Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap

Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science

The importance of feeding during milking and take off level for milking efficiency and milk production

Larissa Stadtmüller

Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, **502**

Uppsala 2014

Degree project / Swedish University of Agricultural Sciences,
Department of Animal Nutrition and Management, **502**

Examensarbete, 30 hp
Masterarbete
Husdjursvetenskap
Degree project, 30 hp
Master Thesis
Animal Science

Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management

The importance of feeding during milking and take off level for milking efficiency and milk production

Larissa Stadtmüller

Handledare:

Supervisor: Prof. Dr. Thomas Jungbluth, University of Hohenheim, Livestock System Engineering

Prof. Dr. Kerstin Svennersten-Sjaunja, SLU, Department of Animal Nutrition and Management

Examinator:

Examiner: Prof. Dr. Kjell Holtenius, Department of Animal Nutrition and Management

Omfattning:

Extent: 30 hp

Kurstitel:

Course title: Degree Project in Animal Science

Kurskod:

Course code: EX0551

Nivå:

Level: Advanced A2E

Utgivningsort:

Place of publication: Uppsala

Utgivningsår:

Year of publication: 2014

Serienamn, delnr: Examensarbete / Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, 502

Series name, part No:

On-line publicering: http://epsilon.slu.se

On-line published:

Nyckelord:

Key words: Feeding during milking, Take off level, milk flow, milking time, udder emptying

THE IMPORTANCE OF FEEDING DURING MILKING AND TAKE OFF LEVEL FOR MILKING EFFICIENCY AND MILK PRODUCTION

A Thesis

Submitted to the University of Hohenheim

And the Swedish University of Agricultural Sciences in

Partial fulfillment of the requirements

For the degree of

Master of Science

In

Animal Science

By

Larissa Stadtmüller

Matriculation Number: 476841

Swedish University of Agricultural Sciences

August 2014

Supervisor	Prof. Dr. Thomas Jungbluth (Hohenheim)
Co–Supervisor	Prof. Dr. Kerstin Svennersten–Sjaunja (SLU)
Examiner	Prof. Dr. Kjell Holtenius (SLU)
Credits	30 ECTS
Level	Advanced E / Master level
Course Title	Degree project in Animal Sciences E30
Course Code	EX0551/0551
Programme/education	Animal Sciences
Place of publication	Uppsala
Year of publication	2014

Acknowledgements

I would like to take this opportunity to express my profound gratitude to Kerstin, my Swedish supervisor, who made it possible for me to write my Master's Thesis abroad in the first place. She welcomed me very warmly in Sweden, which made it easy for me to immerse myself in the project without any problems. She always had the time for a nice chat and, more importantly, to discuss all my questions about the project together. I am particularly grateful for the assistance given by my German supervisor Prof. Jungbluth for his support and for managing all the regulations concerning my thesis at the University of Hohenheim.

Much appreciation goes to Sabine for her exemplary guidance through the whole project and who was never tired of explaining me different procedures two or three times. She helped me with answering every question I had during my time in Uppsala.

I would like to offer my special thanks to all the people in the HUV-department for their very warm and friendly welcome and for helping me to improve my Swedish (listening) skills.

I would like to express my very great appreciation to the whole barn staff in Lövsta for their excellent work. Without their help it would not have been possible to perform the experiment. Furthermore, thanks to the De Laval Team, especially to Sofia, who fixed all the problems we had in the first weeks with the settings of the AMS.

Moreover, I would like to thank all the people who analyzed our samples, especially Lucia from the Food Science Department, who even helped me during her maternity leave and explained all the analysis procedures to me.

My special thanks are extended to the Swedish Farmers Foundation for their financial support of the project. I wish to acknowledge the help provided by Prof. Grimm in the beginning of the project with all his ideas and valuable suggestions.

I am also very grateful for my friend Franziska, my personal facebook, who was in a large part responsible for a magnificent and unforgettable semester abroad. A special thanks to my boyfriend Benedikt, who has always been there for me and has always helped with anything I needed.

I would also like to give my sincere gratitude to my whole family for their support, especially my parents as it would not have been possible to realize my semester abroad without their support. Lastly, I thank all the people who made an allowance in one form or another to the implementation of this project.

The study was financed by the Swedish Farmers Association for Agricultural Research.

List of Contents

List of Tables

List of Abbreviations

AMS	Automatic milking system
DIM	Days in milk
FAC	Fatty acid composition
FFA	Free fatty acids
FGS	Fat globule stability
GC	Gas chromatography
LCFA	Long chain fatty acids
MCFA	Middle chain fatty acids
MFG	Milk fat globule
MFGM	Milk fat globule membrane
MFGS	Milk fat globule size
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SCC	Somatic cell count
SCFA	Short chain fatty acids
VMS™	Voluntary Milking System

Abstract

Milking efficiency is of importance in the modern dairy production with increasing herd sizes and growing automation, such as automatic milking systems. Since the first implementation of AMS previous studies tested different take off level settings for improving milking efficiency. However, more knowledge about optimal settings for completion of milking is still relevant. The objective of this study was to determine how the combination of feeding during milking and cluster take off level shall be practiced for efficient milking in sustainable dairy production. This was tested by examining two different take off level settings combined with improved pre-stimulation by the use of feeding concentrate during milking or no feeding during milking over a four-week experiment. A 4x4 Latin square experimental design was used with four different treatments and four periods. Each period had a duration of seven days. 32 mixed-age Swedish red cows were divided into four groups balanced for milk yield, lactation number and lactation stage. Each cow went through all of the following treatments at whole udder level: cluster take off at a milk flow at 800 g/min and feeding concentrate during milking (800/f), cluster take off at 800 g/min and no feeding during milking (800/nf), cluster take off at 200 g/min and feeding during milking (200/f) and cluster take off at 200 g/min and no feeding during milking (200/nf). Measurements included individual cow milk yield, milking duration, average peak and mean milk flow and milking interval. At the end of each treatment period milk samples were collected and analysed on milk composition, SCC, Na, K, FFA content, MFG size, MFG stability and FAC. For determination of udder emptying residual milk was collected on the last day of each period and analysed on the same parameter as regular milk. The determination of residual milk took place with an intramuscular oxytocin injection after the regular milking. The combination of feeding during milking and take off level affected only the milking interval, where cows had the longest milking interval in the 200/nf treatment. Cows that received the feeding during milking treatment had a significantly higher daily milk yield a lower milking interval and a higher fat yield/milking. FFA content and MFG stability were decreased with the feeding concentrate during milking treatment. Residual milk yield increased with higher take off level. Treatments with higher take off level showed as well a milking time reduction of 42 seconds per cow. A higher take off level setting combined with feeding during milking had no negative effects on milk yield, milk quality and udder emptying. Due to a reduced milking time and elevated milk yield of that treatment it has to be considered as possibility to improve the milking efficiency.

1 Introduction

1.1 Background

In the last 20 years the implementation of automatic milking systems (AMS) has increased continuously in countries where the dairy production is characterised by high labour costs, high-yielding cows and low milk prices. The first AMS was installed 1992 in the Netherlands. Since then automatic milking has been accepted worldwide and the number of established AMS have increased from 250 units worldwide in 1998 to over 8000 units in the end of 2009 (DE KONING, 2010). Nowadays AMS is an established management system and has become a very important system in the dairy production due to decreased milk prices and increased input costs (SVENNERSTEN-SJAUNJA & PETTERSON, 2008). Ninety percent of the 8000 AM farms are found in north-western Europe, including Scandinavia.

The trend of increasing automation in dairy production is caused by the aims of a modern and sustainable milk production with high milking efficiency, high milk yield and persistent lactation. Installing an AMS makes it possible to achieve these aims and to reduce labor, increase the labor flexibility, and hence increasing the social life for the farmer. In addition it is possible to increase the milk yield with AMS through more frequent milking compared with conventional milking. Through reduced labor and increased milk yield a decrease in the fixed costs per kg milk is possible (DE KONING & RODENBURG, 2004). Other benefits with AMS are that the cows have better possibilities to perform natural behavior and thereby the welfare might increase, as an example to some extent the cows are able to choose when they will be milked. Furthermore the milking routine is less stressful due to a more consistent and predictable milking process (SVENNERSTEN-SJAUNJA & PETTERSON, 2008). Thus the cow can voluntary decide daily rhythms and activities.

Despite the mentioned benefits with AMS there also exist some disadvantages regarding the milk quality (JACOBS & SIEGFORD, 2012). Previous studies have reported a higher content of free fatty acids (FFA) in the milk and an alteration in udder health (KLUNGEL, SLAGHUIS, & HOGEVEEN, 2000; DE KONING, SLAGHUIS, & VAN DER VORST, 2003). The effect on udder health has frequently been discussed in previous studies related to the implementation of AMS. Some studies which compared udder health in AMS to conventional milking systems reported no differences in milk somatic cell count (SCC) with AMS. Instead a better teat status and reduced bacterial transfer from one teat to another due to quarter based milking in comparison with udder based milking in a milking parlour was observed (BERGLUND, PETTERSSON, & SVENNERSTEN-SJAUNJA, 2002). Nevertheless, according to HOVINEN & PYÖRÄLÄ (2011) the udder health has deteriorated after the introduction of AMS, especially the individual cow SCC and the rate of new high - SCC cows were higher in their study.

However, AMS is not just a milking tool but it is a complete management system which needs a lot of attention and knowledge by the farmer. Although the AMS is established all over the world nowadays, there is still more development to be done like the right settings for

the removal of the milking cluster at the end of the milking (take off level) and the effect of feeding or not during milking.

1.2 Literature review

1.2.1 Milking efficiency

Most of the labour resources (33-57 %) on a dairy farm are needed for the milking process. Therefore it is important to have a milk harvesting process as efficient as possible (EDWARDS, JAGO, & LOPEZ-VILLALOBOS, 2013). Milking efficiency is defined as the amount of milk (litre) harvested per hour or 24 hours and depends on many factors such as individual cow production and milking duration, which among others is affected by the take off level of the milking cluster and pre-stimulation efficiency.

