



Milk quality on farms with an automatic milking system

Free fatty acids and automatic milking systems

April 2004

Information

This report is produced within the EU project *Implications of the introduction of automatic milking on dairy farms* (QLK5 -2000-31006) as part of the EU-program 'Quality of Life and Management of Living resources'.

Liability

The content of this publication is the sole responsibility of its publisher, and does not necessarily represent the views of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use, which might be made of the following information.

Availability

This report is freely available for every person interested at:
<http://www.automaticmilking.nl>

Milk quality on farms with an automatic milking system

Free fatty acids and automatic milking systems

Ir. Betsie Slaghuis

Ir. Oene de Jong

Ing. Kees Bos

Ing. Judith Verstappen-Boerekamp

Ing. Reina Ferwerda-van Zonneveld

*Applied Research of the Animal Sciences Group of Wageningen UR,
Lelystad, The Netherlands*

April 2004

Deliverable D10

Correspondence:

Ir. Betsie Slaghuis

Runderweg 6

8219 PK Lelystad

betsie.slaghuis@wur.nl

Abstract

With the introduction of automatic milking (AM) systems, increased levels of free fatty acids (FFA) in milk were observed, which might result in off-flavours in milk and dairy products.

The aim of this study was to investigate the factors contributing to elevated FFA levels: influence of the milking frequency, technical parameters of the automatic milking system, and finally, farm management aspects.

Milking frequency was studied in a Latin square design with milking intervals of 4, 8 and 12 hours and showed increased FFA -levels for the shorter intervals. Milk fat and protein percentages of milk were equal.

Milking frequencies are of more importance than technical parameters of the AM system, because FFA levels for farms milking three times per day and AM systems are more or less comparable. However, technical aspects cannot be excluded. Compared to conventional milking, the air/milk ratio is higher, probably resulting in more disruption of milk fat globule membranes. Technical factors were studied in a laboratory study using milking machine components of two AM-systems and of a conventional system. The milking machine components of one AM-system caused an extra increase of 0,09 mmol/100 g fat compared with the conventional milking system. These differences were found using susceptible milk. Susceptible milk is defined as milk with a high initial FFA level, caused by e.g. short milking interval or end of lactation of the cow. The other AM milking machine also caused a significant increase in FFA levels compared to a conventional system, but this increase was due to different FFA levels at the start of the experiment.

FFA levels of samples taken before, during and after milking indicated that taking mixed milk samples per cow was the most representative way of sampling. Therefore mixed milk samples were taken to determine FFA levels during the rest of the experiments. Fat globule size diameters were determined in milk of six cows. Relations between average globule size and FFA were not found. A linear relation between fat production and average globule size was indicated ($P=0.051$) with a percentage variance accounted for of 56,9%. Linear regression showed no significant relations between FFA and fatty acid composition of the milk of the six cows sampled. For milk yield and lactation stage of the cows significant relations were found for some fatty acids. However, results should be interpreted with care, they were based on six cows from one farm.

Apart from milking frequencies and technical parameters, management aspects probably play a role. In a study on variation of FFA levels, 12 farms were monthly sampled. Results showed more variation at higher FFA levels, as was expected. But on conventional farms milking twice a day (2x) high FFA levels were caused by technical factors (high level milk lines in stanchion barns). On farms milking three times a day (3x) FFA levels were relatively high and causes could not be defined in all cases. Similarity between grazing and high FFA levels was found on two of the four investigated farms. On AM farms, low FFA levels were possible throughout the year, depending on the farm tested. On one farm, high FFA levels were due to a high percentage of failed milkings. Reduction of the number of failed milkings reduced the high FFA level to an acceptable level.

In a study on management factors on eight AM farms and on six farms milking 3x with determination of FFA level on two moments (within six weeks in autumn), the differences between high and low FFA level farms were bigger for farms milking 3x than for AM farms with the same AM brand and cooling system. On farms milking 3x with high FFA levels, quota and number of cows on the farm were significant lower than on farms with low FFA levels. These differences were not found on AM farms.

Susceptibility of cows for lipolysis is very important, but it is a complex matter. Known causes are lactation stage, pregnancy stage and milking frequency. Although feeding and animal health play a role in increased FFA levels, more fundamental research is needed regarding this susceptibility of cows.

Although several FFA problems were solved by adjusting milking frequencies and technical adjustments, in some situations high FFA levels remained. Other farm management aspects, like feeding regime, breeding and animal health are still subject of ongoing research and results will be incorporated in future papers.

Table of contents

1	Introduction	1
2	Literature review.....	2
2.1	Possible mechanism of lipolysis	2
2.2	Factors influencing induced lipolysis.....	2
2.2.1	Milking system.....	3
2.2.2	Pumping	3
2.2.3	Cooling.....	3
2.3	Factors influencing spontaneous lipolysis.....	3
2.3.1	Milking frequency	3
2.3.2	Cow factors	3
2.3.3	Nutritional factors	4
2.4	Determination of FFA	4
3	Material and methods	5
3.1	Experiments.....	5
3.1.1	Effect of AM-system on FFA level.....	5
3.1.2	Sampling of milk.....	5
3.1.3	Milking frequency	6
3.1.4	Variation in FFA during the year	6
3.1.5	Influence of management factors on FFA.....	6
3.2	Analytical methods.....	6
3.3	Statistical evaluation	7
3.3.1	Effect of AM-system on FFA level.....	7
3.3.2	Sampling of milk.....	7
3.3.3	Milking frequency	7
3.3.4	Variation study	7
3.3.5	Management study	8
4	Results	9
4.1	Effect of AM-system on FFA level.....	9
4.2	Sampling of milk.....	9
4.3	Milking frequency	12
4.4	Variation of FFA during the year	12
4.5	Influences of management factors.....	14
5	Discussion.....	15
6	Conclusions and recommendations	16
	References	17
	Acknowledgements.....	19
	Abbreviations.....	20

1 Introduction

Milk quality on farms with an automatic milking system (AM system) has been subject to several studies in the past years (Kungel et al, 2000, Rasmussen, 2001, Rasmussen et al, 2002, Van der Vorst and Hogeveen, 2000, Van der Vorst et al, 2002). From these studies it is known that milk quality is affected by changing from conventional milking to automatic milking. The levels of total plate count and free fatty acids (FFA) increase after introduction of an AM system. With regard to somatic cell count and freezing point contradicting results have been found. To gain more insight in the milk quality courses after introduction of an AM system an extended analysis was performed on milk quality data from three countries over a four year period (Van der Vorst et al., 2002). This study showed that after introduction total plate count and somatic cell count improved again. The freezing point remained stable around the level it reached soon after introduction. The levels of free fatty acids did not recover after some time, nor was there a clear balance. Therefore in this study the FFA issue was studied in more detail.

The FFA issue is not new. In the seventies of the last century milk lines and bulk milk tanks were introduced resulting in problems with high acid degree values of the milk fat or free fatty acids. These problems were tackled practically by low level milk lines, no blind pumping, no air leakage and the introduction of periodically maintenance of the milking equipment. The mechanism of lipolysis however was not clarified.

With the introduction of automatic milking systems, the problem of higher levels of free fatty acids occurred again (Klungel et al., 2000; Vorst and Koning, 2002).

When milking three or more times a day instead of two times a day in conventional parlours, an increase in milk yield, a decrease in fat and protein and an increase in free fatty acid levels has been reported (Ipema and Schuil- ing, 1992; Jellema, 1986; Klei et al., 1997).

