred into milk fat (Ashes *et al*., 1997). The low percentage is much due to the alteration of FA that occur before and during ruminal fermentation. However, the composition of FA in the milk can differ depending on the composition and amount of FA present in the feed. This was demonstrated in present experiment where addition of UFA to diet decreased the content of SFA in milk. These results confirm that it is to some extent possible to affect the FA composition in milk by modifying and supplementing feed and diets.

Milk fat has during many decades been an important key ingredient in many foods. It is unique in many ways, much due to its content and composition of different fatty acids, which all have their own characteristics in milk. The physical properties of milk fat can both depend on factors affecting the milk from inside of the udder, such as size of MFG, amount of fat, saturation etc. However, milk fat can also be affected by external factors on the farm or at the dairy plant, e.g. temperature changes, transport or air fluctuations. Due to the many factors affecting content of FFA and size of MFG in milk it may be possible to reduce these non-wanted substances in milk, already on the farm. Technical and management factors including air inlet during transport, cooling time, but also cleanliness of the equipment may impair the milk. By using a good management these disturbing factors may be decreased. FFA content can also vary much during a year. By adjustments within individual cows by increasing MF during early- and late lactation or by adjustments in management of AMS, content of FFA can be reduced (de Koning *et al*., 2004).

Feed intake

Small effects from concentrates were observed in P diet where silage intake was lower compared to silage intake in C diet. Several studies have demonstrated that a diet containing more than 5% supplemented fat generally

has a negative effect on DMI and digestibility of carbohydrates, in particular fibre (Christie, 1979; Palmquist & Jenkins, 1980; Grummer, 1988; Jenkins & Jenny, 1989; Pantoja *et al*., 1994; Potu *et al*., 2011). Other claims the opposite, that supplemented fat has little or no effect on DMI. The variation in diet and fat source may explain this discrepancy between literature sources. In the present experiment the lower intake of silage among cows fed P diet could be a cause of the supplemented fat since there were more than 5% of fat in the diet (7.75% for P diet and 7.73% for R diet). However the added fat in the concentrates had a content of less than 5% in this study. In the present study small variations in milk fat could be seen between groups assigned to different diets. These small variations could verify the well-adjusted amount of fat in the diet to the individual cows with different MY and energy requirements. This could be explained by previous findings, where a diet with more than 5% of fat, especially LCFA, would usually result in a higher production of propionate and a lower production of acetate and butyrate, which are the precursors to milk fat (Chilliard *et al*., 1991; Mosley *et al*., 2007; Weisbjerg *et al*., 2008).

Milk parameters

The results of the current study show that MY in kg per day and cow was in general not affected by diet treatment. Diets with different FA composition did not significantly affect fat-, protein- or lactose content and yield. Usually the protein content in milk would decrease with an increase in fat content in ruminant diets. This is explained to occur because of the decreased content of casein (Doreau & Chilliard, 1997). It is worth noticing that even though diet C and P contained a higher content of saturated fat compared to R diet the fat yield was not higher in milk from cows fed these diets. As discussed, previous studies has not been unanimous considering the increase of fat in milk with a fat supplemented diet. Wiking (2005) demonstrated that an increased fat content in milk is most visible with saturated fats, which was not seen in current study. Further the availability of the supplemented fat can affect how much of the dietary fat that will appear in the milk (Sejrsen et al., 2007). Transfer efficiency may be due to treatment of added fat, different kinds of feedstuff and if the added fat is encapsulated. In present study the concentrate included oil from palm and rapeseed which was not treated or encapsulated.

Concentrate treatments in the current study included both saturated and unsaturated FA, though with a higher content of UFA. It is well known that addition of stearic acid (C16:0) and palmitic acid (C18:0) to diet will decrease the production of C4:0 to C16:0. Along with the biohydrogenation in the rumen the unsaturated C16 and C18 contribute to the decreased amount of FA ≤C14:0. In the present experiment milk from cows fed P diet and R diet had a decreased content of SFA from C4:0 to C16:0 and an increase in both MUFA and PUFA. This is consistent with Wiking *et al*. (2005) that registered a low average fat content in milk fat when feeding cows a diet rich in C18:2. This former study together with present results indicates that a supplement with a high concentration of PUFA, such as rapeseed, will inhibit the formation of precursors for milk fat in the rumen, and further the *de novo* synthesis. These results agrees with previous findings from Patton & Jensen (1975), Grummer (1991) and Mosley *et al*. (2007), showing a linear decrease of *de novo* fatty acid synthesis when a supplementation of dietary fat increases. The individual UFA C18:1, C18:2 (CLA) and C18:3 in this experiment did increase in diets supplemented with fat, P and R diet, compared to C diet.