An improved milking efficiency can be achieved by decreasing the individual cow milking time which influences the herd milking duration. Recent studies mention that it is possible to reduce the milking time by increasing the settings of the cluster take off level without affecting milk yield or udder health. Table 1 presents the results of three studies where different take off levels were tested. All studies reported a reduction in the milking time with higher automatic cluster remover settings.

Table 1. Overview of previous studies which tested different take off levels (g/min) and the effect on milking time (time reduction).

Take off level (g/min)	Time reduction	Reference
480 / 600 / 800	11.1 % between 480 and 800	MAGLIARO & KENSINGER (2005)
200 / 400 / 600 / 800	18 – 26 % from 200 to 800	EDWARDS, JAGO & LOPEZ-VILLALOBOS (2013)
500 – 640 / 730 – 820	Less 10.2 to 15.6 s per cow	STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER (2002)

EDWARDS, JAGO, & LOPEZ-VILLALOBOS (2013) and STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER (2002) investigated the impact of higher take off level on milk flow. Both studies indicated a higher average milk flow rate with increasing take off level.

Detailed information about milking efficiency and milk flow are dealt with in a separate Bachelor thesis written by Carlos Prieto Jimenez and are not focused in the present thesis.

1.2.2 Pre-stimulation and feeding during milking

To obtain optimal milk ejection pre-stimulation of the udder is needed. This can take place in different ways as for instance manual and automatic pre-stimulation through premilking and udder cleaning or as feeding during milking. In the present study the focus is on pre-stimulation by feeding concentrate during milking.

The pre-stimulation of the teats causes the activation of a neuroendocrine reflex which leads to a release of the hormones oxytocin and prolactin from the pituitary gland (SAGI, GOREWIT, MERRILL, & WILSON, 1980; MAYER, SCHAMS, WORSTORFF, & PROKOPP, 1984; UVNÄS-MOBERG, JOHANSSON, LUPOLI, & SVENNERSTEN-SJAUNJA, 2001). Additionally to the milking based oxytocin release previous studies have showed that feeding during milking also stimulates the oxytocin release (SAMUELSSON, WAHLBERG, & SVENNERSTEN, 1993; SVENNERSTEN, GOREWIT, SJAUNJA, & UVNÄS-MOBERG, 1995). It is not yet investigated why this effect occurs. However, it is presumed that impulses from the gastrointestinal tract effect the milking – related oxytocin secretion (VERBALIS, MC CANN, MC HALE, & STRICKER, 1986; SVENNERSTEN, GOREWIT, SJAUNJA, & UVNÄS-MOBERG, 1995).

In AMS feeding concentrate during milking is an important part mainly to motivate the cows to come to the milking unit (PRESCOTT, MOTTRAM, & WEBSTER, 1998). Furthermore, it influences the milking efficiency and the milk yield due to a better stimulation. Both milk flow and udder emptying are better with additional stimulation performed by feeding during milking; in addition, the milk yield increases (SAMUELSSON, WAHLBERG, & SVENNERSTEN, 1993; SVENNERSTEN, GOREWIT, SJAUNJA, & UVNÄS-MOBERG, 1995).

1.2.3 Take off level

The first study about automatic milking cluster removal was published by ARMSTRONG, BICKERT, GERISH, & SPIKE (1970). They tested the possibility of using milk flow measurements as a signal for the end of milking. Since then it has been an established method in modern milking systems. As soon as the milk flow drops below a predetermined level the cluster is detached. The typical default settings for cluster take off level in most of the AMS are historically based on results since that time, with take off levels at a milk flow rate around 200 g/min (SAGI, 1978). But they are addicted to the recommendations in the country and the used trademark. The Swedish take off level settings, for example, are set at a milk flow around 210 g/min at each quarter whereas Denmark and the United states detach the cluster at a quarter-based milk flow level between 300 g/min and 400 g/min (DeLaval, personal communication).

However, a lot of studies thereafter have tested the implementation of automatic cluster remover in milking parlours regarding higher cluster take off level and the impact on milk yield, milking duration, strip yield, udder health and udder emptying. RASMUSSEN (1993) compared a take off level at 200 g/min with a take off level at 400 g/min. The author discovered that a higher take off level had no negative effect on milk yield and milk

composition. Instead he detected reduced milking time, improved teat condition and reduced change in teat-end thickness. Related results were detected by STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER (2002). Take off level between 500 and 640 g/min and between 730 and 820 g/min in five dairy herds were tested. In four herds no effect on milk yield was reported, but a reduction in milking time and an increase in average milk flow were described. In one herd a trend for higher milk production with higher take off level was observed; nevertheless, it could not be proved whether the increase in milk production was directly correlated with the higher take off level. On the contrary MAGLIARO & KENSINGER (2005) found a decreasing milk yield with increasing take off level. They tested three take off level settings at a milk flow at 480 g/min, 600 g/min and 800 g/min.

The effect of higher take off level on udder emptying was not investigated extensively in the previous mentioned studies. However, EDWARDS, JAGO, & LOPEZ-VILLALOBOS (2013) measured strip yield in their study and reported an increase in strip yield from 300 g when they compared a take off level at 200 g/min with a take off level at 800 g/min.

Udder health in combination with higher take off level was also partly tested in some of the previous mentioned studies. EDWARDS, JAGO, & LOPEZ-VILLALOBOS (2013) and RASMUSSEN (1993) found no effect on udder health with higher take off level settings. Only JAGO, BURKE, & WILLIAMSON (2010) examined a higher somatic cell count (SCC) with higher take off level settings whereas the teat condition and the incidence of clinical mastitis were not affected by the higher levels of cluster remover settings. The length of the previous mentioned experiments varied from 11 (EDWARDS, JAGO, & LOPEZ-VILLALOBOS, 2013) to 35 weeks (JAGO, BURKE, & WILLIAMSON, 2010). Conducting the experiments for a different amount of time may have also influenced the udder health.

1.2.4 Fat quality

Milk quality, especially fat quality and fat content, is a very important part of the dairy production worldwide concerning the payment of the milk to the farmers, the dairy factories' further processing of the milk, e.g. cheese–making properties, and the effects on human health (ABENI, DEGANO, GIANGIACOMO, SPERONI, & PIRLO, 2003; PALMQUIST, STELWAGEN, & ROBINSON, 2006). Several studies in the last few years have reported that the adoption of AMS may influence the milk quality negatively in different ways. The most negatively effect on milk harvested in AMS is an increase in the FFA content which can cause rancid flavours in dairy products (ABENI, DEGANO, CALZA, GIANGIACOMO, & PIRLO, 2005; DE KONING, SLAGHUIS, & VAN DER VORST, 2003). The assemblage of FFA in raw milk depends on breed, feed, milking routines and techniques and arises by the hydrolysis of the fat lipids (WALSTRA, WOUTERS, & GEURTS, 2006).

The largest differences between traditional milking in a milking parlour and milking in an AMS are the increase in milking frequencies and altered milking intervals. In a traditional twice-daily milking system the cows have the same milking intervals all the time. Usually, they have a shorter milking interval from the morning to the evening milking followed by a longer interval from the evening to the morning milking. In AMS the cows are able to choose

their milking times by themselves which makes it possible to have more than two milkings per cow per day. The higher milking frequencies influence the milk composition and quality reflected mainly in a higher FFA content, a higher freezing point, higher milk yield, less natural creaming of the milk, lower fat content and greater milk fat globules (KLUNGEL, SLAGHUIS, & HOGEVEEN, 2000). Using goats as test animals, BOUTINAUD, ROUSSEAU, KEISLER, & JAMMES (2003) demonstrated that milking frequencies had an effect on epithelial cell number, alveolar diameter and thus weight of the mammary gland. With a higher milking frequency the proliferation of epithelial cells, the alveolar diameter and the weight of the mammary gland increased. In addition the dispersion of the milk in the udder is directly correlated to the milking frequency. With shorter intervals between the milkings the percentage of milk from the alveoli increased compared to cistern milk. Moreover the alveolar milk has a higher milk fat content as opposed to cisternal milk (ABENI, DEGANO, GIANGIACOMO, SPERONI, & PIRLO, 2003).

Hence the fat secretion rate, especially the secretion of FFA, rises with higher milking frequencies due to an increase in the activity of two fatty acid synthesizing enzymes (acetyl-coenzyme A carboxylase and fatty acid synthetase) according to ABENI, DEGANO, CALZA, GIANGIACOMO, & PIRLO (2005). They suggested an increase mainly in the de novo sythesis of short chain fatty acids (SCFA) while other studies (WIKING, NIELSEN, BÅVIUS, EDVARDSSON, & SVENNERSTEN-SJAUNJA, 2006; SVENNERSTEN-SJAUNJA, PERSSON, & WIKTORSSON, 2002) reported no effect on the de novo synthesis of milk fat with higher milking frequencies. WIKING, NIELSEN, BÅVIUS, EDVARDSSON, & SVENNERSTEN-SJAUNJA (2006) reported a decrease of polyunsaturated fatty acids in milk from cows with higher milking frequencies.

The de novo synthesis of SCFA occurs in the mammary gland in the udder from circulating acids (ß-hydroxybutyrate, acetate) from the rumen while long chain fatty acids (LCFA) were synthesized from blood triglycerides. The different acids are attached at a certain place on the triglycerides. This place is for the SCFA predominantly the position sn-3. Likewise the enzyme lipase prefers position sn-3 and the additional position sn-1 to start hydrolysis of the triglycerides. Hence, if the de novo synthesis is influenced by milking frequency, the content of SCFA might increase with higher milking frequencies as well as the content of FFA due to enhanced lipolysis (WALSTRA, WOUTERS, & GEURTS, 2006; WIKING, 2005; ABENI, DEGANO, CALZA, GIANGIACOMO, & PIRLO, 2005; WIKING, NIELSEN, BÅVIUS, EDVARDSSON, & SVENNERSTEN-SJAUNJA, 2006).