But with automatic milking, not all cows are milked three times a day. An average of 2,1 –2,9 milkings per day has been reported (Klungel et al., 2000; Abeni et al., 2002; Barth et al., 2000; Land et al., 2000; Artmann and Bohlsen, 2000, Koning & Ouweltjes, 2000). So increased milking frequency is not the only explanation for the increase of free fatty acids. Therefore some literature about lipolysis will be described, including possible mechanisms and factors influencing lipolysis.

The objective of this study was to identify risk factors related to high or low levels of FFA on farms milking with AM system and on farms milking three times a day.

2 Literature review

2.1 Possible mechanism of lipolysis

Lipolysis results from the enzymatic hydrolysis of milk fat, causing an accumulation of free fatty acids (FFA) some of which are responsible for the rancid flavour of milk. Results from bacterial lipolysis of milk fat are not considered here, as high counts of psychrotrophs are needed to produce bacterial lipase (Walstra et al, 1999). Those high counts ($>5 \times 10^5$ cfu/ml) are normally not present in bulk tank milk.

The milk fat present in milk fat globules is protected against the action of milk lipoprotein lipase by the milk fat globule membrane. If this membrane is disrupted by e.g. agitation, fatty acids can be splitted from the triglycerides by milk lipoprotein lipase (mLPL).

Another aspect of lipolysis is spontaneous lipolysis which results from the action of mLPL and takes place when milk is cooled after milking. The milk fat globule is probably not disrupted, but some factors present in milk may favour the interaction of mLPL with milk fat, resulting in higher degrees of FFA (figure 1.)

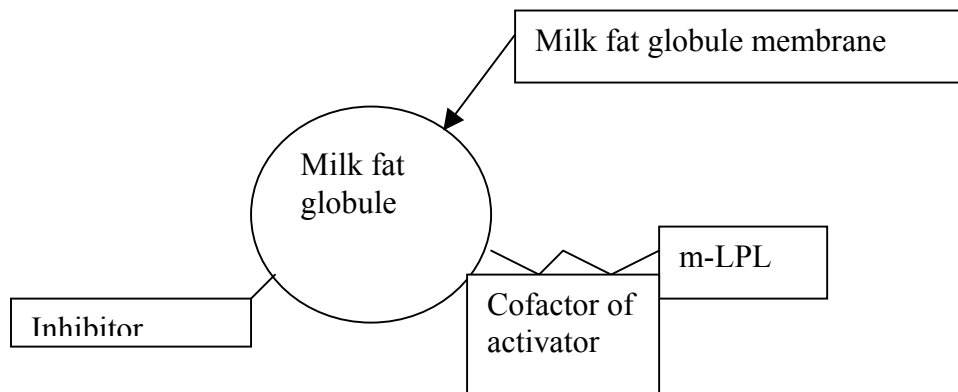


Figure 1. Schematic view of possible mechanism of spontaneous lipolysis in milk.

Several treatments, referred to as activation treatments, enhance lipolysis (induced lipolysis) and are often used to study lipolysis of milk fat by mLPL. Treatments which cause activation include agitation, homogenisation, temperature changes and the addition of blood serum or heparin to milk. Milk from individual cows differ in their susceptibility to these treatments. Correlations between spontaneous and induced lipolysis have been reported (Jellema, 1986; Chazal and Chilliard, 1987; Cartier and Chilliard, 1989).

The mechanisms of these treatments that promote lipolysis are not clearly understood. But some conclusions can be made.

- In most cases the action of mLPL on cold stored milk fat (commonly named spontaneous lipolysis) does not depend on the amount of mLPL that is present in whole milk (Cartier and Chilliard, 1990).
- A correlation was found between spontaneous lipolysis and the activator-inhibitor balance in skim milk. A lower content of inhibitors in skimmed milk was linked to a higher amount of cream LPL (Cartier and Chilliard, 1990).
- Activators are related to blood serum components, as addition of blood serum to raw milk may increase the level of free fatty acids (Jellema, 1975, 1986). Inhibitors have been determined as proteose pepton component 3 fraction (Cartier et al., 1990).

Factors influencing induced and spontaneous lipolysis will be discussed.

2.2 Factors influencing induced lipolysis

All factors influencing induced lipolysis are based on damaging of the milk fat globule membrane. Mechanical agitation (e.g. by leakage or stirring too fast with low milk levels in bulk cool tank) and certain temperature fluctuations as for instance freezing and subsequent thawing will damage the globule membrane.

After removing milk from the udder, milk is transported through tubes and pipes to the bulk tank with vacuum and air. Intensive mixing of milk and air may lead to damage of the fat globule membrane. Different parts of the milking process and their influences on damage of milk fat globule membrane will be discussed.

2.2.1 *Milking system*

When changing over from bucket milking machines into pipeline milking machines in the late sixties, increased FFA levels were found (Jellema, 1973). The design of the equipment was important, although with good machine installation, maintenance and operation, some design differences were possible. Especially high pipe line milking systems showed increased FFA levels. Lifting milk by air in vertical pipe sections, especially with a high air to milk flow ratio, increases FFA (Fleming, 1980). An air admission of 13 l/min in the milking cluster increased FFA levels significantly compared with 6 l/min (O'Brien et al., 1998). The air to milk flow ratio in automatic milking systems is rather high (8-10:1) compared with conventional milking (3:1). This is caused by the different design of the systems: no milk claws, but four separate, although with a smaller diameter but longer tubes per quarter to transport the milk from teat cup to milk line or receiver.

2.2.2 *Pumping*

Milk pump operation also influence FFA levels in milk: continuous milk pumping resulted in an increase (O'Brien et al., 1998). Escobar & Bradley (1990) found higher FFA levels in raw milk pumped by 3500 rpm compared to 1750 rpm. Raw milk with high milk fat content and larger milk fat globules was less stable to pumping (Wiking et al., 2003). Also pumping milk at 31°C compared to 4°C increased FFA.

2.2.3 *Cooling*

The storage in the bulk tank is not the cause of the increase FFA levels (Jellema, 1973), but the rate of cooling might be of influence. FFA levels can be increased by so called temperature activation: cooling to 5 °C, warming to 30°C and subsequent cooling below 10°C (Krukovsky and Herrington, 1939). However, although during conventional milking milk is warmed and cooled again, this phenomenon does not occur. Temperatures after mixing warm and cold milk, never exceeds 15°C in general.

Freezing of milk will enhance lipolysis, because when thawing milk fat globule membranes will be damaged because milk fat is still crystallised.

2.3 **Factors influencing spontaneous lipolysis**

In the literature several definitions for spontaneous lipolysis can be found. Spontaneous lipolysis can be defined as FFA level in milk not treated other than cooling soon after milking (Deeth and Fitz-Gerald, 1983).

Cartier and Chilliard (1990) defined spontaneous lipolysis as the difference between FFA content after 22 h storage at 4°C and 'initial' FFA (immediately inactivated). The effect of spontaneous lipolysis and susceptibility are closely linked. Jellema (1986) defined susceptibility as FFA level in untreated milk after 24 hours storage at 4°C. So different definitions are comparable and mean the same: FFA level after cooled storage of raw milk without any other treatment.

2.3.1 *Milking frequency*

Increase in FFA due to increasing milking frequency has been reported (Jellema, 1986; Ipema and Schuiling, 1992; Klei et al., 1997; Svennersten-Sjauna et al, 2002). This increase was however found after storage of samples at low temperatures (4-6°C) during a maximum storage time of 24 hours. Svennersten-Sjauna et al. (2002) also determined FFA immediately after sampling and found higher FFA levels for cows milked twice a day compared to six times a day.

2.3.2 *Cow factors*

Within farms differences in susceptibility for lipolysis between cows are normal. Between farms differences also exist, but more factors like for instance milking frequency may contribute to this difference than only cow factors (Chazal and Chilliard, 1987).

