Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap

Swedish University of Agricultural Sciences
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Frequency of unsuccessful milkings in automatic milking rotary

Effect on milk yield, lactose content and somatic cell count at udder quarter level

Photo: DeLaval

Ida Ljunggren

Examensarbete / Sveriges lantbruksuniversitet,
Institutionen för husdjurens utfodring och vård, **515**

Examensarbete, 45 hp
Masterarbete

Uppsala 2015

Husdjursvetenskap

Degree project / Swedish University of Agricultural Sciences,
Department of Animal Nutrition and Management, **515**

Degree project, 45 hp
Master Thesis
Animal Science

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Frekvensen av misslyckade mjölkningar i automatisk mjölkningskarusell
Effekten på mjölkavkastning, laktoshalt och celltal på juverdelsnivå

Ida Ljunggren

Supervisor:	Kerstin Svennersten-Sjaunja, SLU, Department of Animal Nutrition and Management
Examiner:	Kjell Holtenius, SLU, Department of Animal Nutrition and Management
Extent:	45 HEC
Course title:	Degree Project in Animal Science
Course code:	EX0653
Programme:	Agricultural Science Programme – Animal Science
Level:	Second cycle A2E
Place of publication:	Uppsala
Year of publication:	2015
Series name, part No:	Degree project / Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, 515
On-line published:	http://stud.epsilon.slu.se
Nyckelord:	**AMR, misslyckad mjölkning, ofullständig mjölkning, avsiktligt överhoppad mjölkning, juverdelsnivå, juverhälsa, mjölkmängd, laktos, SCC**
Keywords:	AMR, unsuccessful milking, incomplete milking, omitted milking, udder quarter level, udder health, milk yield, lactose, SCC

List of abbreviations

AM	Automatic milking
am	Morning
AMR	Automatic milking rotary
AMS	Automatic milking system
IMF	Increased milking frequency
MF	Milking frequency
MI	Milking interval
OM	Omitted milking
pm	Afternoon
SCC	Somatic cell count
UL	Udder level
UQ	Udder quarter
UQL	Udder quarter level

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Abstract

Developments in milk production are heading towards fewer but larger herds where the milking process is often fully automated. Automatic milking systems were launched in the 1990's and in the year 2010 the Automatic Milking Rotary (AMR) was introduced. As a rule there are no supervision personnel present during the milking event in systems with automatic milking. This means that there is a risk that cows can be incompletely milked in one or more udder quarters, for example if the robots fail in attaching the teat cups or if the cow kicks off the milking unit. Incomplete or missed milking can lead to reduced milk yield and/or milk leakage, which can later lead to mastitis. This is important to each dairy farmer's milk production as well as the udder health of each individual cow.

The present study was composed of two experiments. The purpose of Experiment 1 was to investigate the frequency and cause of unsuccessful or incomplete milking in the AMR. Experiment 1 was carried out on a herd of 171 cows and included eight milkings (four days) and one day for recording udder conformation and assessing teat position and condition. The frequency and causes of unsuccessful or incomplete milking were registered in a template. In addition, the possibility of a relationship between milk somatic cell count (SCC) and kick off of the teat cups was investigated. The results of Experiment 1 showed a relationship between udder conformation and failed attachment of teat cups. The results also showed that 82.6% of the milkings in AMR were successful in the 1st milking attempt. A proportions of 37.9% of the failures at cluster/quarter level at 1st milking were repeated at the 2nd milking attempt. The results also showed that 95% of the individual quarters were successfully attached at the 1st milking attempt and that approximately 96% of the milkings, at udder level, were successful when outcome of the 1st and 2nd milking attempts were combined. The primary cause of unsuccessful milkings in the 1st and 2nd milking attempts was failed attachment of the teat cups, mainly on the right back teat. A problem with the automatically recorded data was that 43 milkings were noted as approved by the AMR during the 1st milking attempt even though disturbances, e.g. failed attachment of teat cups, kick off of milking unit, and/or tramp on milking tube, occurred according to the manual monitoring. When these 43 milkings were added to the unsuccessful milkings after the 2nd attempt (60 milkings), the final milking result was 93.1% successful milkings at udder level.