Generally, the FFA content in the milk can be used as a measure of damage of the fat globules. The main reason for the increment of the FFA content is an increase in lipolysis, particularly enzymatic hydrolysis of the milk fat catalyzed by mainly lipoprotein lipase.

The typical composition of fatty acids (FAC) in milk fat is shown in Table 2. LCFA dominate, largely palmitic acid (C16:0), oleic acid (C18:1) and stearic acid (C18:0). However, the composition of FAC in milk depends on feed, breed and stage of lactation (WALSTRA, WOUTERS, & GEURTS, 2006).

Table 2. Distribution of the major fatty acids in bovine milk fat. Assumed from WIKING (2005), adapted from JENSEN, FERRIS, & LAMMI-KEEFE (1991).

Fatty acid	Average range (wt%)
C 4:0	2 – 5
C 6:0	1 – 5
C 8:0	1 – 3
C 10:0	2 – 4
C 12:0	2 – 5
C 14:0	8 – 14
C 15:0	1 – 2
C 16:0	22 – 35
C 16:1	1 – 3
C 17:0	0.5 – 1.5
C 18:0	9 – 14
C 18:1	20 – 30
C 18:2	1 – 3
C 18:3	0.5 – 2

Most of the fat lipids are presented as triglycerides in milk fat globules. The triglycerides are synthesized as globules in the lumen of the endoplasmatic reticulum in the epithelial cells of the mammary gland. The milk fat globule (MFG) is afterwards released into the cytoplasma covered with a surface of phospholipids. While the globules are moving in the direction of the apical cytoplasma they are fusing so that an enlargement occures. The globules are released into the alveolar lumen through the apical plasma membrane and get enveloped by an outer bilayer membrane (MATHER & KEENAN, 1998; MESILATI-STAHY & ARGOV-ARGAMAN, 2014). This membrane, termed milk fat globule membrane (MFGM), protects the MFG against lipolysis.

The size of the globules (diameter) ranges from 0.1–10 µm according to breed and lactation stage. If the membrane globule stability declines it can be ripped and fatty acids can be seperated from the triglycerides (DE KONING, SLAGHUIS, & VAN DER VORST, 2003). According to WIKING (2005) the fat globule size enlarges with higher milking frequencies, whereas the FFA content elevates with larger globules being more vulnerable to lipolysis. In addition, WIKING (2005) observed a coherence between the average size of milk fat globules and the daily fat yield. The milk fat globules grew larger with raising fat synthesis due to a lower production of MFGM. The stability of the milk fat globule membrane (FGS) is the main protection against lipolysis and is mainly effected by the fat content of the milk and the milk fat globule size.

There are two different ways of lipolysis: spontaneous lipolysis effected by milking frequency, udder health and stage of lactation and induced lipolysis effected by mechanical treatments of the milk e.g. homogenisation, pumping and temperature fluctuations (WALSTRA, WOUTERS, & GEURTS, 2006). AMS differ in the transport and storage systems of the milk compared to traditional milking systems and overall the milk is being exposed to harsher mechanical treatments with AMS.

In modern milking systems there are longer distances from the milking unit to the bulk tank and greater temperature fluctuations in the bulk tank. As WIKING, BJÖRCK, & NIELSEN (2003) have noted the presence of air also raises the lipolyses by lowering the stability of the milk fat globule membrane. In milking systems air is used as a transport medium; it is mixed with milk and is pumped together through the milk pipeline. The damage of the milk fat globule membrane is caused by contact between the air bubble and the milk fat globule (WIKING, 2005). There has not been a recent study which investigates different take off levels in AMS and the influence on FFA content. The earlier cluster removal at a higher take off level might effect the FFA content due to the fat content being higher at the end of milking and the fat globules being bigger. Hence, there might be an effect on FFA content if the cluster is detached before the big globules are harvested.

1.3 Hypothesis and research aim

It can be hypothesized that milking efficiency can be improved when the cows receive concentrate during milking as well as the cluster take off occurs at a higher milk flow rate. To test this hypothesis two different take off levels - at a milk flow at 200 g/min and 800 g/min - at whole udder level were combined with feeding during milking as an extra stimulation for milk let down or no feeding during milking. As it was noted before, feeding during milking enhances the oxytocin release and further improves the udder evacuation. As a result milk production is increased. Considering that the improved pre-stimulation could affect the udder emptying it should be possible to test higher take off levels without having negative effects on udder emptying.

Most of the previous mentioned studies focused on milk yield, milk flow, milking time, milk composition and SCC in combination with higher take off level and concentrated lesser on measuring udder emptying. As a consequence the present study measures udder emptying by measuring residual milk yield. Due to the major importance of fat quality in milk from AMS and to get a better understanding of the relevance of the different milking routines the effects on milk composition, especially milk fat and milk fat quality was investigated.

Despite the fact that the number of implemented automatic milking systems is continuously raising worldwide the knowledge of optimal use can be improved; additional research is needed to establish the best possible settings concerning the improvement of milking efficiency and the optimization of milk production without endangering the animals' health.

The main purpose of this study was to investigate how improved pre-stimulation by feeding concentrate during milking in combination with cluster take off in modern milking systems shall be practiced for a milking process as efficiently as possible in a sustainable production with special focus on fat quality parameters.

2 Materials and Methods

2.1 Animals and feeding

The study was performed at the Swedish Livestock Research Centre in Lövsta, Swedish University of Agricultural Sciences, Uppsala, from the 13th March until the 23rd April and included 32 lactating cows of the Swedish red breed. Cows were in lactation 1 to 4 and averaged 145 ± 51 d (mean ±SD) in milk at the onset of the study as presented in Table 3.

Table 3. Status of all cows before the start of the study (n = 34)[1], status of cows which were selected for analysis on fat parameters (n = 24) and cows which were selected for determine residual milk (n = 16).

	Mean	SD	Min – Max
All cows		7.46	15.80 - 47.37
Milk yield (kg/cow)	30.03		
Lactation stage (d)	146	51	77 – 226
Lactation number	1.82	0.94	1 – 4
Cows selected for fat analysis			
Milk yield (kg/cow)	32.05	6.49	24.05 - 47.37
Lactation stage (d)	143	50.47	77 – 225
Lactation number	1.88	1.03	1 – 4
Cows selected for residual milk determination			
Milk yield (kg/cow)	32.36	7.48	24.05 – 47.37
Lactation stage (d)	135	51.52	77 – 212
Lactation number	1.94	1.12	1 – 4

[1]with the data of 2 cows which were excluded after the first period.

The cows were housed in a loose housing system with concrete floor, 62 cubicles and were milked in an AMS (Voluntary Milking System™, DeLaval, Tumba, Sweden). Water and grass silage (basic ration) were supplied *ad libitum* during the study. The feeding troughs were refilled several times a day depending on the amount the cows ate daily. Concentrate was offered individually in three automatic feeding stations in the laying area according to the milk yield. The ratio between roughage and concentrate depended on the milk yield of each cow. The composition of the feeds used in the study is shown in
Table **4**.

The cows had free access to the feeding area at all times but on their way back to the lying area they had to pass a selection gate. If they had the permission to get milked the gate to the waiting area in front of the AMS opened, if they had no milking permission the cow could go to the lying area (DeLaval FeedFirst™).

For the selection of the cows for the experiment udder health status (the criteria was a somatic cell count below 100,000 cells/ml), milk yield and lactation stage were considered. A 4x4 Latin square experimental design was used, thus four groups with eight cows each were built. To get representative results every group contained four multiparous and four primiparous cows with similar average milk yield and similar lactation stage.

After the first period two cows had to be replaced. One cow was replaced because of many incomplete milkings in the first period. At this juncture the teat position caused failure of the teat cup attachment. Thus she was very often milked just on three teats instead on four teats. As a consequence there were not all four teats included in the removal which is not comparable with the results of the other cows nor is it representative for the aim of the study. The second cow that was replaced was exposed to a treatment without feeding during milking in the first period; hence she blocked the AMS at all times and therefore disturbed the whole cow traffic.

Table 4. Average values (± SD) of measurements of the feeds used in the study.

Measure	Concentrate[1]		Grass silage
	(Solid 120)	(Unik 82)	
Metabolizable Energy, MJ/kg of DM	13.20	14	10.80 ± 0.12
CP, g/kg of DM	194 ± 2.75	322 ± 6.40	129 ± 1.41
DM, % of DM	88 ± 0.38	88 ± 1.40	31.78 ± 0.72
pH	-	-	3.86 ± 0.02

[1]the cows got a mixture of two different concentrates, the ratio between solid 120 and unik 82 was 50:50.

2.2 Treatments

As previously mentioned the design of the trial was a 4x4 Latin square design, accordingly four periods and four different treatments were chosen. Table 5 indicates the four different treatments which were used for the experiment.

Table 5. List of treatments with the different automatic cluster take off levels (milk flow g/min at whole udder level) and the stimulation treatment for milk let down (feeding during milking yes/no).

	Treatment	
Treatment name	Cluster take off level (g/min)	Stimulation for milk let down
800/f	800	Feeding
800/nf	800	no feeding
200/f	200	Feeding
200/nf	200	no feeding

Two different cluster take off levels were investigated at a milk flow rate of 200 g/min and 800 g/min both at whole udder level. These take off levels were combined with improved stimulation for milk let down (feeding during milking) and no feeding during milking. The cows which obtained treatment 800/f and 200/f (feeding during milking) received maximum 2 kg of concentrate in the AMS per day.