Milk from cows late in lactation is more susceptible to lipolysis than milk from cows early in lactation (Jellema, 1986). Relation with milk production is obvious: lower milk yield at end of lactation is common. Cartier and Chilliard (1990) suggest two explanations for this spontaneous lipolysis. The first is that the substrate surface may be larger as a result of smaller fat globules. The average size of fat droplets decreases and milk fat content increases as lactation and pregnancy advance (Mulder and Walstra, 1974), so the total surface to be covered by membrane increases. The second is that susceptible milk is more susceptible for damage of the globule membrane, during mechanical milking and cooling. A combination of stage of pregnancy and milk yield seemed to be important, but stage of pregnancy may have been enhanced by management factors as feeding conditions (Chazal and Chilliard, 1986). However, this pregnancy stage effect was linked to hormone status of the cow (Chilliard et al., 2003).

Although a clear correlation was not to be expected between the level of FFA and cell count, problems with high FFA levels appeared more frequently on farms with a higher average cell count (300.000-500.000 cells/ml) than on farms with a low cell count (Jellema, 1975).

A breed effect between Friesian and Montbéliarde on susceptibility for spontaneous lipolysis could not be concluded (Chazal and Chilliard, 1987). But Mulder and Walstra (1974) reported different average globule size diameters of fat globules for Friesians and Guernseys.

Not only decreasing milk yields at the end of lactation may enhance lipolysis, also decreases in milk yield during lactation may increase lipolysis. This could be due feeding management or health problems of cows.

2.3.3 *Nutritional factors*

Effects of feeding regimes of cows have been found for poor quality grass silage (Jellema, 1975, Chazal and Chilliard, 1987), resulting in underfeeding (energy shortage) of cows. But, when grass silage was of good quality, still increase of FFA in milk occurred compared to milk from cows being fed hay of grass with the same high nutritive value (Chazal et al., 1987).

Milk lipolysis was lower for cows fed diets based on concentrates or corn silage than for diets based on grass silage, natural grassland hay or pasture (Ferlay et al., 2002). For pasture, underfeeding could be a reason for higher lipolysis.

This could be an explanation for the fact that in the Netherlands FFA levels (personal communication Van den Bijgaart, 2003) in 2002 in autumn were slightly higher in the western part of the country, where in general no or less corn silage is fed. Other factors could also play a role in this (soil type, differences in parlour equipment, other differences in management).

Chilliard et al. (2003) reported that lipid supplementation in diets resulted in changes in fatty acid composition of the milk and decrease of FFA levels. Level of spontaneous lipolysis and goat milk LPL activity decreased when protected sunflower oil was distributed. This was confirmed by feeding unprotected linseed oil, high oleic sunflower oil and sunflower oil (all 5-6% of diet DM) to goats, but interaction with other diet components might influence the overall effect. When corn silage was fed to goats, no positive effect of oil supplementation was found on goat milk fat content, whereas oil supplementation increased sharply milk fat content when hay was used.

Zinc supplementation to 8 cows resulted in decreased development of free fatty acids due to spontaneous lipolysis during cold storage of raw milk (Hermansen et al., 1995). The effect of Zn may be associated with an effect in the milk fat globule membrane, but more extensive research is needed in this case.

2.4 **Determination of FFA**

Determination of FFA is possible with different methods (Kuzdzal-Savoie, 1980), resulting in different levels of FFA depending on the type of FFA being determined.

With the BDI-method (IDF, 1991), used in this study, the short chain fatty acids are not determined as these acids are not present in the fat.

3 Material and methods

3.1 Experiments

3.1.1 Effect of AM-system on FFA level

The effect of technical factors on FFA levels was investigated by passing milk through a conventional and simulated AM systems. Damage of fat globule membranes by transport through the equipment results in elevated FFA levels. By comparing conventional and AM systems the level of damage of the membrane can be determined.

Laboratory experiments

For this experiments equipment for conventional milking consisting of milking cluster, milk tubes and milk recorder jar, was connected with an artificial udder and teats. Milk flow could be regulated, resulting in a milking velocity of about 2 kg/min. Air inlet at the milking cluster was 8 l/min and milk vacuum was 42 kPa. Equipment for automatic milking of one brand was consisting of teatcups, milk tubes and milk receiver part. This was also connected to the same artificial udder and teats. Milk flow velocity was also about 2 kg/min. Air inlet at the teat cups was 24 l/min. Milk vacuum was also 42 kPa.

Distances between teat cups and milk receiver part was about the same for both systems.

Fifty liters of raw warm milk from cows late in lactation or known to have high FFA levels (see 3.1.2.) was used for laboratory experiments. Raw warm milk was divided in two equal portions and sampled for FFA.

One part was divided again in two equal portions. These two portions were passed through the equipment according to the vigouring milking circumstances, one after each other. The other two portions were passed through the remaining equipment. Duplo samples were taken from the milk after passing the equipments. All milk was mixed together and the procedure was repeated again. This repetition was done to have milk with higher FFA levels, probably resulting in more effects of milking equipment on FFA levels.

This procedure was repeated on two other days for brand 1.

Experiments in practice

For AM brand 2, this procedure was performed partly on a system on an experimental farm and partly on the laboratory (conventional milking).

For this experiments equipment for conventional milking was the same as described in laboratory experiments. Equipment for automatic milking of brand 2 was consisting of teatcups, milk tubes and milk receiver part. This was also connected to the artificial udder and teats. Milk flow velocity was also about 2 kg/min.

Samples of the raw milk before passing the system were taken from milk being divided in two portions.

Brand 1 was also compared in a milking parlour, where on one stand teat cups, milk lines and milk receiver part was placed instead of the normal milking cluster. For this study 26 cows were divided into two groups (A and B). Group A was milked with the conventional milking system on day 1 and with the AM constructed system on day 2. Group B was milked with the AM system on day 1 and with the conventional system on day 2. Duplo samples were taken after milking (post milk) and from the recorder jar (mixed milk). Milk production was noted. Milk samples were stored at 5°C.

3.1.2 Sampling of milk

The moment of sampling, storage time and the effect of inactivation was subject of this study

The moment of taking samples during the milking process was studied by taking three samples from six cows during one milking. Six cows were selected: two were at the beginning of the lactation (7-100 days), two were halfway the lactation (100-200 days) and two were at the end of lactation (>200 days).

Samples were taken:

- Before milking after pre squirting (by hand)
- Halfway the milking process cluster was removed and cow was milked by hand
- After removal of the cluster, stripped milk was milked by hand
- From the recorder jar (mixed milk)

Each sample of minimum 100ml was splitted in two parts of 50 ml. One half was inactivated immediately by adding hydrogen peroxide (0,02% end concentration, Jellema, 1979). The other half was stored for 24 h at 4°C and inactivated after the 24 h period. Sampling was done on two days, to determine day variability of cows.

From the recorder jar two extra samples were taken for determination of fat globule size diameter and fatty acid composition. Samples for fat globule size diameter were stored at 4°C and transported for immediate determination to NIZO Food Research. Samples for fatty acid composition were cooled, frozen, transported to and analysed by NIZO Food Research.

In a separate experiment influence of storage at 4°C on FFA level was studied. Milk of one susceptible cow was inactivated after different times of storage. After 0, 19, 21, 23, 25, 27, 29 and 31 hours at 4°C milk was inactivated with hydrogen peroxide. FFA was determined of duplo samples.

3.1.3 Milking frequency

Influence of milking frequency on FFA levels was investigated. The effect of lipolysis was determined in a Latin square design experiment with 12 cows, three periods, three milking intervals (4, 8 and 12 hours corresponding to 6, 3 and 2 times milking a day) and 4 cows per group (table 1.). Cows were milked according to this scheme for 4 days and on day 4: all cows were milked and sampled. Milk samples were divided into two sub samples: one was inactivated by hydrogen peroxide immediately (0,02%, Jellema, 1979), the other one was stored at 5°C ±1°C (cooling facility could not reach 4°C) for 24 hours and inactivated with hydrogen peroxide.