Experiment 2 was based on two different treatments, Treatment A and Treatment B, with 11 cows in each treatment group. The cows in the study had SCC $\leq$ 150 000 cells/ml milk per udder quarter. The purpose of the study was to investigate how one or three purposely omitted milkings on one udder quarter impacted milk yield, lactose content, and milk SCC. The design was a within udder comparison. One udder quarter (right front) was exposed to a treatment, while the adjacent quarter (left front) was the control quarter. Differences between treated and control udder quarter was calculated and tested if the difference differed from zero. Treatment A was carried out for 28 milkings (14 days) and Treatment B for 36 milkings (18 days). Treatment A was based on *one* purposely omitted milking on right front udder quarter and Treatment B *three* omitted milkings on right front right udder quarter with three milkings between the omitted milkings. At udder quarter level, a significant difference between front udder quarters were observed in milk yield, lactose content and SCC during the first days after treatment, where milk yield and lactose content decreased while SCC increased in the treated udder quarter. The difference between quarters disappeared at the end of the experimental period.

The present study shows the importance for all four udder quarters to be completely milked on every milking occasion. Further development of the AMR and further research on the effects and recovery period of unsuccessful and incomplete milking is needed. Future research should involve a greater number of cows and a wider variation in udder health.

Sammanfattning

Utvecklingen inom mjölkproduktionen går mot färre men större besättningar där mjölkningen ofta är helt automatiserad. Automatiska mjölkningssystem lanserades under 1990-talet och år 2010 lanserades automatisk mjölkningskarusell (AMR). I regel finns inte någon övervakande personal närvarande vid automatisk mjölkning, vilket kan medföra att kon riskerar att bli ofullständigt mjölkad på en eller flera juverdelar om exempelvis maskinen misslyckas med spenkoppspåsättning eller om kon sparkar av mjölkningsorganet. Ofullständig eller missad mjölkning kan leda till minskad mjölkmängd och/eller mjölkläckage som i senare skede kan orsaka mastit. Detta har betydelse för den enskilda mjölkbondens mjölkproduktion och den enskilda kons juverhälsa.

Studien bestod av två delstudier. Syftet med delstudie 1 var att undersöka frekvens och anledning till misslyckad eller ofullständig mjölkning i AMR. Delstudie 1 genomfördes på 171 kor och inkluderade 8 mjölkningar (4 dagar) samt en dag för registrering av juverform och spenbedömning. Antal och orsak till misslyckade mjölkningar noterades. Dessutom undersöktes ett eventuellt samband mellan mjölkens celltal (SCC) och avspark av mjölkningskoppar. Resultatet av delstudie 1 visade att ett samband mellan juverform och misslyckad påsättning av mjölkningsorganet kunde noteras. Resultatet visade även att ca 85,5% av mjölkningarna i AMR var lyckade vid första försöket och att 95 % av de individuella spenarna var korrekt påsatta vid första försöket. Den främsta anledningen till misslyckade mjölkningar i första och andra mjölkningsförsöket var misslyckad påsättning av mjölkningsorgan, främst på höger bakspene. Vidare visade studien att 37.9 % av de misslyckade försöken upprepades vid andra försöket. När antalet lyckade mjölkningar vid andra mjölkningsförsöket lades till dem som lyckades vid första försöket blev resultatet ca 96% lyckade mjölkningar på heljuvernivå. Dock noterades 43 mjölkningar som godkända av AMR vid första försöket även då misslyckad påsättning av mjölkningskoppar, avspark av mjölkningsorgan eller tramp på mjölkslang förekom, enligt den manuella övervakningen av mjölkningarna i försöket. När dessa 43 mjölkningar adderas till de misslyckade mjölkningarna efter andra mjölkningstillfället (60 mjölkningar), blev slutresultat 93,1% lyckade mjölkningar.