Table 6 illustrates the Latin square with the four periods and the corresponding treatments for each cow group for the whole experiment. Each period consisted of seven days and every cow was exposed to all treatments. To counteract carry over effects the design was arranged in a way where the treatments did not follow each other in a row.

Table 6. Treatment plan with the four periods, the four cow groups and the corresponding treatments.

	Period			
Group	1	2	3	4
1	200/f	800/nf	800/f	200/nf
2	800/nf	200/nf	200/f	800/f
3	800/f	200/f	200/nf	800/nf
4	200/nf	800/f	800/nf	200/f

The last period had to be stopped after three days because of a diarrhea outbreak in the whole dairy barn. The diarrhea was caused by a corona-virus infection of the cows. During this virus infection the cows received additional hay and straw *ad libitum* to their basic ration.

The third period was onset one more time after a week after the diarrhea outbreak to have the same requirements in the last period. However, milk samples were not taken during the repetition of period three, thus the last period was performed two weeks after the virus infection.

The settings for the new treatments for the next period were always started in the afternoon on the last day of each period after the milk sampling.

2.3 Milking

All cows were milked in an AMS (Voluntary Milking System™, DeLaval, Tumba, Sweden) with a milking interval from 9.1 ± 1.6 h (mean ± SD) and a daily milking frequency of around 2.6 milkings/cow. Accordingly the cows got their milking permission after seven hours. In the last period the cows received their milking permission already after six hours due to an accidently defective setting in the AMS program (Delpro™, DeLaval, Tumba, Sweden). To keep these intervals the cows with a milking interval above 8h that have not voluntarily entered the AMS were fetched to milking during the day (from 0600 until 2000h).

Whereas the treatments were tested at whole udder level the teat cups were automatically removed all together at a milk flow rate of 200 g/min and 800 g/min respectively. The vacuum level was 44 kPa, the pulsation rate was 60 cycles per minute, the pulsation ratio was 65:35. The liners used were 927 259-01 (DeLaval, Tumba, Sweden) in the AMS. For the determination of the residual milk a Harmony™ milking cluster (DeLaval, Tumba, Sweden) with 964 420-80 liners was used. The automatical cleaning of the AMS amounted in total 1.5 hours distributed over the whole day.

To determine the residual milk yield the cows received an oxytocin injection (2.5 ml Partoxin®, Pharmaxim AB, Helsingborg; 1 ml Partoxin® conforming 10 IU oxytocin) immediately after their normal milking in the AMS. They were injected either in the left thigh muscle or in the neck if it was not possible to inject it in the thigh muscle. After two minutes a Harmony™ milking cluster was attached and the residual milk was milked in an extra milk bucket for maximal eight minutes. If there was a visible milk flow udder massage and downward pressure were started after six minutes for maximal two minutes. In case there was no visible milk flow at an earlier time downward pressure plus udder massage were started before the first six minutes and the milking cluster was detached after maximum two minutes massage. The collected residual milk yield was measured with a measuring cylinder (1 L volume) and was analyzed on milk composition, SCC, sodium, potassium, FFA, FAC, MFG and FGS.

2.4 Milk sampling

The milk samples were collected during the last three days (day 5, 6 and 7) of each period. The number of cows from which milk samples were collected and the parameters analysed are presented in the sampling protocol in Table 7.

The samples for the analysis on milk composition and somatic cell count (SCC) were taken automatically from the AMS. For the other milk components milk samples were gathered from the AMS-sampler by using a litre measure and were afterwards distributed into different tubes.

Table 7. Sampling protocol with number of collected samples per sampling day (5,6 and 7) and per milking (one milking = 1, two milkings = 2, all milkings = all), further the analysed parameters of the samples.

	Sampling day							
	5		6		7			
					Regular milk		Residual milk	
Analyzed Parameters	milkings (m)	samples (s) [1]	m	s	m	S	m	s
MC[2], SCC	all	32	all	32	1	32	1	16
Na, K	-	-	2	32	1	32	1	16
MFG, FGS	-	-	1	24	1	24	1	16
FFA, FAC	-	-	-	-	1	16	1	16

[1]Number of samples taken per milking;
[2]milk composition (MC): fat, protein, lactose.

During the three sampling days milk samples from all 32 cows from all milkings were collected for the analysis on milk composition (fat, protein, lactose) and SCC. Samples for the analysis on fat globule parameters (MFG size, FGS) were collected on day 6 and 7 from 24 cows (6 cows of each group). Additionally on day 7, milk samples were collected from 16 of these 24 cows and analyzed on FFA and FAC, plus residual milk was determined from these 16 cows (4 cows of each group).

The 6 and 4 cows, respectively of each group which were chose for the analysis of fat parameters and the determination of residual milk were selected from their group of 8 cows by milk yield, lactation stage and lactation number.

The freshly taken samples were brought to the refrigerator after every second hour because of the surrounding temperature in the barn (~10 °C).

2.5 Milk analysis

The fresh milk samples were stored in a refrigerator at 4°C for maximum five days (120 hours) until they were analyzed on the different parameters.

2.5.1 Milking parameters

Registrations on individual cow milk yield, individual milking time, peak milk flow and average milk flow from each quarter were recorded every day during the whole experiment by the software of the AMS (Delpro™, DeLaval, Tumba, Sweden).

2.5.2 Milk composition and SCC

The milk composition and the SCC of the collected fresh milk samples were analyzed at the Department of Animal Nutrition and Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, Uppsala. The samples were preserved with Bronopol. The SCC was analyzed by flow-cytometry by a Fossomatic 5000 (Foss Electric, Hillerød, Denmark); the milk composition was analyzed using Fourier transform infrared (FTIR) spectroscopy (MilkoScan™ FT120, Foss Electric, Hillerød, Denmark).

2.5.3 Sodium and potassium

The content of sodium and potassium were used as an indicator to control if the treatments affect the leakiness of the tight junctions. The analysis was assayed by Agrilab AB, Uppsala, using inductively coupled plasma optical emission spectroscopy (ICP-OES). For preparation the samples were diluted 100 times with distillated water and directly analyzed with the Spectroblue FMX26 ICP-OES (Spectro Analytical Instruments GmbH, Ametek Inc., US). The ICP-OES was linear multilevel calibrated by injecting solutions containing amounts of the respective minerals to be analyzed.

2.5.4 Fat quality parameters

Two samples were collected for the analyses of the fat quality parameters. One collected sample for the assay of the content of FFA and the FAC in the milk and another sample to determine the size of the MFG and their stability.

2.5.4.1 Free fatty acids and fatty acid composition

The FFA content and FAC of the milk fat were assayed in the Food Science Department, Swedish University of Agricultural Sciences, Uppsala. These samples were also stored in a refrigerator at 4 °C during 48 hours after the collection and thereafter stored frozen in minus 21 °C until the analysis took place. To determine the FFA content a modified method of the method of DEETH, FITZ-GERALD, & WOOD (1975) was used. The method involves an extraction of milk with two solvents (extraction mixture: isopropanol: petroleum ether: 4N H_2SO_4, 40:10:1 and petroleum ether) to separate the fat. The titration of the released fat was conducted with 0.002 N methanolic KOH and phenol as indicator.

In order to determine the FAC of the samples a gas chromatograph (CP 3800, Varian, Walnut Creek, CA) with helium as carrier gas and a flame ionization detector were applied. Prior to gas chromatography (GC) the milk was extracted with diethyl ether and hexane, gravimetric determination of the fat was conducted using microbalance (UMT2 ultramicro, Mettler-Toledo, AG Grifensee, Switzerland). Finally, the samples were trans-esterified to methyl esters involving methanolic sodium methoxide (DEETH, FITZ-GERALD, & WOOD, 1975).

2.5.4.2 Milk fat globules and fat globule stability

The samples which were analyzed on MFG and FGS were sent as fresh samples to the Department of Food Science, Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Denmark on the last sampling day. To obtain the particle size distribution of

milk fat globules according to WIKING, STAGSTED, BJÖRCK, & NIELSEN (2004) integrated light scattering by a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK) was employed.

The fat globule stability was analysed by measuring the activity of the protein γ-glutamyl transpeptidase in the milk fat globule membrane (WIKING, STAGSTED, BJÖRCK, & NIELSEN, 2004).

2.6 Statistical analyses

Milking data was captured, summarized and analyzed from 34 cows (data of 32 cows used over the whole experiment plus data of the two excluded cows) by using mixed models in SAS (Statistical Analysis system version 9.3, SAS Inst.Inc., Cary, NC). Milk yields per milking were summed to produce a total daily milk yield for each animal. Fat yield per milking for regular milk was calculated from the composite test samples of each milking in the last three days of each period and fat yield per milking for residual milk was calculated using test samples from the last day of each period. The amount of residual milk was correlated with total yield due to a big variation of the actual yield. SCC data was transformed with a $\log_{10}$ transformation before analysis because it is not normally distributed.

Overall 16 different mixed models were tested with 7 different factors, but not each model included all 7 factors. Considering that sample type was significant ($P < 0.05$) in the models for milk composition, SCC, sodium, potassium and in the models for the fat parameter, two models were calculated for each sample type, one model for regular milk and one model for residual milk. All the models contained the factors: feeding, take off level, the interaction of feeding x take off level, parity (lactation number) and lactation stage (DIM = days in milk). Models that included just these factors were daily milk yield, residual milk yield, yield/milking, protein content, SCC, mean milk flow, peak milk flow, milking duration and milking interval. The models for fat yield/milking and fat content contained additional the factor milking interval. The models for lactose content, MFG size, MFG stability, K and Na included instead of milking interval the factor period. In the beginning the factor period was included in each model but it was excluded in every model where it was not significant. The model for FFA content contained both factors period and milking interval. FAC was not analyzed with a mixed model but averages were calculated by SAS.