A higher MF resulted in an elevated MY. An increase in MF from two milkings to four milkings per day gave an increased MY among all three diets. This is consistent with earlier experiments (Erdman & Varner, 1995; Klei *et al*., 1997; Stelwagen, 2001; Svennersten-Sjaunja & Pettersson, 2007; Soberon *et al*., 2011) where a clear increase in MY was observed. As can be seen in Table 10 a higher MY did increase the milk component yields but decreased the concentrations of components in milk which can be explained by the dilution effect (Klei *et al*., 1997; Harvatine & Allen, 2005; Løvendahl & Chagunda, 2011). Overall, the IMF did not have an effect on FA composition in milk. The content of PUFA in milk gave significant (P=0.047) differences when MF was changed. With an IMF the content of PUFA in milk decreased. This is comparable with results from Wiking (2005) where the proportion of PUFA was lower in the milk from the udder half milked four times a day compared to the udder half milked twice a day. Also some individual FA were significantly increased or decreased due to a change in MF. The content of C4:0 was significantly increased with an IMF which can be explained by a stimulated production of SCFA when the cow is milked more than twice daily. A higher MF significantly decreased C12:0 and C18:3 (n-6).

In the present study registrations from lactating cows had a DIM between 130 and 148 within each treatment group and a DIM between 68 and 211 at individual level at start of experiment. All registrations were based on this range in DIM where both early and late lactating cows been excluded. Since there were overall significant effects of stage of lactation on MY, it is to be considered as valid factors to include in future experiments. The research claims that a higher MF can be used for early lactating cows in order to receive a higher MY which would have been interesting to study. If early lactating cows would be included in the experiment it is crucial to adjust the dietary fats individually since milk fat content may vary between different stages of lactation.

FFA and MFG size

In the present study milk from cows in all treatment groups had levels of FFA high enough to be associated to the occurrence of off-flavours. Several studies have reported a threshold level of <2.0 mEq/100 g fat before a rancidity will appear (Tuckey & Stadhouders, 1967; Pillay *et al*., 1980; Fox & McSweeney, 1998). A significant effect of diet was however not shown in present study. Content of FFA in milk was approximately doubled after introduction of an IMF. This is consistent with numerous studies made by different researchers. The cause of an increased content of FFA with a higher MF can be because of an increased MY that in general causes larger MFG with thinner MFGM. Another explanation can also be the elevated production of SCFA, which are more susceptible for lipolysis due to the placement on the outer positions in the triglyceride molecule (Quattara *et al*., 2004). The size of MFG is affected by the FA derived from diet (mostly LCFA), such as C16:0, C18:0 and C18:1 (Fox & McSweeney, 1998; Wiking *et al*., 2003; Weisbjerg *et al*., 2008). Correlations between average size of MFG and the FA C16:0, C16:1, C18 and C18:1 was found by Wiking *et al*., (2004). In contrast no correlations were found between average size of MFG and shorter FA (<C16) or between C18:2 and C18:3. According to Wiking *et al*. (2003 & 2004) the *de novo* fatty acid synthesis does

not affect the size of MFG. This is agreeable with present results showing that none of the diets had a significant effect on size of the MFG.

Higher MF tended (P=0.096) to have an effect on MFG size, where an introduction of four milkings per day resulted in slightly larger MFG. Earlier studies have demonstrated a more clear effect on globule size by an IMF and decreased milking intervals (Wiking *et al*., 2003 & 2006). The low effect in current study could be due to the low amount of SFA added in diet. The size of MFG is crucial for the stability and technological properties of the milk where a smaller size is proved to be more stable. The knowledge, achieved from present study and earlier studies make it possible to affect the size of the fat globules through feed with a content of specific FA. A diet rich in UFA and not SFA may be an alternative to avoid the negative effects on milk quality when an IMF is practiced.