Delstudie 2 bestod av två behandlingar, A och B, med 11 kor i vardera behandlingsgruppen. Korna som ingick i studien hade mjölk SCC på ≤ 150 000 celler/ml i enskild juverdel. Syftet var att undersöka hur en eller tre avsiktligt överhoppade mjölkningar på en juverdel påverkar mjölkmängd, laktoshalt samt mjölkens SCC. Designen på delstudie 2 var en jämförelse mellan juverdelarna. En juverdel (höger fram) utsattes för en behandling och motsvarande juverdel (vänster fram) var kontroll. Differensen mellan den behandlande juverdelen och kontrolljuverdelen testades för att se om den skilde sig från noll. Behandling A pågick över 28 mjölkningar (14 dagar) och för behandling B över 36 mjölkningar (18 dagar). Behandling A bestod av *en* avsiktligt överhoppad mjölkning och behandling B bestod av *tre* avsiktligt överhoppade mjölkningar med tre mjölkningar mellan de överhoppade mjölkningarna. Resultat för både behandling A och B visade att när jämförelser gjordes på juverdelsnivå, kunde en signifikant skillnad ses mellan de främre juverdelarna för mjölkmängd, laktoshalt och SCC under de första dagarna efter behandlingen, där mjölkmängd och laktoshalt minskades och SCC ökade i den behandlade juverdelen. Skillnaden mellan juverdelarna försvann vid slutet av försöksperioden.

Studien visar betydelsen av att varje juverdel blir fullständigt mjölkade vid varje mjölkningstillfälle. Vidare utveckling av AMR samt vidare forskning på effekterna och återhämtningsperiod vid misslyckad eller ofullständig mjölkning behövs. Framtida forskning bör omfatta fler antal kor samt större variation i juverhälsa.

Introduction

Automatic milking systems (AMS) have been in the European market since 1992 (de Koning *et al.,* 2002). One of the major advantages with AMS is the reduction of manual labor. Thus, AMS prevent problems that are usually associated with manual handling during the milking occasion. However, problems that usually do not occur during conventional milking might occur in systems with automatic milking (AM). One or more udder quarters can be incompletely milked or un-milked due to failed attachment of teat cups or kick off during the milking since there is no manual handling during the milking process. The udder quarter will in such cases remain incompletely milked if the cow is not directed to a new milking immediately after the milking with omitted milking. Studies have reported that the milk yield and milk somatic cell count (SCC) are negatively influenced when one omitted milking (during 24 hours) at udder level (UL) was carried out on cows milked in conventional systems (Lakic *et al.,* 2011). A decrease in milk yield could be seen for up to ten days and SCC was elevated for several days. It is not fully evaluated whether similar effects occur when one quarter remains incompletely milked or if the adjacent quarter is affected.

A study reported that the average attachment failure was 7.6% of total milkings in the AMS. A 26% decrease in milk production in the affected udder quarter due to the extended milking interval, compared to the regular milk yields, was observed (Bach and Busto, 2005). Studies have also reported that front teats are easier to attach than back teats (Hamann *et al.,* 2004) and that the level of udder fill has a major importance in successful attachment (Kolbach *et al.,* 2012). No reports have been given about the effect of more than one failed attachment. Since milk SCC is a major indicator of mastitis (Hamann and Krömker, 1997), it is of interest to find out if SCC is affected during omitted milking or incomplete milking. Already during moderate increase in SCC the lactose concentration in milk decreases and can therefore also be used as an indicator of mastitis or udder disturbance (Berglund *et al.,* 2007).

The automated milking rotary (AMR) is the newest development in automatic milking and is now on the market. Recent studies have reported that about 19% of the milkings were incomplete in a prototype of AMR during the 1st milking attempt. After two attempts 90% of the milkings were complete (Kolbach *et al.,* 2012). The left back udder quarter also had the highest probability of failed attachments. The AMR used for the present study was a commercial 24 bail unit with five robotic arms (two teat preparation modules, two automatic cup attachers and one arm for teat spray).

Aim and Hypothesis

The first aim of the present study was to register the frequency of unsuccessful and incomplete milkings in a commercial AMR. The hypothesis related to the first aim was that the AMR has a milking efficiency rate of 80%, in other words less than 20% of the milkings are unsuccessful.

The second aim was to evaluate the effect of one omitted milking or three omitted milkings on milk yield, lactose content and milk SCC at udder quarter level. The hypothesis related to the second aim was that one or three omitted milkings at udder quarter level have a negative effect on milk yield and milk SCC at udder quarter level.

Literature Review

Development of Milking Technology

The dairy industry has undergone a major technological evolution to become more and more sustainable with regard to the financial profits for the dairy farmer and the health and welfare of each individual cow. Milking techniques have improved and today do not require as much manual effort as before. The number of dairy farms worldwide is decreasing but at the same time the dairy herds are becoming larger and cows are able to produce more milk.