As earlier mentioned the present thesis had its focus not on the analysis of milk flow, milking duration and milking interval because these parameter were dealt with in a Bachelor thesis. However, during the statistical analysis the specific analysis of milk flow, milking duration and milking interval with mixed models was found to be necessary for the discussion.

3 Results

3.1 Regular milk analysis

DIM was significant in each model except in the models for MFG size, milking interval and milking duration. Primiparous cows had the highest mean values for fat, lactose, FFA content and MFG size and additional the shortest milking intervals and milking duration compared with multiparous cows whereas multiparous cows had greater mean values for daily milk yield, milk flow, protein, sodium and potassium content, fat yield/milking, SCC and for MFG stability.

3.1.1 Milk yield and milk composition

Mean DIM at the end of the experiment were 186 ± 51 d (mean ± SD). Daily milk yield is presented in Table 8 and shows that feeding concentrate during milking had a significant effect ($P < 0.05$) on daily milk yield. The treatments 800/f and 200/f had a higher milk yield than the treatments without feeding.

Referring to the data in Table 8 it is apparent that fat, protein and lactose content were not associated with the interaction of feeding x take off level ($P > 0.05$) but there was a tendency found for SCC ($P = 0.08$). Take off level had significant effects on protein content ($P < 0.05$). An increasing take off level setting was associated with a significant decrease in protein content (3.50 % vs. 3.53 %). However, this statistical significant difference is of no practical importance. Fat yield/milking was significantly increased with the feeding concentrate during milking treatments ($P < 0.05$). Cows that received concentrate during milking had besides a higher milk yield a slightly higher fat yield/milking than cows that were not fed during milking (0.496 kg vs. 0.475 kg).

Table 8. Milk yield, milk composition and SCC for the tested treatments with standard error (SE) and *P* – values for feeding, take off level and the interaction of feeding x take off level. [1]

Item	Treatment				*P* - values			SE
	800/f	800/nf	200/f	200/nf	feeding	Take off	f x t[2]	
Milk yield (kg/d/cow)	30.24	29.04	31.51	29.01	<0.05	0.48	0.46	1.06
Yield/milking (kg/cow)	11.96	11.93	11.97	11.98	0.77	0.31	0.62	1.06
Fat (%)	4.29	4.29	4.32	4.38	0.63	0.22	0.51	0.05
Fat yield/ milking (kg)	0.50	0.48	0.51	0.49	<0.05	0.18	0.50	0.01
Protein (%)	3.51	3.49	3.55	3.52	0.13	<0.05	0.68	0.02
Lactose (%)	4.78	4.77	4.78	4.79	0.39	0.13	0.25	0.01
$Log_{10}SCC$[3]	1.51	1.54	1.53	1.48	0.46	0.32	0.08	0.03
	(32.36)	(34.67)	(33.88)	(30.20)				

[1]Values are means;
[2] f x t = interaction of feeding x take off level;
[3]$Log_{10}SCC$ = log_{10} transformation of the SCC (back transformed SCC x 1000/ml).

3.1.2 Milk flow, milking duration and milking interval

Average mean and average peak milk flow were analysed in two models and both showed no significant differences due to treatments. Peak milk flow was not affected by feeding or take off level whereas a tendency was found on the effect of mean milk flow due to take off level ($P = 0.06$). As expected milking duration was significantly different between the two take off level settings ($P < 0.05$). Increasing take off level settings from 200 g/min to 800 g/min resulted in shorter milking times (800 g/min: 7.07 min, 200 g/min: 7.82 min). Milking interval was significantly affected by the feeding concentrate during milking treatment ($P < 0.05$) and additional by the interaction of feeding x take off level ($P < 0.05$). Cows that received the feeding treatments had a shorter milking interval (552 min) than cows with no feeding treatments (566 min). The longest milking intervals were detected in the 200/nf treatment (Table 9).

Table 9. Milk flow (kg/min), milking duration (min) and milking interval (min) of regular milk for the tested treatments with SE and *P* – values for feeding, take off level and the interaction of feeding x take off level. [1]

Item	Treatment				*P* - values			SE
	800/f	800/nf	200/f	200/nf	feeding	Takeoff	f x t[3]	
Peak flow [2]	1.15	1.16	1.13	1.14	0.49	0.18	0.98	0.02
Mean flow	0.77	0.78	0.75	0.76	0.33	0.06	0.88	0.01
Milking interval	558	550	545	583	<0.05	0.12	<0.05	7.12
Milking duration	7.07	7.07	7.72	7.93	0.55	<0.05	0.55	0.19

[1]Values are means;
[2]Peak and mean milk flow = kg/min per udder quarter;
[3]f x t = interaction of feeding x take off level.

3.1.3 Sodium and potassium

The detected average of sodium and potassium content in regular milk was 390 mg/l and 1805 mg/l, respectively. Both minerals were found not to be significant among the four tested treatments but they were affected by period, parity and lactation stage ($P < 0.05$). A description of the mean values for the tested treatments and the corresponding *P* – values are provided in Table 10.

Table 10. Sodium and potassium content (mg/l) in regular milk for the tested treatments with SE and *P* – values for feeding, take off level and the interaction of feeding x take off level (f x t). [1]

Item	Treatment				*P* – Values			SE
	800/f	800/nf	200/f	200/nf	feeding	take off	f x t[2]	
Na	397	389	387	389	0.64	0.34	0.31	5.59
K	1811	1817	1805	1787	0.69	0.26	0.44	17.09

[1]Values are means;
[2]f x t = interaction of feeding x take off level.

3.1.4 Fat parameters

The analysis of FFA content, MFG size and MFG stability in milk showed no significant difference between the four treatments 800/f, 800/nf, 200/f and 200/nf (Table 11). However FFA content and MFG stability were both affected by the feeding concentrate during milking treatments. Cows that received the feeding treatments had a significantly ($P < 0.05$) lower FFA content and a lower activity of γ-glutamyl transpeptidase than cows with no feeding

treatments (FFA: 0.11 mEq/ml vs. 0.13 mEq/ml; MFG stability: 108.29 Δ abs/min vs. 110.15 Δ abs/min).

Table 11. FFA content, MFG size and MFG stability in regular milk for the tested treatments with SE and *P* – values for feeding, take off level and the interaction of feeding x take off level. [1]

Item[3]	Treatment				*P* - values			SE
	800/f	800/nf	200/f	200/nf	feeding	takeoff	f x t[2]	
FFA content	0.11	0.13	0.11	0.13	<0.05	0.77	0.96	0.01
MFG size	4015	3995	4000	4016	0.93	0.89	0.42	29.30
MFG stability	108	110	109	110	<0.05	0.62	0.94	1.18

[1]Values are means;
[2]f x t = interaction of feeding x take off level;
[3]FFA content (mEq/ml), MFG size (µm) = Average volume-weighted diameter ($d_{(4,3)}$),
MFG stability = Activity of γ-glutamyl transpeptidase (Δ abs/min).

A summary of the analysed major fatty acids and the percentage of SCFA, MCFA, LCFA, MUFA and PUFA in regular milk are given in Table 12. The milk fat contained most of palmitic acid (C16:0) and oleic acid (C18:1 (n – 9)) with an average of 35.44 wt % and 20.61 wt % respectively, as expected. The MCFA had the greatest proportion of fatty acids in regular milk with an average of 35.14 wt% followed by LCFA with 30.93 wt% and SCFA with 28.01 wt% Significances between the treatments for the FAC were not investigated in the present thesis due to too less time in the end of the completion of the thesis.

Table 12. Major fatty acids, SCFA, MCFA, LCFA, MUFA and PUFA (wt %) in regular milk for the tested treatments. [1]

Item[2]	Treatment			
	800/f	800/nf	200/f	200/nf
C4:0	3.35	3.63	3.43	3.36
C6:0	2.27	2.42	2.25	2.22
C8:0	1.38	1.45	1.33	1.36
C10:0	3.47	3.60	3.37	3.57
C12:0	4.28	4.39	4.10	4.44
C14:0	13.45	13.24	12.19	13.47
C16:0	33.46	36.96	36.60	33.55
C18:0	9.28	10.79	10.59	10.57
C18:1 (n – 9)	22.47	16.70	20.49	21.27
C18:2 (c9t13)	0.09	0.08	0.04	0.05
C18:3 (n – 9)	0.28	0.33	0.15	0.18
SCFA	28.20	28.73	26.67	28.42
MCFA	33.46	36.96	36.60	33.55
LCFA	32.25	28.04	31.34	32.10
MUFA	23.88	19.22	22.58	23.35
PUFA	1.08	1.01	0.77	0.76

[1]Values are means;
[2]SCFA = short chain fatty acid, MCFA = middle chain fatty acid, LCFA = long chain fatty acid, MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

3.2 Residual milk analysis

As mentioned the analysed models for milk composition, SCC, fat parameter, sodium and potassium showed all significant differences ($P < 0.05$) between regular and residual milk. Fat content, SCC, FFA content, MFG size, MFG stability and sodium content were higher whereas lactose, protein and potassium content were lower in residual milk compared with regular milk. Parity was significant in each residual milk model except the model for residual milk yield and fat yield/milking. Fat content, MFG size, MFG stability and potassium were not affected by DIM. Period was also found not to be significant in the potassium and the lactose model.

3.2.1 Milk yield and milk composition in residual milk

The average residual milk yield was 0.81 kg/cow or 6.77 %/cow of total yield, respectively. Mean residual milk yields per treatment are presented in Table 13.