Table 1. Latin square design of spontaneous lipolysis study, with groups of four cows defined as A, B and C.

Period		1	2	3
Milking interval	Milking frequency			
12 h	2x per day	A	B	C
8 h	3x per day	B	C	A
4 h	6x per day	C	A	B

3.1.4 Variation in FFA during the year

Because FFA is only determined twice a year (in spring and in autumn), variation through the year on farms was unknown. Therefore twelve farms were selected, based on FFA levels of the previous year (two determinations). Four farms were milking conventionally two times a day (2x). Four were milking conventionally three times a day (3x) and four were milking with AM systems.

From these four farms per group, two were selected as high and two as low. The high farms had high FFA levels in the previous year (>0.80 mmol/100 g fat), the low farms had low FFA levels (<0.70 mmol/100 g fat; table 2.). FFA levels of bulk tank milk were determined monthly and in two periods of 14 days, one in autumn and one in spring, from every bulk tank. Sampling was performed from April 2003 until April 2004.

Table 2. Number of farms in design of study on variation of FFA levels

FFA level	high	low
Type of farm	(>0,80 mmol/100 g fat)	(<0,70 mmol/100 g fat)
Conventional 2x per day	2	2
Conventional 3x per day	2	2
AM	2	2

3.1.5 Influence of management factors on FFA

Also other factors than technical en milking frequency were studied, but influence of milking frequency could not be excluded.

The effect of farm management aspects was studied at 8 farms using the same brand of AM-system and the same cooling system and 6 farms milking three times a day (Table 3.). Farms with AM systems used their system at least one year, but these systems were relative new as was the cooling system. Half of the farms were classified as high (FFA >0.75 mmol/ 100 g fat during at least the last year) and the other half as low (<0.70 mmol/100 g fat).

Bulk tank milk was sampled twice within six weeks in autumn and analysed for FFA. Detailed farm and AM characteristics including milk frequencies and management information regarding feeding, housing conditions and animal health was obtained by a questionnaire during a visit at the farm and correlations among these factors with FFA levels were calculated.

Table 3. Number of farms in design of study on farm management.

FFA level	high	low	remarks
Type of farm	(>0,75)	(<0,70 mmol/100 g fat)	
Conventional 3x per day	3	3	different brands, same cooling system
AM	4	4	same brand, same cooling system

3.2 Analytical methods

All raw milk samples were analysed according to the BDI-method (IDF, 1991).

Globule size diameter were measured using a Malvern Instruments SB 80. A mean volume surface diameter was used for calculations. Fatty acid composition was After thawing samples for fatty acid composition fat was separated and treated. Analysis was performed using capillary gas chromatograph in combination with polar phase (100% cyanopropyl).

3.3 Statistical evaluation

For statistical evaluation part of the FFA results were transformed to log, in order to get a better normal distribution. For differences no log transformation was performed.

3.3.1 Effect of AM-system on FFA level

Analysis of variance was performed by using the model:

$$\text{FFA}_{24} - \text{FFA}_0 = \text{delta FFA} = \mu + I_{ij} + M_{kl} + D_{mno} \times I_{ij} + \varepsilon$$

where:

$\text{FFA}_{24} - \text{FFA}_0 = \text{delta FFA}$ = difference in FFA level after 24 h of incubation at 5°C and immediately after sampling

μ = total mean

I_{ij} = effect of equipment

M_{kl} = deviation of milk used for the first or the second time

D_{mno} = deviation of day of experiment

ε = residual error

3.3.2 Sampling of milk

Analysis of variance was performed by using the model:

$$\text{LOGFFA} = \mu + S_{ijkl} + G_{ijk} + C_{ijkl} + I_{ij} + D_{ij} + \varepsilon$$

With: μ = total mean

S_{ijkl} = effect of sample

G_{ijk} = group deviances

C_{ijklmn} = cow deviances

I_{ij} inactivation deviance

D_{ij} = date deviance

ε = residual error

3.3.3 Milking frequency

Analysis of variance was done with the model:

$$\text{FFA}_{24} - \text{FFA}_0 = \mu + I_{ijk} + C_{ijkl} + D_{ijklm} + C_{ijkl} * D_{ijklm} + \varepsilon$$

With:

FFA_{24} = FFA after 24 h incubation at 5°C

FFA_0 = FFA immediately inactivated after sampling

μ = total mean

I_{ijk} = effect of milking interval

C_{ijkl} = cow deviances

D_{ijk} = day deviances

ε = residual error

3.3.4 Variation study

Analysis of variance was performed using the model:

$$\text{LogFFA} = \mu + T_{ijk} + L_{ij} \times T_{ijk} + \varepsilon$$

With:

μ = total mean

T_{ijk} = effect of type of farm

L_{ij} = level of FFA (high or low)

ε = residual error

3.3.5 *Management study*

Two types of analysis were carried out on the data. Variables that could be expressed numerically, for instance milk yield, were analysed retrospectively with the following ANOVA-model:

$$Y = \mu + \text{CLASS}_i + \varepsilon$$

Y= response variable (factors studied on farm level)

μ =overall mean

CLASS = effect i of different strata (low,high) of FFA

ε = residual error

Variables that could not be expressed numerically, were analysed by changing variables to class and effects to variables.

4 Results

4.1 Effect of AM-system on FFA level

Results of the laboratory trial on brand 1 and of the trial in practice on brand 2 are given in table 4. Differences between brand 1 and conventional were significant over three test days. Day effects existed and were due to the FFA level of the milk used: FFA level of milk on day 2 was lower than on day 1 and 3. Susceptible milk on day 2 was not as susceptible as on the other days.

FFA levels before passing the equipment in test 2 were higher for the conventional system, but difference in levels between conventional and brand 2 were difficult to explain. Probably some mixing and/or sampling problems were due to this difference. Levels were equal after passing the system and increase in FFA showed significant differences. But because of the different levels before passing the system, this increase was difficult to validate. For both AM brands a higher increase in FFA levels can be concluded compared to a conventional system.

Table 4. Mean FFA level before and after passing through a conventional or an AM system

Milking system	n	mean FFA before (mmol/100 g fat)	mean FFA after (mmol/100 g fat)	increase FFA (mmol/100 g fat)
Test 1				
Conventional	12	0.43 ^a	0.50 ^a	0.07 ^a
Day 1	4	0.48	0.53	
Day 2	4	0.36	0.41	
Day 3	4	0.45	0.56	
AM brand 1	12	0.42 ^a	0.58 ^b	0.16 ^b
Day 1	4	0.47	0.58	
Day 2	4	0.36	0.42	
Day	4	0.45	0.63	
Test 2				
Conventional	8	0.58 ^a	0.65 ^a	0.07 ^a
AM brand 2	8	0.45 ^b	0.65 ^a	0.21 ^b

Different superscripts mean significant difference ($P < 0.05$), n = number of tests, 4 tests per day, figures with superscripts are least square means.

Brand 1 was also tested in a milking parlour and compared to this parlour (Table 5). Significant differences were due to differences in milking intervals: cows milked conventional had a mean milking interval of 14 hours, while cows milked with the AM system had a mean interval of 15 h 45min. Differences between stripped milk sampling and mixed milk sampling could not be concluded (see also 4.2).

Table 5. Backtransformed least square mean FFA in mixed milk and in post milk per cow milked in a conventional system and in a simulated part of AM system of brand 1.