History of Automatic Milking System

In the 1980's, the cost of labour increased in many countries with dairy production. Because of this, the need for improved labour efficiency grew, which is one of the main reasons for automatic milking (AM) (Rossing & Hogewerf, 1997). Automatic milking systems (AMS) are systems where cows are milked by robotic units without direct human input during the milking process (De Koning & Van de Vorst, 2002) and have evolved through many stages. It started with a single milking machine and continued with milking parlours provided with automatic cluster removers. The last step to an automated milking process was the automatic attachment of the teat cup and automatic teat cleaning. All these development steps, along with new milking technology, led to reduced manual labour input during milking, which in turn resulted in a higher output per man-hour (Rossing & Hogewerf, 1997).

Since the first AMS was launched commercially in the Netherlands in 1992, interest in AM has increased (De Koning & Rodenburg, 2004) (Figure 1) and AM is now established in many European countries, and in North America and Japan. In 2011, there were over 3,000 dairy herds in the Scandinavian countries that were automatically milked and every fifth cow was milked in an AMS (Gyllenswärd, 2012). In 2012, there were approximately 25,000 milking robots in operation around the world on about 18,000 farms. These numbers include all robot brands sold in the world (personal message, Perrotin, 2014).

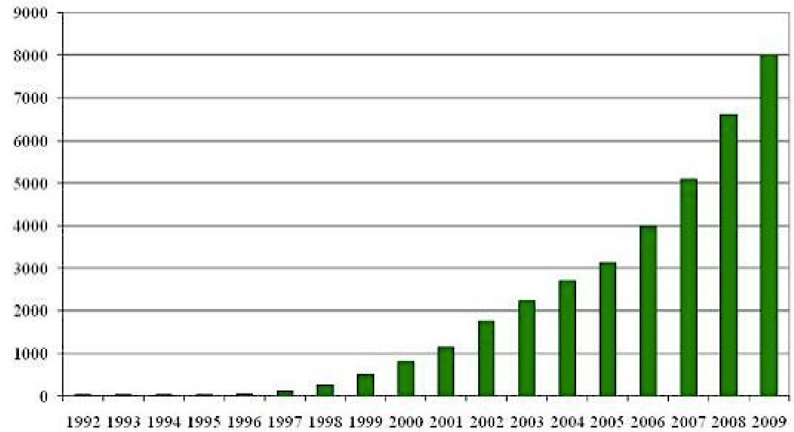


Figure 1. Growth of AM farms worldwide (1992-2009) since AMS was introduced in 1992 (De Koning, 2010).

Equipment and Cow Traffic in Systems with Automatic Milking

The expanding implementation of the AMS has continued. AM has a robotic arm to detect and put on the teat cup and to carry out teat cleaning, and also has a control system with sensors and software (De Koning, 2010). The efficiency of the AMS has the potential both to benefit milk production and to decrease labour. It has been projected that use of the AMS can increase milk production by up to 12% due to the possibility for increased milking frequency, can decrease labour by as much as 18%, and can also improve dairy cow wellbeing by allowing cows to decide when to be milked (Siegford & Jacobs, 2012).

The AMS includes both single and multi-stall systems. The single stall systems have integrated robotic milking functions, and can milk 55-65 cows more than two times a day. Multi-stall systems however, with 2-4 stalls, are able to milk 80-150 cows up to three times per day (Rotz *et al.*, 2003).

The AMS relies on the cow's own motivation to visit the feeding area and milking unit several times per day. The main motive for the cow to visit the milking unit is the supply of concentrates dispensed in a feed manger in the milking box during the milking process (Prescott *et al.*, 1998). Every cow in the AMS barn has an electronic cow-collar, which is part of a sophisticated cow identification system. There are sensors in the gates to read the collars. This is to guide the cows into the different areas in the barn, for example into the milking unit if she has milking permission, otherwise into the feeding or resting areas. The collars also ensure that the robot only attaches teat cups to cows considered acceptable for milking. Milk that is considered acceptable is transported into the bulk milk tank, otherwise milk that is not considered acceptable is transported to a separate system. This prevents milk containing antibiotics from entering the tank. As soon as the milk flow reaches the pre-programmed level of milk flow for detachment of teat cups they are detached. This is to avoid over-milking (DeLaval International AB, 2014), which can cause oedema in the teat and can have a negative effect on udder health (Hillerton *et al.*, 2002).