Table 13. Residual milk yield, milk composition and SCC for the tested treatments with SE and *P* – values for feeding, take off level and the interaction of feeding x take off level. [1]

Item	Treatment				*P* - values			SE
	800/f	800/nf	200/f	200/nf	feeding	takeoff	f x t[2]	
Milk yield (%)[3]	8.54 (1.02)	8.04 (0.96)	4.89 (0.59)	5.61 (0.67)	0.93	<0.05	0.62	0.02
Milk yield (kg)								
Fat (%)	9.91	10.26	10.59	10.91	0.50	0.18	0.98	0.62
Protein (%)	3.28	3.22	3.20	3.24	0.91	0.63	0.49	0.07
Lactose (%)	4.56	4.56	4.60	4.61	0.92	0.36	0.99	0.06
$Log_{10}SCC$[4]	1.79	1.81	1.79	1.80	0.80	0.99	0.97	0.09
	(61.66)	(64.57)	(61.66)	(63.10)				

[1]Values are means;
[2] f x t = interaction of feeding x take off level;
[3] residual milk yield in % of total milk yield;
[4] $Log_{10}SCC = log_{10}$ transformation of the SCC (back transformed SCC x 1000/ml).

The residual milk yield was affected by lactation stage and take off level ($P < 0.05$). The treatments with a higher take off level revealed a significantly higher residual milk yield (200 g/min: 5.25 % (0.63 kg), 800 g/min: 8.29 % (0.99 kg)). SCC, fat yield/milking, fat, protein and lactose content were not different among the tested treatments.

3.2.2 Sodium and potassium in residual milk

There were no significances detected in the residual milk for the analysis of sodium and potassium among the treatments neither for feeding nor for take off level. The average sodium and potassium content in residual milk were 457 mg/l and 1731 mg/l, respectively over the whole experiment (data not shown).

3.2.3 Fat parameters in residual milk

As shown in Table 14, none of the fat parameters were affected by feeding, take off level or the interaction of feeding x take off level.

Table 14. FFA content, MFG size and MFG stability in residual milk for the tested treatments with SE and *P* – values for feeding, take off level and the interaction of feeding x take off level. [1]

Item[3]	Treatment				*P*-values			SE
	800/f	800/nf	200/f	200/nf	feeding	takeoff	f x t[2]	
FFA content	0.16	0.18	0.24	0.18	0.42	0.18	0.16	0.03
MFG size	4310	4283	4329	4374	0.82	0.16	0.36	49.59
MFG stability	129	130	129	131	0.42	0.82	0.86	3.10

[1]Values are means;
[2]f x t = interaction of feeding x take off level;
[3]FFA content (mEq/ml), MFG size (µm) = Average volume-weighted diameter ($d_{(4,3)}$), MFG stability = Activity of γ-glutamyl transpeptidase (Δ abs./min).

Nevertheless, the factor period had significant effects on all fat parameters ($P < 0.05$). The mean percentages of the major fatty acids, SCFA, MCFA, LCFA, MUFA and PUFA in residual milk are given in table 15 and were similar compared with these in regular milk. The averages of MCFA, LCFA, SCFA and were 35.44 wt%, 31.14 wt%, 27.37 wt%.

Table 15. Major fatty acids, SCFA, MCFA, LCFA, MUFA and PUFA (wt %) for the tested treatments in residual milk. [1]

Item[2]	Treatment			
	800/f	800/nf	200/f	200/nf
C4:0	3,31	3,66	3,41	3,37
C6:0	2,20	2,38	2,30	2,21
C8:0	1,33	1,38	1,40	1,37
C10:0	3,46	3,49	3,55	3,53
C12:0	4,25	4,31	4,37	4,38
C14:0	12,09	13,82	10,65	13,24
C16:0	36,95	31,75	38,40	34,66
C18:0	10,74	10,69	9,28	10,42
C18:1 (n – 9)	19,49	22,26	20,18	20,49
C18:2 (c9t13)	0,04	0,05	0,03	0,06
C18:3 (n – 9)	0,10	0,24	0,17	0,09
SCFA	26.64	29.04	25.68	28.10
MCFA	36.95	31.75	38.40	34.66
LCFA	30.45	33.19	29.84	31.06
MUFA	21.23	24.45	22.41	22.89
PUFA	0.70	0.72	0.78	0.65

[1]Values are means;
[2]SCFA = short chain fatty acid, MCFA = middle chain fatty acid, LCFA = long chain fatty acid, MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

4 Discussion

4.1 Milk yield and milk composition

The results of this experiment have shown that the combination of feeding during milking or no feeding during milking and cluster take off level settings had no significant effects on milk yield, milk composition and SCC ($P > 0.05$). There was no difference found in daily milk yield between a cluster take off level at 200 g/min and 800 g/min at whole udder level. This coincides with previous studies. STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER (2002) tested take off level settings between 500 and 640 g/min and 730 and 820 g/min. The author reported no change in milk yield only a tendency for higher milk production with higher take off level. Four different take off level settings (200 g/min, 400 g/min, 600 g/min and 800 g/min) were tested by EDWARDS, JAGO, & LOPEZ-VILLALOBOS (2013). No change in milking production between the take off level settings was found. In contrast MAGLIARO & KENSINGER (2005) compared take off level settings at 480 g/min, 600 g/min and 800 g/min and discovered a reduced milk production at a higher take off level. Several issues may be responsible for the differences between the present study and the studies of STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER (2002); MAGLIARO & KENSINGER (2005) and EDWARDS, JAGO, & LOPEZ-VILLALOBOS (2013) including distinctions in experimental duration, milking equipment, milking frequency and cow performance. All three mentioned studies milked twice a day in a milking parlour. Thus the milking frequency was lower than in our study, where the cows were milked in AMS with an average milking frequency about 2.6 milkings/day.

The present study tested an improved stimulation by feeding or no feeding during milking. Previous studies showed that feeding during milking inreases the milking–related oxytocin levels in the circulation and consequently, improves the milk production (SAMUELSSON, WAHLBERG, & SVENNERSTEN, 1993; SVENNERSTEN, GOREWIT, SJAUNJA, & UVNÄS-MOBERG, 1995; JOHANSSON, 2000). This was confirmed in this study and appeared in a significant effect of the feeding concentrate during milking treatment on daily milk yield. The treatments with feeding concentrate during milking had an increased average daily milk yield of 1.80 kg compared with the treatments without feeding. SAMUELSSON, WAHLBERG, & SVENNERSTEN (1993) reported a lower difference (0.2 kg) in daily milk production when they compared no feeding during milking and feeding concentrate while milking. JOHANSSON (2000) tested feeding before milking, during milking and after milking. In agreement with the present study and SAMUELSSON, WAHLBERG, & SVENNERSTEN (1993) an elevated milk production was reported when cows were fed during milking compared when they were milked without feeding. This demonstrates that milk removal and milk production are stimulated when cows were fed during milking.

Fat yield/milking increased additional with feeding during milking in the present study. This can be seen as a result of the increased daily milk yield. The amount of fat globules increases with higher milk production. SAMUELSSON, WAHLBERG, & SVENNERSTEN (1993) and JOHANSSON (2000) also mentioned a higher fat yield/day with higher milk yield when the cows obtained feed during milking in their studies. Fat, protein and lactose content were

not affected by feeding which is also in line with the results of SAMUELSSON, WAHLBERG & SVENNERSTEN (1993). JOHANSSON (2000) reported instead higher daily fat and lactose contents in milk from cows which were fed during milking.

Increasing take off level settings had no effect on fat and lactose, which is in accordance with EDWARDS, JAGO & LOPEZ-VILLALOBOS (2013) and JAGO, BURKE & WILLIAMSON (2010). No effect on fat content with higher take off level shows that milk ejection and milk removal were not incomplete. Due to an increase in fat content from the beginning of milking till the end of milking (JOHANSSON, KORKMAN, & NELSON, 1952) there would be a decrease in fat content with higher take off level (ONTSOUKA, BRUCKMAIER, & BLUM, 2001). A significant effect on protein content with increasing take off level settings as it was detected in the present study was not reported in the studies from EDWARDS, JAGO & LOPEZ-VILLALOBOS (2013) and JAGO, BURKE & WILLIAMSON (2010). However, the difference in protein content in the present study was visible in the second decimal thus it is an unimportant difference and not expressive.

SCC was not affected by feeding or take off level settings in the present study. These findings are conform to the study of EDWARDS, JAGO & LOPEZ-VILLALOBOS (2013). However, JAGO, BURKE & WILLIAMSON (2010) reported a slightly higher SCC with increasing take off level. There was only a tendency found that the interaction of feeding x take off could have an effect on SCC. The treatment with higher take off level and no feeding as well as the treatment with lower take off level and feeding had an slightly higher SCC than the other two treatments. One explanation might be the udder emptying being lower with higher take off and no feeding during milking thus there is more milk left in the udder for the bacteria. Considering the low take off/feeding treatment the udder emptying is higher with a lower take off and feeding during milking, hence the percentage of residual milk in regular milk is higher. Due to a higher amount of SCC in residual milk the SCC in regular milk is higher (BRUCKMAIER, ONTSOUKA, & BLUM, 2004). Considering the experimental length which may have an effect on udder health the present study was a short term study for only four weeks. STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER (2002) tested the different take off level settings for the same experimental length as the present study. However, MAGLIARO & KENSINGER (2005) used an experimantal duration of 12 weeks with two week periods and EDWARDS, JAGO & LOPEZ-VILLALOBOS (2013) tested their treatments for 11 weeks. Nevertheless, there was only a tendency found.