	Mean FFA in mixed milk per cow after 24h at 5°C (mmol/100 g fat)	Mean FFA in stripped milk per cow after 24h at 5°C (mmol/100 g fat)
Conventional	0.39 ^a	0.40 ^a
Brand 1	0.33 ^b	0.33 ^b

Different superscripts per column mean significant difference ($P < 0.05$)

4.2 Sampling of milk

FFA levels of samples taken before, during and after milking are presented in table 6. Unfortunately no results of pre squirts are available, due to the shortage of fat present in this milk to perform the BDI test. Influence of way of sampling, inactivation and interaction of inactivation and group was significant. The interaction of inactivation and group was due to cow 4 who was susceptible for lipolysis in group 100-300 days in milk, resulting in higher FFA levels after 24 h at 5°C. All levels were rather low, due to rather insusceptible cows sampled. Although differences were found for FFA levels between mixed milk and stripped milk after 24 h at 5°C, these differences were not found in table 5. Therefore mixed milk samples were taken to determine FFA levels during the following experiments.

Fat globule size diameters are given in table 7. Relations between average globule size and FFA were not found. A slight relation between globule size and days in milk was indicated ($P = 0.068$). A linear relation between fat production and average globule size was also indicated ($P = 0.051$) with a percentage variance accounted for of

56,9%. Cow 4 seemed to be rather susceptible to lipolysis (see table 6.) and fat content of the milk was rather low compared to the other cows. Also total fat production was the lowest of all cows and the average globule size was also rather small. This cow had a disease history and was removed from the farm. More cows need to be sampled to indicate a possible relation between FFA and average globule size.

Table 6. Backtransformed least square mean initial FFA (mmol/100 g fat) and FFA (mmol/100 g fat) after 24 h at 5°C in different milk samples of six cows, divided into three groups.

Groups (DIM) Samples	Initial FFA				FFA after 24 h at 5°C				total mean
	<100	100-300	>300	mean	<100	100-300	>300	mean	
Pre squirts	-	-	-	-	-	-	-	-	-
Halfway milking	0.14	0.09	0.12	0.11	0.18	0.34	0.23	0.24	0.17 ^a
Stripped milking	0.19	0.11	0.13	0.14	0.14	0.39	0.21	0.22	0.18 ^a
Mixed milk	0.20	0.15	0.14	0.16	0.28	0.48	0.25	0.32	0.23 ^b
Mean	0.17	0.11	0.13	0.14 ^c	0.19 ^x	0.40 ^y	0.23 ^x	0.26 ^d	

DIM = days in milk , - = no result, a and b, c and d and x and y mean significant differences (P<0.05).

Table 7. Average globule size d_{vs} (μm) and FFA (mmol/100 g fat) of mixed milk of six cows, sampled on two days and determined in duplo.

Cow	d_{vs} (μm)	FFA (mmol/100 g fat)	days in milk	fat (g/kg)	milk yield (kg/milking)	fat production (kg/milking)
1	4.20	0.29	17	52.0	22.1	1.15
2	5.41	0.31	18	47.6	21.6	1.04
3	3.49	0.42	169	44.8	18.5	0.83
4	2.91	0.56	180	31.5	18.2	0.56
5	3.31	0.21	373	48.7	16.0	0.78
6	2.75	0.30	395	51.9	13.3	0.70

Fatty acid composition is given in table 8 for six cows, sampled once. Linear regression showed no significant relations between FFA and all fatty acids. For milk yield and lactation stage of the cows significant relations were found for C14, C16, C17, C18:1 t others and C18:3 c9c12c15. For lactation stage significant relations were found for C18:1 c9 and C6. For milk yield relations were found for C10:1 and C18. Although significant linear relations were found, results should be interpreted with care, they are based on six cows from one farm.

FFA levels of stored susceptible milk are reported in figure 2. After 24 hours storage hardly any change of FFA level can be concluded, indicating that 24 h storage is a good indication of FFA levels in stored milk.

Table 8. Fatty acid composition (mol/mol%), milk yield and FFA levels of six cows, sampled during morning milking.

Cow	1	2	3	4	5	6
Milk yield (kg/milking)	22.1	21.6	18.5	18.2	16.0	13.3
Days in milk	17	18	169	180	373	395
Fat content (g/kg)	52.0	47.6	44.8	31.5	48.7	51.9
FFA (mmol/100 g fat)	0.29	0.31	0.42	0.56	0.21	0.30
Fatty acids (w% of total FA)						
C4:0	4.92	3.87	4.16	4.38	4.94	4.08
C6:0	1.63	1.63	2.75	2.25	2.79	2.52
C8:0	0.71	0.82	1.65	1.30	1.61	1.45
C10:0	1.19	1.41	3.56	2.56	3.09	2.96
C10:1	0.05	0.10	0.39	0.36	0.32	0.38
C5/C7/C9/C11	0.18	0.12	0.39	0.27	0.22	0.29
C12:0	1.58	2.09	5.28	4.68	3.82	5.30
C12:1	0.04	0.04	0.14	0.18	0.10	0.19
C13	0.05	0.05	0.25	0.21	0.14	0.25
C14	6.27	6.98	12.93	11.42	11.86	12.54
C14:1	0.24	0.55	0.94	1.27	0.82	1.18
C15	1.03	1.04	2.22	2.12	1.90	1.97
C16	26.58	25.73	31.68	31.73	34.21	33.06
C16:1	1.91	2.60	1.44	1.86	1.89	2.53
C17	1.75	1.62	1.41	1.25	1.14	1.25
C18	15.97	12.8	29.00	7.56	9.15	6.84
C18:1 t9	0.28	0.22	0.20	0.23	0.17	0.18
C18:1 t11	1.39	1.06	1.08	1.29	0.85	0.77
C18 :1 t other	2.79	3.41	2.39	2.62	1.88	1.63
C18 :1 c total	27.77	29.49	14.95	19.03	16.22	17.25
C18 :2 c9c12	1.41	1.88	1.11	1.25	0.93	1.22
C18 :2 others	0.69	0.72	0.61	0.61	0.52	0.68
C20:0	0.12	0.10	0.12	0.08	0.12	0.06
C20:1	0.06	0.07	0.02	0.03	0.03	0.03
C18:3 c912c15	0.61	0.57	0.32	0.30	0.32	0.29
C18:2 c9t11	0.37	0.41	0.40	0.53	0.41	0.48
C21:0	0.05	0.03	0.02	0.01	0.03	0.01
C18:2 t10c12	0.03	0.04	0.03	0.07	0.05	0.05
C20 :2	0.08	0.06	0.09	0.05	0.08	0.06
C22 :0	0.02	0.04	0.10	0.07	0.08	0.10
Unkown total	0.24	0.42	0.35	0.43	0.35	0.39
Sum	100	100	100	100	100	100

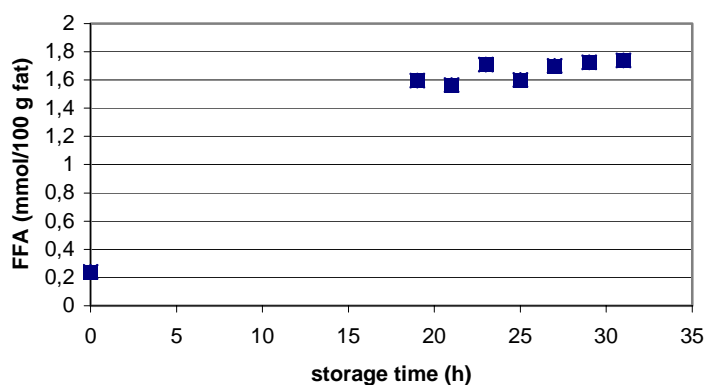


Figure 2. Influence of storage time at 4°C on FFA level of susceptible milk of one cow. Points represent mean of two samples.