The AMS is equipped with sensors that detect and monitor abnormalities in the milk, which is necessary for the system to satisfy legislation and hygiene standards set by the dairy industry. Furthermore, the AMS is equipped with a teat cleaning system that also includes teat cup cleaning. The AMS sensors monitor and control the milking process, while storing data in a database. The dairy farmer has access to this database through a management program and is able to read or print attention lists and other reports displayed on the screen. If some sort of intervention is needed, the dairy farmer is informed quickly by a remote notification system (De Koning, 2010).

There are different forms of cow traffic in barns with AM. The free cow traffic enables cows to freely move around in the system, which allows the cows to set their own daily routine moving back and forth between the milking unit and the feeding and resting area (Ketelaar de Lauwere *et al.*, 2000; Thune *et al.*, 2002; Halachmi *et al.*, 2003). Forsberg (2008) describes two types of cow traffic, i.e. forced and selective. Cows in forced cow traffic must all pass through the milking unit in order to reach the feeding area. After feeding, the cows then pass through one-way gates to reach the resting area where they can lie down. Cows in selective cow traffic must all pass through a selection gate. This gate identifies and sorts the cows, directing cows with milking permission into the milking unit while allowing cows without milking permission to immediately enter the feeding area. Cows with milking permission are directed into the feeding area after milking.

Automatic Milking Rotary

The world’s first automatic milking rotary (AMR) was launched by the Swedish company DeLaval AB in 2010. The first commercial AMR was a carousel with 24 milking stations (Figure 2) and a maximum milking capacity of 90 cows per hour. It was designed to serve a herd size of 300-800 cows (Siegford & Jacobs, 2012).

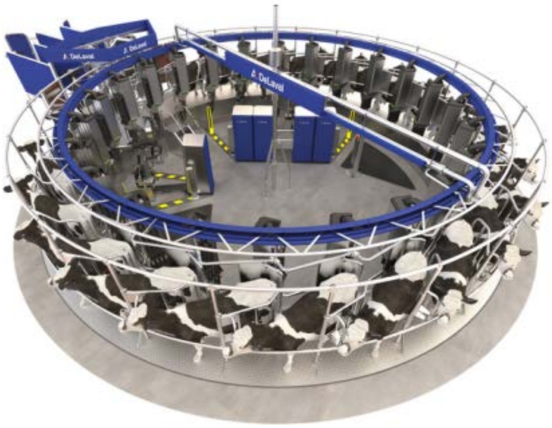


Figure 2. Computer image of AMR with 24 milking stations.
http://www.delaval.com/en/About-DeLaval/DeLaval-Newsroom/?nid=2718 (2014-06-09)

The AMR consists of three components: the teat preparation module, with one or two robotic arms, for cleaning and stimulating the teats (Figure 3), the automatic cup attacher with one or two robotic arms (Figure 3), and the robotic teat spray module for disinfecting the teats after milking. Each process (teat preparation, teat cup attachment, teat disinfection) takes 20-30 seconds per robot. The system uses historical records to determine expected milk production per udder quarter (UQ), while also regularly updating the coordinates of each teat. This speeds up the process of locating the teat. A laser camera is used to detect the cow’s precise position and detect the teats so that the teat cups can be attached (Hunter Nilsson, 2014). The teat preparations module and the automatic cup attachers stay in the stationary position after preparing the cow for milking. The carousel platform continues to rotate the cows from the entry point to the exit point.