Important to mention is also that it is not only the proportion of fat added to diet or MF that affect the final results. In the present study there are many factors not included in the analysis such as feeding routines, the ratio of the feeds (concentrate vs silage) and the degree of ruminal degradation that can change the final result, which have not been statistical analyzed and evaluated in current study. In further experiments it would also be interesting to see how the concentrates used in this study would affect FFA and FA composition if they were protected from alteration in the rumen.

The opinion among researchers is inconsistent regarding which threshold value for concentration of FFA that should be used for quality assurance of milk. This discrepancy makes it difficult for future research to obtain a result that is reliable. According to Wiking *et al*. (2005) there is still a risk of rancidity in milk when a low threshold is exceeded. And when the risk is present there are many factors affecting to which extent and how the milk quality will be decreased. It is also important to point out that certain off-flavours in milk may be desirable in some manufacturing of dairy products, such as cheeses. The measurement method used in the determination of FFA may also contribute to the studies' different results.

The analytic methods measuring FA composition have been very different during the past decades. The discrepancy in which method or analysis that has been used, when measuring different milk parameters, in different research papers makes it difficult to compare values that will generate reliable results. For several years, the technology has evolved with new developed methods and analysis. By using this fairly new analytic method with a gas liquid chromatography reliable results can be made. More research is needed where the same analytic method has been used throughout a high number of experiments to be able to compare these previous results with newer results but also to reduce the error sources.

The choice of the additional fat in the feed depends on what the milk should be used for. A certain composition is desirable in the manufacturing of a product while another composition is required for another product. In order to get milk with the right or desirable FA composition for the right purpose it may be possible to select milk already on the farm. By using a measurement technique in the AMS and by using special bulk tanks for raw milk with different compositions this can be achieved.

Results concerning the effect of supplemental vegetable oils on the milk fat composition have varied among researchers. Overall the present study could not present any differences concerning the use of a supplement rich in palm oil or rich in rapeseed oil. These results break the rumors that added rapeseed, rich in UFA can impair milk quality in a worse manner than palm oil supplement. Moreover it is, with present results, possible to almost exclude the impact on milk by a combination of MF and a given feed. The results from current study could not reveal any significant differences on composition in milk fat between the three diets when an IMF was performed. Neither were concentration of FFA or size of MFG affected by MF. Though it should be mentioned that MFG had a tendency to increase in size when a IMF was performed. A higher MF can therefore be used at farms where fat is added in the diet, without affecting the milk negatively. However it is important to note that milk quality may be impaired with a higher MF if management and technology is poorly administered on the farm.

Conclusion

Dietary oil supplements at a total of 4% fat (DM basis) to lactating cows did have small, but significant, effect on DMI. Diet did not decrease or increase MY. In milk, FA composition was altered to some extent depending on FA present in fat supplement where a higher content of UFA in diet showed to decrease the content of SFA in milk.

A higher MF resulted in a higher MY and elevated concentration of FFA in milk. Size of MFG did not significantly change with an IMF but had a tendency to increase. Some individual FA were affected by MF where the content of C4:0 was increased and C12:0 and C18:3 (n-6) were decreased when the cows were milked four times per day instead of two times per day. he effect of a combination of experimental diets and a higher MF did not affect the composition in milk.

A combination of a diets supplemented with fat consisting of different FA compositions and a higher MF did not have any effect on the milk parameters. This indicates that rapeseed oil, as well as, palm oil can be used in a AMS without impairing milk quality.

Conclusively, with the present results, locally produced rapeseed oil in feed can be a good alternative for palm oil enrichment, not only in a conventional farm but also at a farm using AMS. Together with this important aspect locally produced rapeseed oil could help slow down the deforestation that is a main climate issue from the palm oil industry. Further, the hypothesis that rapeseed oil would give similar results as palm oil regarding milk composition, FA composition, content of FFA and size of MFG was supported and rapeseed oil could therefore be a good alternative as supplement in feed to lactating cows.

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