4.3 Milking frequency

Analysis of variance of log transformed FFA showed (Table 9) small differences between intervals after 0 hour storage and significant differences after 24 hour storage. The increase after 24 hours storage defined as spontaneous lipolysis was significant for the different milking intervals.

Table 9. Effect of milking interval on FFA contents (meq/100 g fat) in raw milk after 0 hours and 24 hours storage after sampling.

Interval (h)	4	8	12
0 hours	0.20 ^a	0.19 ^{ab}	0.15 ^b
24 hours	1.23 ^a	0.71 ^b	0.42 ^c
Delta FFA	0.97 ^a	0.49 ^b	0.25 ^c
Milk yield (kg/milking)	6.0 ^a	11.7 ^b	16.9 ^c

^{a,b,c} statistically significant difference on the same row P<0.05)

No significant differences between the milking intervals were found for fat, protein, lactose content and cell counts.

4.4 Variation of FFA during the year

Some characteristics per farm are given in Table 10.

Results until April 2004 showed more variation for high than for low FFA levels on different farms (figure 3). Also on farms milking three times a day (3x), selection based on low levels in the previous year, was not adequate. Seasonality and/or differences in feeding regimes or other factors seemed to influence the FFA levels and the variation on these farms. For farms milking two times a day (2x) the selection was appropriate: farms with high FFA levels had high milk lines and tied stalls, where it is more difficult to achieve low FFA levels. Construction of milking equipment is important to avoid high FFA levels. One of the farms milking 3x also had a tied stall with a high milk line (farm 6). On two of the four farms milking 3x (one high and one low) FFA levels increased when cows were pastured. For automatic milking (AM) differences between high and low were not extreme, but still more variation in farms with higher levels could be concluded. However, levels for AM farms were relative lower than on the other type of farms.

Table 10. Characteristics of 12 farms with regularly sampling for FFA.

Farm	Type	Level	Cows	Milk yield (kg)	Housing system	Grazing (months)	Mastitis cases* (%/year)	BMSCC (x 1000cells/ml)	TBC (x1000/ml)
1	2x	high	44	6973	Tied stall	0	0-10	480	10
2	2x	high	50	-	Tied stall	6	0-10	±300	-
3	2x	low	187	8900	Loose	2	0-10	187	5
4	2x	low	60	8000	Loose	6	10-20	165	9
5	3x	high	55	11000	Loose	6	0-10	104	5
6	3x	high	45	8500	Tied stall	6	0-10	202	11
7	3x	low	135	9000	Loose	0	10-20	-	-
8	3x	low	38	9500	Loose	5	0-10	±200	±7
9	AM	high	100	8100	Loose	0	10-20	±300	-
10	AM	high	55	7500	Loose	0	20-30	400	9
11	AM	low	60	7000	Loose	6	0-10	250	10
12	AM	low	53	9500	Loose	0	-	194	12

- = not known, * = reported by the farmer, ± = estimated, based on what farmer mentioned

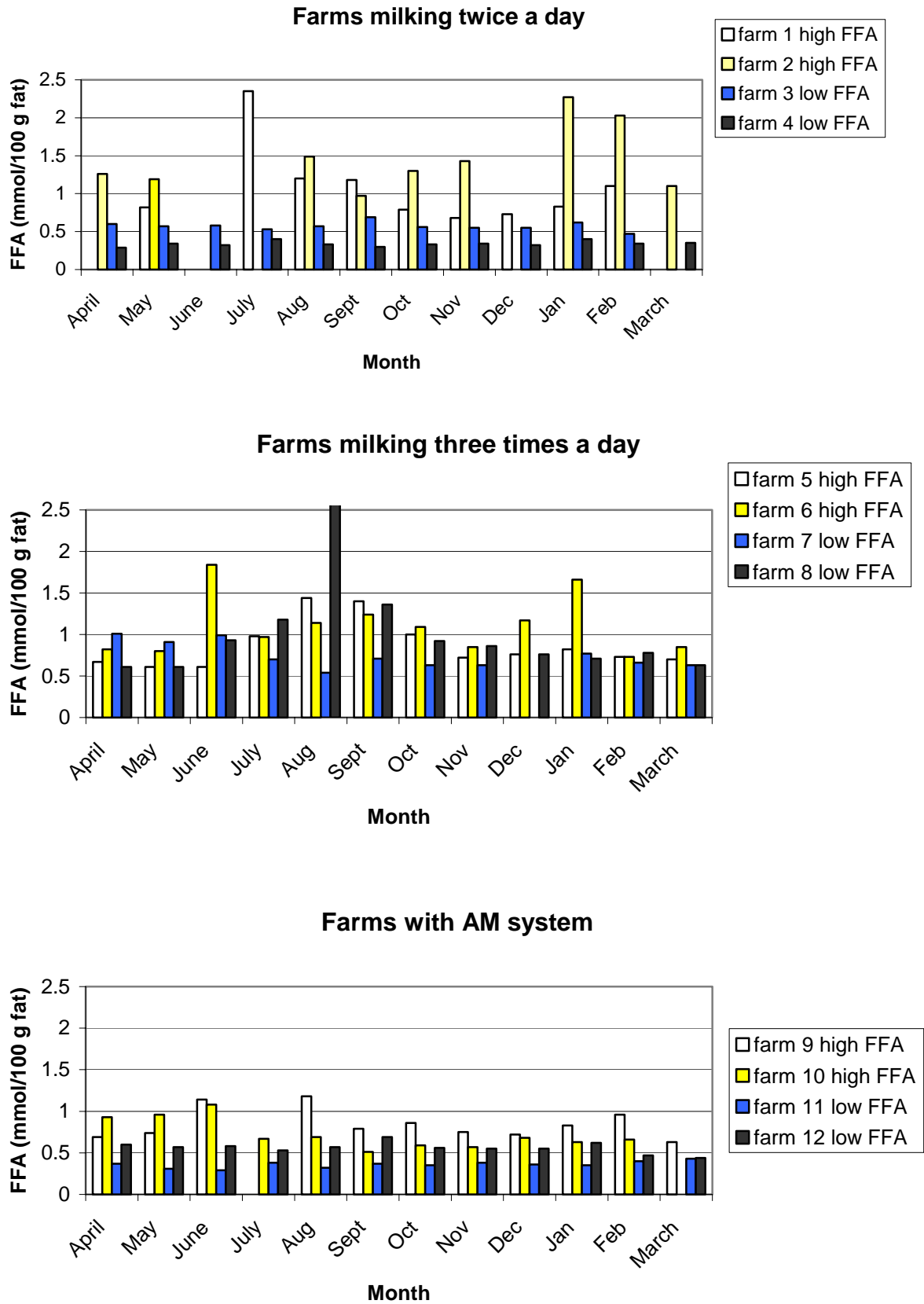


Figure 3. FFA levels on twelve farms based on monthly sampling.

4.5 Influences of management factors

Selection of farms, based on FFA levels in the previous year, in the study on farm management aspects seemed to be adequate for 3x milking per day but not for AM-systems (Table 11.).

On farms milking 3x with high FFA levels quota and number of cows on the farm were significant lower than on farms with low FFA levels. This has to be studied in more detail. No differences between high and low FFA level on farms milking 3x were found for average milk production per cow and fat percentage of the milk.

For AM farms the selection was made on the same brand and cooling systems, all quite recently installed, but FFA results for at least one year were available. After solving some starting problems most of the FFA levels decreased and although significant difference was found between AM farms with high and low FFA levels, the levels were not as high as in the previous year as the selection was based on $> 0,75$ mmol/100 g fat. On the AM farm with the highest FFA levels, the milking frequency was not adequately adjusted. Cows producing less (7 kg per milking) were milked too often.