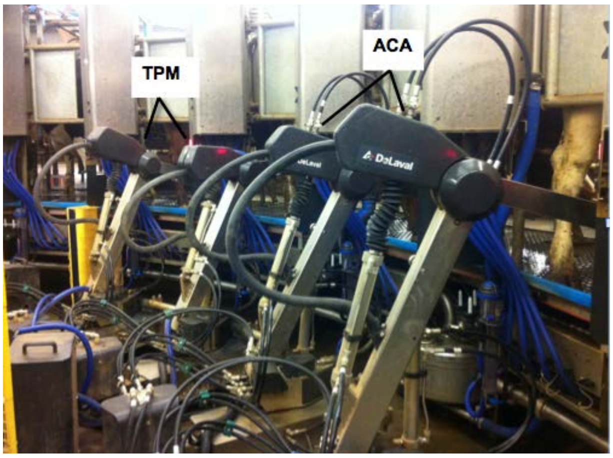


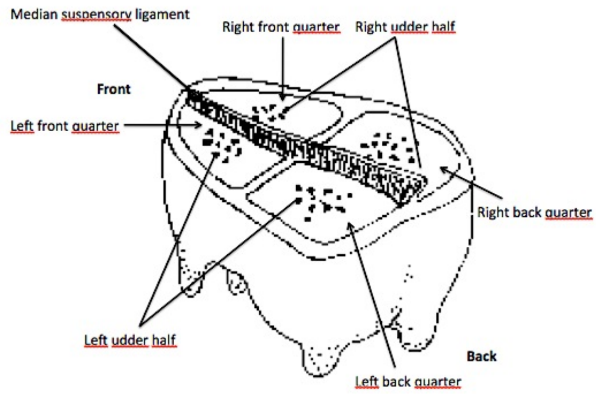
Figure 3. The four robot arms in the 24-station AMR. Two arms are the teat preparation module (TPM) and two arms are the automatic cup attacher (ACA). (Photo: Ida Ljunggren).

Anatomy and Physiology of Milking

To understand the process of milking and the prerequisites for the AM, it is of importance to have knowledge about the biology of lactation. Therefore an overview of the anatomy and physiology of the bovine udder that is relevant for understanding milking is presented here.

Anatomy of the Udder

The bovine udder consists of four quarters: Right Front - RF, Left Front - LF, Left Back - LB, Right Back - RB, each with its own mammary gland. The bovine mammary gland consists of alveoli, milk ducts, a gland cistern, sphincter muscle, and the associated teat, which consists of a teat cistern and a streak canal (Figure 4a). The four udder quarters are separated by connective tissue and the median suspensory ligament divides the udder in left and right udder halves (Figure 4b).



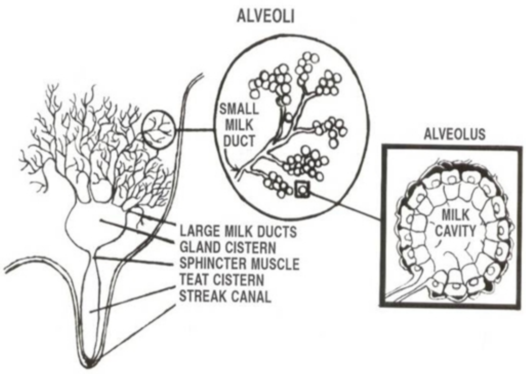


Figure 4a. Anatomy of one udder quarter. http://www.milksmart.co.nz/How-to-Improve/The-milking-process/Milk-let-down/Milk-let-down/

Figure 4b. A cross-section of an udder Modified from: http://www.fao.org/docrep/t0690e/t0690e05.htm

Each udder half receives its blood supply through the respective artery, which branches into smaller arteries out into the udder tissue. The veins run parallel to the arteries and the two major veins of the udder halves are connected to each other through a shunt that allows blood to pass from one udder half to the other (Sandholm, 1995). The back udder quarters are usually larger than the front udder quarters. This means that the back quarters have a higher milk yield, longer milking time and higher peak flow (Tancin *et al.*, 2006). In a study by Berglund *et al.* (2007) it was found that there was no difference in milk yield or the milk components fat, protein and lactose in respective pairs of healthy front and back quarters.

Milk Formation

Milk synthesis is an independent process in each quarter and in a healthy udder no milk from one quarter can pass over to another. In high-yielding dairy breeds, the total milk volume at UL may exceed 25 liters (Sjaastad *et al.*, 2003b). At udder quarter level (UQL), there is a high correlation between the blood flow to the mammary gland and milk yield i.e. good blood flow will lead to high milk yield (Davis & Collier, 1985).

Milk is a water-soluble liquid consisting of many components, where the major components are lactose (4.6%), milk fat (4.0%) and protein (3.3%) (Walstra *et al.*, 2006). Many factors have an impact on milk composition, for instance breed, age, lactation stage, season, nutritional management and mammary gland health (Wolfson & Sumner, 1993).