Residual milk analysis showed no significant difference in milk composition and SCC with different take off level settings or the feeding concentrate during milking treatments. Certainly the residual milk yield increased significantly with higher take off level settings due to a shorter milking time, plus at an earlier take off more milk was left in the udder. Residual milk is typically 10–20 % of total milk left in the udder after completed milking (JOHANSSON, KORKMAN, & NELSON, 1952) and as long as this threshold is not exceeded there is no risk of incomplete milking (MEIN, 2001). The results of this study showed an average residual milk yield between 4.5 and 8.5 % of total milk, accordingly not higher than the typical threshold. Determination of residual milk was not performed in earlier mentioned studies where different take off level were tested. EDWARDS, JAGO & LOPEZ-VILLALOBOS

(2013) and JAGO, BURKE & WILLIAMSON (2010) collected both strip yield after regular milking for investigating udder emptying with higher take off level. JAGO, BURKE & WILLIAMSON (2010) reported no negative effect on udder emptying with higher take off level. This difference may be due to that the highest take off level was 400 g/min in their study and not 800 g/min as it was in the present study. However, similar to our findings EDWARDS, JAGO & LOPEZ-VILLALOBOS (2013) examined a slightly increase in strip yield (0.3 kg) with higher take off level (800 g/min). The differences in milk composition and SCC between residual and regular milk were as expected. Fat content and SCC were higher, protein and lactose content were lower in residual milk. SVENNERSTEN & CLAESSON (1990) showed that fat content is very different in different milk fractions. Furthermore, fat content increases during milking (JOHANSSON, KORKMAN, & NELSON, 1952) and reaches its highest point in residual milk. This is caused by the lower specific gravity of milk fat and slowly movements of the fat droplets (ONTSOUKA, BRUCKMAIER, & BLUM, 2001).

4.2 Milk flow, milking duration and milking interval

Peak milk flow, mean milk flow and milking duration were not affected by the interaction of feeding x take off. Average mean milk flow showed a tendency for a positive interaction with higher take off level settings ($P = 0.06$). This result is in conformity with earlier mentioned studies (EDWARDS, JAGO, & LOPEZ-VILLALOBOS, 2013; STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER, 2002; RASMUSSEN, 1993). All of them reported an increased average milk flow with higher take off level settings.

Milking duration was further affected by increasing take off level settings. A take off level setting at a milk flow at 800 g/min resulted in a decreased milking time of 42 seconds per cow. A shorter milking time with a higher take off level was expected ever since the previous studies from RASMUSSEN (1993), STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER (2002), EDWARDS, JAGO & LOPEZ-VILLALOBOS (2013) MAGLIARO & KENSINGER (2005). The latter reported a similar result than the present study. Milking duration declined of 0.70 min per cow with a take off level setting change from 480 g/min to 800 g/min. In the studies of EDWARDS, JAGO & LOPEZ-VILLALOBOS (2013) a time reduction of 0.80 min per cow was detected when they compared take off level settings of 200 g/min and 800 g/min. In the study of STEWARD, GODDEN, RAPNICKI, REID, JOHNSON, & EICKER (2002) the time reduction was lower (10 and 15 seconds per cow) with take off level settings between 500 g/min and 820 g/min.

Despite, a defined milking interval between 7 and 10 hours was conducted in the present study feeding no concentrate during milking let the milking interval increase. Besides a significant effect on milking interval with the feeding concentrate during milking treatment, an interaction effect of feeding x take off was detected. The cows that obtained the treatment 200/nf showed the longest milking interval. Hence the cows in the treatment with the longest milking time and no feeding waited longer in the waiting area in front of the AMS than cows in other treatments. That result is in line with the investigations of SCOTT, THOMSON, KERRISK, & GARCIA (2014) who reported a shorter waiting time with feeding during milking due to a greater motivation of the cows.

4.3 Sodium and potassium

Sodium and potassium were neither in regular milk nor in residual milk affected by feeding, take off or the interaction of feeding x take off. The average values of both minerals conform the normal level in bovine milk (GAUCHERON, 2005). Thus, the tight junctions in the mammary gland were not affected by the treatments. Leaky tight junctions are marked by an increase and decrease in the Na and K concentrations (STELWAGEN, FARR, & MC FADDEN, 1999). Considering that the composition of minerals in milk is relatively constant there were no big differences detected among the four treatments. The significant effect of DIM on sodium and potassium reveal their natural variety in milk (GAUCHERON, 2005).

4.4 Fat parameters

Take off level and the interaction of feeding x take off had no significant effects on the fat parameters neither in regular nor in residual milk. Nevertheless the feeding concentrate during milking treatments had an effect on FFA content and MFG stability in regular milk. A lower FFA content was detected when cows received concentrate during milking. FFA content is affected by the milking process, mainly by air inlet, as well as by milk transfer to the bulk tank. In AMS the air inlet is often higher compared with traditional milking systems (DE KONING & RODENBURG, 2004). Therefore, increased FFA content with no feeding during milking could be caused through a higher air inlet in the teat cups due to more stress for the cows. Normally, the cows receive concentrate during milking in the AMS. If they do not receive feeding, as expected, they may be more restless and stand in a different position in the AMS. Hence the AMS need longer time to attach the teat cups, moreover there could be a higher air inlet as a result of a pulsation start before attaching the teat cup. It was earlier hypothesized that take off level could influence FFA content when the cluster take off occurred before the big fat globules are harvested in the end of milking. Indeed, take off level had no effects on FFA content in the present study. The distribution of fatty acids did not differ from their normal range in bovine milk (JENSEN, FERRIS, & LAMMI-KEEFE, 1991).

MFG stability is a measuring tool for the resistance of MFG against outside influences.The MFGM protects the MFG from influences like lipolysis. Hence, it is important to know the quantity of the MFGM which was measured by using the activity of γ-glutamyl transpeptidase, an enzym in the MFGM, as a marker. The activity of γ-glutamyl transpeptidase showed a decrease in the treatments with feeding concentrate during milking. In the present study there could be a coherence between the significant higher fat yield/milking and the declined activity of γ-glutamyl transpeptidase in regular milk in the present study. WIKING (2005) reported that the MFG size increases with increasing fat yield. Furthermore, with bigger MFG the amount of membrane material is confined and the activity of γ-glutamyl transpeptidase decreases. However, the MFG size was not significantly affected by feeding during milking such as fat yield/milking and MFG stability. Nevertheless, with a higher milk fat yield the amount of fat globules increases, thus more membrane material is needed whereby the membrane of the single MFG become thinner. Accordingly the MFG stability decreases.

The statistical analysis of fat parameter in residual milk revealed no significances among the treatments. Indeed, sample type was significant in all fat parameter, hence mean values of FFA content, MFG size and MFG stability were higher in residual milk due to a higher fat content. As mentioned earlier and approved in the present study, fat content and fat yield increase during milking and are highest in the residual fraction of the milk (ONTSOUKA, BRUCKMAIER, & BLUM, 2001). Thus, the size of the globules increases during the milking process and the MFGM is more vulnerable for disruption and lipolysis. As a result of higher lipolyses FFA content increases.

5 Conclusion

Increasing take off level settings in AMS improves the milking efficiency apparent from reduced milking duration around one minute per cow. Thus, higher take off levels can be an opportunity on many farms for elevating milking efficiency. Feeding during milking was approved as a useful treatment which enhances udder evacuation and milk production, respectively due to extra stimulation for milk let down. Although there were no significant effects determined with the interaction of feeding x take off, best results (high milk yield, short milking time) were detected at a higher take off level combined with feeding during milking. As a result of this study low take off level combined with no feeding cannot be suggested due to the longest milking time plus a lower milk yield. In addition, the motivation of the cows to come to the AMS is lower when they receive no concentrate during milking, therefore the milking interval increases. Feeding was further correlated with a lower FFA content and a decreased MFG stability. To observe udder emptying residual milk yield was measured. The udder health of the cows was not negatively impaired despite of a higher take off level.

In summary, milk yield and milk quality were not negatively influenced with a higher take off level. Nevertheless, more calculations can be done. Furthermore, little attention has been paid in previous studies to investigate fat parameter with different take off level settings. Hence more research is necessary to confirm the results of the present study.

6 References

ABENI, F., DEGANO, L., CALZA, F., GIANGIACOMO, R., & PIRLO, G. (2005). Milk quality and automatic milking: fat globule size, natural creaming, and lipolysis. *Journal of Dairy Science 88*, pp. 3519-3529.

ABENI, F., DEGANO, L., GIANGIACOMO, R., SPERONI, M., & PIRLO, G. (2003). Robotic milking and milk quality: Effects on the cheese-making properties of milk. *Italian Journal of Animal Science Vol.2*, pp. 301-312.

ARMSTRONG, D., BICKERT, W., GERISH, J., & SPIKE, P. (1970). Automatic milking machine removal. *Journal of Dairy Science 53 (Abstract)*, p. 658.

BERGLUND, I., PETTERSSON, G., & SVENNERSTEN-SJAUNJA, K. (2002). Automatic milking: effects on somatic cell count and teat end-quality. *Livestock Production Science 78*, pp. 115-124.

BOUTINAUD, M., ROUSSEAU, C., KEISLER, D., & JAMMES, H. (2003). Growth hormone and milking frequency act differently on goat mammary gland in late lactation. *Journal of Dairy Science 86*, pp. 509-520.

BRUCKMAIER, R., ONTSOUKA, C., & BLUM, J. (2004). Fractionized milk composition in dairy cows with subclinical mastitis. *Veterinary Medecin Czech 49*, pp. 283-290.

CASTRO, A., PEREIRA, J., AMIAMI, C., & BUENO, J. (2012). Estimating efficiency in automatic milking systems. *Journal of Dairy Science 95*, pp. 929-936.

DE KONING, K. (2010). Automatic milking: common practice on dairy farms. *The First North American Conference on Precision Dairy Management.* Colorado, Canada.

DE KONING, K., & RODENBURG, J. (2004). Automatic milking: State of the art in Europe and North America. In A. MEIJERING, H. HOGEVEEN, & C. DE KONING, *Automatic milking: A better understanding* (pp. 27-37). Wageningen, Netherlands: Wageningen Academic Publishers.

DE KONING, K., SLAGHUIS, B., & VAN DER VORST, V. (2003). Robotic milking and milk quality: Effects on bacterial count, somatic cell count, freezing point and free fatty acids. *Italian Journal of Animal Science Vol.2*, pp. 291-299.