On AM farms no differences in quota, number of cows, milk production and fat percentage of the milk were found for high and low FFA level.

Table 11. Mean FFA and standard deviation (between brackets) of 6 farms milking three times a day and 9 farms milking with the same AM system. All farms are sampled twice within six weeks in autumn (oct-nov 2003).

Farm type	n	FFA (mmol/100 g fat)				
		High FFA		Low FFA		
3x	3	0.87 ^a	(0.17)	3	0.48 ^b	(0.05)
AM	4	0.69 ^a	(0.13)	4	0.53 ^b	(0.12)

^{a,b} Different superscripts within one row means significant difference

5 Discussion

Performing tests in circulating milk through the milking components of the AM system and conventional milking systems showed differences between conventional and AM system (brand 1), although day effects were also found. This was probably due to the susceptibility of the used milk. However, interaction between susceptibility and mechanical disruption of globule membranes during the milking process can be concluded. For brand 2 compared to the conventional system, no differences in FFA levels were found after passing the equipment, but increases were significant, due to differences in FFA levels before passing the equipment. An AM system effect for brand 2 can be concluded.

Compared to conventional milking systems, AM systems differ in number of milk lines transporting the milk to the receiver part: four lines instead of one originating from the teat cluster. The four lines have a smaller diameter than the one in a conventional system, but in general airinlet in AM systems is higher than in normal conventional systems. A higher air inlet resulted in higher FFA levels (O'Brien et al., 1998 and Fleming, 1980). Milking frequency is also important when comparing conventional and AM systems (Table 5.), because differences in milking intervals exceeded the possible effect of the AM system.

Influence of milking frequency on FFA levels was clear, as expected (Table 7), although the initial FFA levels were quite low compared to other studies. But different determination methods might result in different FFA levels, because for example with the BDI-method did not detect low chain fatty acids. Some other methods determined these low chain fatty acids better (Kuzdzal-Savoie, 1980).

A small relation between globule size and days in milk was indicated ($P=0.068$). Mulder and Walstra (1974) indicated decrease of globule size diameter with increasing lactation stage. So this relation was expected, although the number of sampled cows was low. Relation between fat production and average globule size as found by Wiking et al. (2004) could also be found for our results.

Relations between FFA and fatty acid composition were not found, but results of six cows are not very representative. This is partly due to the fact that the BDI-method did not detect low chain fatty acids and to the fact that only six cows were sampled. On the other hand influences of stage of lactation and milk production on some fatty acids are worthwhile for further research (e.g. C10, C12, C15 for the two cows in early lactation were lower than for the other cows, C18:1 c total was higher for the other the two cows in early lactation). Wiking et al. (2004) also found a negative correlation between C18:2 and C18:3 and average globule size, indicating more unsaturated fat resulting in decreased the fat globule sizes. This also resulted in less damage by pumping. On the other hand, Walstra and Jenness (1984) reported a decrease in average globule size with increasing stage of lactation. Also FFA levels increased with increasing stage of lactation, so average globule size might play a role, but mechanism of lipolysis is more complex than suggested.

Variation in FFA levels on different types of farms (2x, 3x and AM) showed different patterns. On farms milking 2x high FFA levels were related to construction of the milking equipment (high level pipe line systems on tied stalls). Technical factors were important on these farms.

Apparently variation in FFA level on farms milking 3x was due to other factors than distinction between high and low (Figure 3.). Farms milking 3x had higher production levels than the other farms. Maybe these high production levels, combined with frequent milking might indicate higher demands from individual cows (e.g. energy metabolism on higher levels). Also late lactation cows are milked three times a day, resulting in extra risk for high FFA levels. But not all farms milking 3x had high FFA levels.

Differences between high and low FFA level AM farms were not as pronounced as for the other type of farms. Farm 10 adjusted the number of failed milkings in June and the FFA level decreased from that time and remained relative low. This was an example of excessive mixing of milk and air. For farm 9 such an easy explanation could not be concluded. Apparently some management factors not easily defined were due to this variable FFA levels. Vorst et al. (2002) found indications that farms with higher milk production might give lower risk for high FFA levels with AM systems.

One factor could be of importance, as the BMSCC of AM farms and farms milking 2x with high FFA levels was also relative high, although not all figures were as precise as possible. For farms milking 3x BMSCC were lower than for the other type of farms. Maybe the high production levels on these farms resulted in these BMSCC's.

Management factors were studied (Table 9.) but showed for AM farms less different FFA levels than for farms milking 3x. AM systems being installed recently are modified compared to earlier systems. Modified new cooling systems have been introduced and modification to the AM system have been applied; not only technical but also on the software part of the systems. Still some starting problems remain, regarding the levels used to select the AM farms (high and low).

6 Conclusions and recommendations

Increased FFA levels are due to increased milking frequencies (both for AM and conventional: 3x milking per day) and to milking machine components of AM systems.

Milking frequencies are of more importance than technical parameters of the AM system, because FFA levels for farms milking three times per day and AM systems are more or less comparable. However, technical aspects cannot be excluded. Compared to conventional milking, the air/milk ratio is higher, probably resulting in more disruption of milk fat globule membranes, especially in the situation with more susceptible milk.

Apart from milking frequencies and technical parameters, management aspects probably play a role. Susceptibility of cows for lipolysis is very important, but it is a complex matter. Known causes are lactation stage, pregnancy stage and milking frequency. Although feeding and animal health play a role in increased FFA levels, some more fundamental research is needed regarding the susceptibility of cows.

Recommendations for the farmer

- Location of the AM system on the farm should be defined in a way that requirements are fulfilled (e.g. proper way to enter the system).
- Preventive maintenance is important. Replacement of liners and tubes should not be delayed (see also D9). A service contract with the supplier is recommended.
- Replace leaking tubes and teat cup liners immediately (excessive mixing of milk and air)
- Adjust software settings if necessary like minimum milking frequency, attentions, but also feeding regime.
 - During increasing lactation stage milking frequency should be reduced: cows > 250 DIM and expected milk yield of less than 7 kg per milking should not be milked more than twice a day.
 - As with conventional milking, also in automatic milking excessive air intake during attachment of the teatcups should be prevented. If the number of failed milkings is increasing and/or more than 5%, the service technician of the AM-system should be contacted.
 - Act on attentions and alerts: replace the cleaning agent when attention is given. Check cows with high conductivity and take measures if necessary.
 - Adjust feeding regime as lactation stage increases. Be aware of changing of feeding regime when cows are starting grazing.
- Check the cooling tank on the occurrence of freezing of milk. This can be done by scraping over the bottom of the tank after the start of the cooling system. Scraping can be done by help of a lengthened bottle scraper.

Recommendation for the AM manufacturer/technician

- The AM system should be designed in a way that milk is transported smoothly in order to prevent disruption of fat globule membranes.
- After installation of the AM system, control of different technical and software parts is necessary. The 'plug and play' principle sometimes leads to starting problems with subsequent milk quality failures (e.g. TBC, see D17).
- Cooling tank and AM system should be placed in accordance with instructions of manufacturers. Communication between different manufacturers e.g. AM-supplier and supplier of cooling tank is important. The cooling should be designed in a proper way, to prevent freezing of milk.
- The post run time of the milk pump should be limited to prevent free fatty acids due to excessive mixing of air and milk. So called 'blind' pumping of the milk pump will result in increased FFA levels as known from conventional milking.
- The adjustment of milking frequencies for individual cows should be easy to perform.
- The service system should be adequate, as maintenance of the system is important.