Milk formation occurs in the alveoli (Figure 5). The main substances that ruminant epithelial cells must receive from the blood in order to synthesize lactose, milk protein and milk fat are glucose, amino acids, volatile fatty acids and long chain fatty acids. Furthermore, certain vitamins and some proteins, e.g. immunoglobulins and plasma albumin, are also transported, unchanged, through epithelial cells into the lumen of the alveoli, where all milk components accumulate and form milk. Lactose is a disaccharide consisting of one glucose molecule and one galactose molecule and is formed by enzymes in the Golgi apparatus. In the same way as proteins, lactose undergoes exocytosis, and is transported out of the secretory cell. On the way out to the lumen, water is absorbed into the vesicle by osmosis (Sjaastad *et al.*, 2003b). The synthesis of major proteins, including the caseins ß-lactoglobulin and a-lactalbumin, occurs in the mammary epithelial cells in the rough endoplasmic reticulum. Another substance that also is secreted from mammary epithelial cells is milk fat which is secreted as fat globules in the lumen of the alveolus (Džidic, 1999).

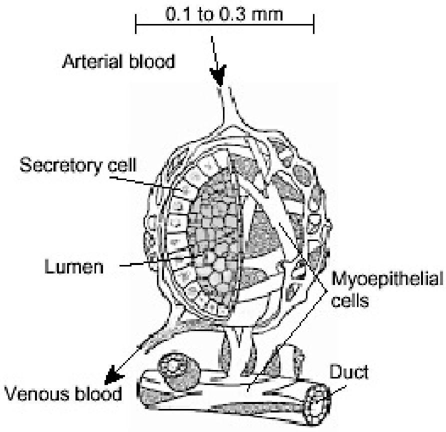


Figure 5. The structure of the alveoli (Wattiaux, 2014)

Hormones Released during Milking

During milking, a variety of hormones that are important for the lactating dairy cow are released. The hormones prolactin and cortisol are released in the blood stream of the dairy cow during milking, along with the milking related release of oxytocin (Gorewit *et al.*, 1992).

Oxytocin is a peptide hormone that is required for successful milk ejection (Mepham, 1987). Oxytocin is produced by neurons in the hypothalamus, and is transported via nerve fibres to the pituitary gland from where it is then secreted into the blood. Oxytocin is transported via the blood to the udder, where it binds to receptors on the myoepithelial cells. Oxytocin triggers the myoepithelial cells around the alveoli to contract so that the milk leaves the alveoli and reaches the udder cistern (Gimpl & Fahrenholz, 2001).

Prolactin is a peptide hormone that is produced in the pituitary gland. The secretion of prolactin into the blood is regulated by the hypothalamus. Prolactin influences milk synthesis and plays an active role in maintaining milk secretion (Knight & Flint, 1995). Prolactin also triggers cell differentiation and cell proliferation (Akers & Lefcourt, 1982; Sjaastad *et al.*, 2003a).

Milk Let-Down Reflex

The main fraction of milk, more than 80%, is stored in the alveoli where it is tightly bound by capillary forces. The remaining fraction of milk (less than 20%) is stored in the udder cistern and the larger milk ducts, and it is immediately available during suckling or milking before the release of oxytocin in the blood stream (Bruckmaier & Blum, 1998).

For the synthesized milk to be available for the calf or the milking machine, the milk has to be expelled from the alveolar compartment to the udder cistern. When milk is shifted from the alveolar compartment to the cisternal compartment, milk ejection or milk let-down occurs (Bruckmaier & Blum 1998). In order for milk let-down to occur, it is required that the oxytocin in the blood is above a certain threshold level.

A well-executed pre-stimulation of the udder and teats as well as other positive stimulation during milking, leads to efficient emptying of the udder, which has been reported to have a positive effect on the milk yield (Rasmussen *et al.*, 1990). The milk ejection reflex is dependent on stimulation of the receptors on the teats and udder that send a signal through the nervous system to introduce the release of oxytocin from the pituitary gland (Akers, 2002). The calf suckling stimulates nerve fibres in the teats, or when the robotic unit is cleaning the teats before milking. Calf suckling is the strongest stimulation, followed by hand milking and then machine milking. However, machine milking is usually enough to trigger the milk ejection reflex. Depending on how filled the udder is, the time required for stimulation of milk ejection varies from 40 seconds up to two minutes. It takes longer time for a semi-filled udder to achieve milk ejection since the myoepithelial cells must contract more to squeeze out the milk (Bruckmaier *et al.*, 1994; Bruckmaier & Hilger, 2001).