DEETH, H., FITZ-GERALD, C., & WOOD, A. (1975). A convenient method for determining the extend of lipolysis in milk. *Australien Journal of Dairy Technology 30*, pp. 109-113.

EDWARDS, J., JAGO, J., & LOPEZ-VILLALOBOS, N. (2013). Milking efficiency for grazing cows can be improved by increasing automatic cluster remover thresholds without applying premilking stimulation. *Journal of Dairy Science 96*, pp. 3766-3773.

EDWARDS, J., JAGO, J., & LOPEZ-VILLALOBOS, N. (2013). Short-term application of prestimulation and increased automatic cluster remover threshold affect milking characteristics of grazing dairy cows in late lactation. *Journal of Dairy Science 96*, pp. 1886-1893.

GAUCHERON, F. (2005). Review: The minerals of milk. *Reproduction Nutrition Development 45*, pp. 473-483.

HOVINEN, M., & PYÖRÄLÄ, S. (2011). Udder health of dairy cows in automatic milking. *Journal of Dairy Science 94*, pp. 547-562.

JACOBS, J., & SIEGFORD, J. (2012). The impact of automatic milking systems on dairy cow management, behavior, health and welfare. *Journal of Dairy Science 95*, pp. 2227-2247.

JAGO, J., BURKE, J., & WILLIAMSON, J. (2010). Effect of automatic cluster remover settings on production, udder health, and milking duration. *Journal of Dairy Science 93*, pp. 2541-2549.

JENSEN, R., FERRIS, A., & LAMMI-KEEFE, C. (1991). The Composition of milk fat. *Journal of Dairy Science 74*, pp. 3228-3243.

JOHANSSON, B. (2000). Effect of milking and feeding routines on milk production, hormone release and behaviour in dairy cattle. *Academic Dissertation no.211* . Swedish University of Agricultural Sciences, Uppsala: Johansson, B.

JOHANSSON, I., KORKMAN, N., & NELSON, N. (1952). Studies on udder evacuation in dairy cows. *Acta Agriculturae Scandinavica* . Institute of Animal Breeding, Royal Agricultural College of Sweden, Uppsala.

KLUNGEL, G., SLAGHUIS, B., & HOGEVEEN, H. (2000). The effect of the introduction of automatic milking systems on milk quality. *Journal of Dairy Science 83*, pp. 1998-2003.

MAGLIARO, A., & KENSINGER, R. (2005). Automatic cluster remover setting affects milk yield and machin-on time in dairy cows. *Journal of Dairy Science 88*, pp. 148-153.

MATHER, I., & KEENAN, T. (1998). Origin and secretion of milk lipids. *Journal of Mammary Gland Biology and Neoplasia 3*, pp. 259-273.

MAYER, H., SCHAMS, D., WORSTORFF, H., & PROKOPP, A. (1984). Secretion of oxytocin and milk removal as affected by milking cows with and without manual stimulation. *Journal of Endocrinology 103*, pp. 355-361.

MEIN, G. (2001). *Completeness of milking.* Lecture notes , Melbourne.

MESILATI-STAHY, R., & ARGOV-ARGAMAN, N. (2014). The relationship between size and lipid composition of the bovine milk fat globule is modulated by lactation stage. *Food Chemistry 145*, pp. 562-570.

ONTSOUKA, C., BRUCKMAIER, R., & BLUM, J. (2001). Fractionized milk composition during removal of colostrum and mature milk. *Journal of Dairy Science 86*, pp. 2005-2011.

PALMQUIST, D., STELWAGEN, K., & ROBINSON, P. (2006). Modifying milk composition to increase use of dairy products in healthy diets. *Animal Feed Science and Technology 131*, pp. 149-153.

PRESCOTT, N., MOTTRAM, T., & WEBSTER, A. (1998). Relative motivations of dairy cows to be milked or fed in a y-maze and an automatic milking system. *Applied Animal Behaviour Science 57*, pp. 23-33.

RASMUSSEN, M. (1993). Influence of switch level of automatic cluster removers on milking performance and udder health. *Journal of Dairy Research 60*, pp. 287-297.

SAGI, R. (1978). Milk flow rate and end of milking detectors. *Proc. National Mastitis Council 17*, pp. 328-334.

SAGI, R., GOREWIT, R., MERRILL, W., & WILSON, D. (1980). Premilking stimulation effects on milking performance and oxytocin and prolactin release in cows. *Journal of Dairy Science*, pp. 800-806.

SAMUELSSON, B., WAHLBERG, E., & SVENNERSTEN, K. (1993). The effect of feeding during milking on milk production and milk flow. *Swedish Journal of Agricultural Research 23*, pp. 101-106.

SCOTT, V., THOMSON, P., KERRISK, K., & GARCIA, S. (2014). Influence of provision of concentrate at milking on voluntary cow traffic in a pasture-based automatic milking system. *Journal of Dairy Science 97*, pp. 1481-1490.

STELWAGEN, K., FARR, V., & MC FADDEN, H. (1999). Alteration of the sodium to potassium ratio in milk and the effect on milk secretion in goats. *Journal of Dairy Science 82*, pp. 52-59.

STEWARD, S., GODDEN, S., RAPNICKI, P., REID, D., JOHNSON, A., & EICKER, S. (2002). Effects of automatic cluster remover settings on average milking duration, milk flow, and milk yield. *Journal of Dairy Science 85*, pp. 818-823.

SVENNERSTEN, K., & CLAESSON, C. (1990). Effect of local stimulation of one quarter on milk production and milk components. *Journal of Dairy Science 73*, pp. 970-974.

SVENNERSTEN, K., GOREWIT, R., SJAUNJA, L.-O., & UVNÄS-MOBERG, K. (1995). Feeding during milking enhances milking-related oxytocin secretion and milk production in dairy cows whereas food deprivation decrease it. *Acta Physiol Scand 153*, pp. 309-310.

SVENNERSTEN-SJAUNJA, K., & PETTERSON, G. (2008). Pros and cons of automatic milking in Europe. *Journal of Animal Science* .

SVENNERSTEN-SJAUNJA, K., PERSSON, S., & WIKTORSSON, H. (2002). The effect of milking interval on milk yield, milk composition, milk yield and raw milk quality. *Proc. Int. Symp. The first North American conference on robotic milking*, (pp. V43-V48). Toronto, ON, Canada.

UVNÄS-MOBERG, K., JOHANSSON, B., LUPOLI, B., & SVENNERSTEN-SJAUNJA, K. (2001). Oxytocin facilitates behavioural, metabolic and physiological adaptions during lactation. *Applied Animal Behaviour Science 72*, 225-234.

VERBALIS, J., MC CANN, M., MC HALE, C., & STRICKER, E. (1986). Oxytocin secretion in response to cholecystokinin and food: differentiation of Nausea from Satiety. *Science 232*, 1417-1419.

WALSTRA, P., WOUTERS, J., & GEURTS, T. (2006). *Dairy Science and Technology - Second Edition.* Boca Raton, Florida: CRC Press, Taylor & Francis Group.

WIKING, L. (2005). Milk fat globule stability: Lipolyses with special reference to AMS. *Academical dissertation*.

WIKING, L., BJÖRCK, L., & NIELSEN, J. (2003). Influence of feed composition on stability of fat globules during pumping of raw milk . *International Dairy Journal 13*, 797-803.

WIKING, L., NIELSEN, J., BÅVIUS, A. K., EDVARDSSON, A., & SVENNERSTEN-SJAUNJA, K. (2006). Impact of milking frequencies on the level of free fatty acids in milk, fat globule size, and fatty acid composition. *Journal of Dairy Science 89*, pp. 1004-1009.

WIKING, L., STAGSTED, J., BJÖRCK, L., & NIELSEN, J. (2004). Milk fat globule size is affected by fat production in dairy cows. *International Dairy Journal 14*, pp. 909-913.

Declaration

I,

Stadtmüller, Larissa

Born on: 30th July 1987

Matriculation number: 476841,

hereby declare on my honor that the attached declaration

☐ Homework/Presentation

☐ Bachelor Thesis

☑ Master Thesis

☐ Diplom Thesis,

has been independently prepared, solely with the support of the listed literature references, and that no information has been presented that has not been officially acknowledged.

Supervisor: Prof. Dr. Thomas Jungbluth (Hohenheim)

Co–Supervisor: Prof. Dr. Kerstin Svennersten–Sjaunja (SLU)

Thesis topic: The importance of feeding during milking and take off level for milking efficiency and milk production

Semester: 4

I declare, here within, that I have transferred the final digital text document (in the format doc, docx, odt, pdf or rtf) to my mentoring supervisor and that the content and wording is entirely my own work. I am aware that the digital version of my document can and/or will be checked for plagiarism with the help of an analyses software program.

Uppsala, 18th August 2014

I denna serie publiceras examensarbeten (motsvarande 15, 30, 45 eller 60 högskolepoäng) vid Institutionen för husdjurens utfodring och vård, Sveriges lantbruksuniversitet. Institutionens examensarbeten finns publicerade på SLUs hemsida *www.slu.se*.

In this series Degree projects (corresponding 15, 30, 45 or 60 credits) at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, are published. The department's degree projects are published on the SLU website *www.slu.se*.

Sveriges lantbruksuniversitet Fakulteten för veterinärmedicin och husdjursvetenskap Institutionen för husdjurens utfodring och vård Box 7024 750 07 Uppsala Tel. 018/67 10 00 Hemsida: www.slu.se/husdjur-utfodring-vard	*Swedish University of Agricultural Sciences* *Faculty of Veterinary Medicine and Animal Science* *Department of Animal Nutrition and Management* *PO Box 7024* *SE-750 07 Uppsala* *Phone +46 (0) 18 67 10 00* *Homepage: www.slu.se/animal-nutrition-management*