References

- Abeni, F., Degano, L., Capelletti, M., 2002. Effect of robotic milking on physicochemical and renneting properties of bovine milk: preliminary report from an Italian experimental farm. Proc. 1st North American Conference on Robotic Milking, Toronto, Canada, V64-V67.
- Artmann, R., Bohlsen, E., 2000. Results from the implementation of automatic milking system (AMS)- multi-box facilities. Proc. International Symposium on Robotic Milking, Lelystad, The Netherlands, 221-231.
- Barth, K., Fischer, R., Worstorff, H., 2000. Evaluation of variation in conductivity during milking to detect sub-clinical mastitis in cows milked by robotic systems. Proc. International Symposium on Robotic Milking, Lelystad, The Netherlands, 89-96.
- Bijgaart, H.J.C.M. van den , 2003 personal communication.
- Cartier, P., Chilliard, Y., 1989. Lipase redistribution in cows' milk during induced lipolysis. I. Activation by agitation, temperature change, blood serum and heparin. *J. Dairy Res.* 56:699-709.
- Cartier, P., Chilliard, Y., 1990. Spontaneous lipolysis in bovine milk : combined effects of nine characteristics in native milk. *J. Dairy Sci.* 73:1178-1186.
- Cartier, P., Chilliard, Y., Paquet, D., 1990. Inhibiting and activating effects of skim milks and proteose-peptone fractions on spontaneous lipolysis and purified lipoprotein lipase activity in bovine milk. *J. Dairy Sci.* 73:1173-1177.
- Chazal, M.P., Chilliard, Y., 1987. Effect of breed of cow (Friesian and Montbéliarde) on spontaneous and induced lipolysis in milk. *J. Dairy Res.* 54:7-11.
- Chazal, M.P., Chilliard, Y., 1986. Effect of stage of lactation, stage of pregnancy, milk yield and herd management on seasonal variation in spontaneous lipolysis in bovine milk. *J. Dairy Res.* 53:529-538.
- Chazal, M.P., Chilliard, Y., Coulon, J.B., 1987. Effect of nature of forage on spontaneous lipolysis in milk from cows in late lactation. *J. Dairy Res.* 54:13-18.
- Chilliard, Y., Ferlay, A., Rouel, J., Lamberet, G., 2003. A review of nutritional and physiological factors affecting goat milk synthesis and lipolysis. *J. Dairy Sci.* 86:1751-1770.
- Ferlay, A., Martin, B., Pradel, P., Chilliard, Y., 2002. Effect of the nature of forages on lipolytic system in cow milk. Poster B4-38 Congrilaït 2002, 26th IDF World Dairy Congress, Paris, France.
- Fleming, M.G., 1980. Mechanical factors associated with milk lipolysis in bovine milk. In IDF bulletin 118 Flavour impairment of milk and milk products due to lipolysis. 41-52.
- Escobar, G.J., Bradley, R.L., 1990. Effect of mechanical treatment on the free fatty acid content of raw milk. *J. Dairy Sci.* 73:2054-2060.
- Hermansen, J.E., Larsen, T., Andersen, J.O., 1995. Does zinc play a role in the resistance of milk to spontaneous lipolysis. *Int. Dairy J.* 5:473-481.
- IDF, 1991. Determination of free fatty acids in milk and milk products. IDF Bulletin No. 265. Brussels, International Dairy Federation.
- Ipema, A.H., Schuiling, E., 1992. Free fatty acids; influence of milking frequency. Proc. Of Prospects for Automatic Milking, Wageningen, The Netherlands, EAAP Publ. 65, 491-496.
- Jellema, A., 1976. Susceptibility of bovine milk to lipolysis. *Neth. Milk Dairy J.* 29:145-152.
- Jellema, A., 1979. Behandeling van monsters i.v.m. vetsplitsingsonderzoek. *Zuivelzicht* 71:24.

- Jellema, A., 1986. Some factors affecting the susceptibility of raw cow milk to lipolysis. *Milchwissenschaft* 41:553-558.
- Klei, L.R., Lynch, J.M., Barbano, D.M., Oltenacu, P.A., Lednor, J., Bandler, D.K., 1997. Influence of milking three times a day on milk quality. *J. Dairy Sci.* 80: 427-
- Klungel, G.H., Slaghuis, B.A., Hogeveen, H., 2000. The effect of the introduction of automatic milking systems on milk quality. *J. Dairy Sci.* 83:1998-2003.
- Krukovsky, V.N., Herrington, B.L., 1939. Studies of lipase action. II The activation of milk lipase by temperature changes. *J. Dairy Sci* 22: 137-147.
- Kuzdzal-Savoie, S., 1980. Determination of free fatty acids in milk and milk products. In: Flavour impairment of milk and milk products due to lipolysis. *Bulletin International Dairy Federation Document* 118. p. 53-66.
- Land, A. van 't, Lenteren, A.C. van, Schooten, E. van, Bouwmans, C., Gravesteyn, D.J., Hink, P., 2000. Effects of husbandry systems on the efficiency and optimisation of robotic milking performance and management. *International Symposium on Robotic Milking, Lelystad, The Netherlands*, 167-176.
- Mulder, H., Walstra, P., 1974. *The Milk fat globule. Emulsion science as applied to milk products and comparable foods.* Commonwealth Agricultural bureaux, Farnham Royal, Bucks., England. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- O'Brien, B., O'Callaghan, E., Dillon, P., 1998. Effect of various milking machine systems and components on free fatty acid levels in milk. *J. Dairy Res.* 65:335-339.
- Rasmussen, M.D., Bjerring, M., Justesen, P., Jepsen, L., 2002. Milk quality on Danish farms with automatic milking systems. *J. Dairy Sci.* 85:2869-2878.
- Svennersten-Sjauna, K., Persson, S., Wiktorsson, H., 2002. The effect of milking interval on milk yield, milk composition and raw milk quality. *Proc. 1st North American Conference on Robotic Milking, Toronto, Canada*, V43 –V48.
- Vorst, Y., Koning, K. de, 2002. Automatic milking systems and milk quality in three European countries. *Proc. 1st North American Conference on Robotic Milking, Toronto, Canada*, V1-V12.
- Walstra, P., Geurts, T.J., Noomen, A., Jellema, A., Boekel, M.A.J.S. van, 1999. In : *Dairy Technology, Principles of Milk Properties and Processes.* Marcel Dekker, Inc. New York Basel. P. 162.
- Walstra, P. and Jenness, R., 1984, *Dairy Chemistry and Physics.* John Wiley & Sons, New York, Chichester, Brisbane, Toronto, Singapore. P 254-278.
- Wiking, L., Nielsen, J.H., 2003. Can strategies for cow feeding and cooling of milk in automatic milking systems improve milk quality? *Ital. J. Anim. Sci.* 2:316.
- Wiking, L., Björck, L. and Nielsen, J.H., 2004. Impact of size distribution of milk fat globules on milk quality affected by pumping. In: *Automatic Milking- a better understanding*, eds A. Meijering, H. Hogeveen, C.J.A.M. de Koning, Wageningen Academic Publishers. p. 348-356.

Acknowledgements

The milking personnel of the experimental farm Waiboerhoeve is gratefully acknowledged for performing the experiments with dairy cows. We want to express our thanks to the farmers who participated in this study.

NIZO Food Research Ltd. is acknowledged for performing the analysis on fatty acid composition and fat globule diameter dispersion. The Milk Control Station in Zupthen is thanked for performing analyses on FFA.

Abbreviations

2x	farms milking two times a day conventionally
3x	farms milking three times a day conventionally
AM	Automatic Milking
DIM	Days in milk
FFA	Free Fatty Acids
h	hour(s)
n.a.	not available
n.d.	not determined
se	Standard error