It is important to pre-stimulate the teat(s) before milking for a steady milk flow. Otherwise, the milk flow can be reduced or completely stop when the milk available in the udder cistern is milked out. To release all the milk in the udder, the oxytocin levels must be maintained by continuous stimulation throughout the whole milking occasion. There will always be some milk left in the alveoli after milking, even when oxytocin levels are high during milking. This remaining amount of milk in the alveoli is called residual milk and consists of 10-30% of the total volume of milk (Bruckmaier & Blum, 1998).

Bruckmaier *et al.* (2001) concluded that there was a critical time limit of two minutes between pre-stimulation and the start of the milking process due to the fact that oxytocin levels decreased after two minutes if stimulation was not continued. Decreased oxytocin levels can lead to increased amounts of residual milk. Bruckmaier *et al.* (2001) considered teat cleaning devices in the AMS to be suitable also for carrying out pre-stimulation to avoid delay in the whole milking process.

Pre-stimulation and continuous stimulation of the teats throughout the whole milking process is important for milk ejection and efficient udder emptying. This is also especially important for cows in a late lactation stage or if a cow is directed into a second milking shortly after the first milking (Bruckmaier & Wellnitz, 2008).

Milking Routine

A high-yielding cow in good health will ensure productivity/profitability and cow wellbeing. To achieve these goals, the correct design of milking routines is crucial.

Milking Preparation

Regardless of milking system, whether it is a conventional milking system or the AMS, the cow has the same needs, in other words a stress-free environment and a rapid and smooth milking process that includes correct teat and udder stimulation. The herdsman’s responsibility in conventional systems and the AMS is to ensure that these needs are satisfied.

The optimum milking process is a fast and complete milk removal to achieve high milk production and maintain good udder health. The milk ejection reflex must be induced and a complete milk ejection must occur during each milking. Prolonged intervals from start of the udder preparation until cluster attachment can have a negative effect on milk yield and milking time (Mayer *et al.*, 1984; Bruckmaier & Wellnitz, 2008).

Animal-human contacts have noticeable effects on the behaviour and productivity of farm animals, including dairy cows (Hemsworth, 1997). This is why the most effective milking routine also depends on the co-operation between the animal and the herdsman. Stepping and kicking by the cow during milking may be a sign of pain or discomfort. Therefore, for monitoring cow wellbeing during milking as well as udder health problems, step and kick behaviour might be a good measurement tool (Rousing *et al.*, 2004). A study by Wenzel *et al.* (2003) compared levels of restless behaviour in the AMS versus the auto-tandem milking parlour where personal manually attach the milking unit, and observed that there were higher levels of stepping, foot-lifting and kicking in the AMS. Studies made with the AMS by Hopster *et al.* (2002) found that the way that the teat cups were detached caused greater step-kick behaviour. If the teat cups were detached one at a time as each udder quarter was finished milking, this caused high step-kick rates at the end of the milking.

A pleasant milking environment together with gentle handling has many positive effects on the cow and the milking occasion. Studies done by Seabrook (1994) showed that, compared with aversive handling, cows milked with pleasant handling entered the milking shed more quickly, produced 13% more milk and were dunging less on the platform.

To avoid the spread of pathogens and bacteria between cows in the herd, it is important to have strict milking routines and good milking hygiene (Bartlett *et al.*, 1992). Infected animals must be separated from healthy animals by grouping and milking sequence. Maintaining good hygiene, keeping teat ends healthy, teat disinfection both before and after milking, and using correctly functioning milking equipment are effective preventative methods to control the spreading of mastitis (Oliver, 2008).

Cow Motivation to visit the Milking System

A desirable milking routine includes incentives for the cow to be highly motivated to be milked and that the milk ejection starts rather quickly. As mentioned above, studies have found little proof to support the assumption that the cows will voluntarily enter the milking unit in the AMS to be milked. Both the hypothesis that an increased milking interval or a full udder would motivate the cows to be milked could not be supported. When cows had the opportunity to choose milking and feeding, they always chose feeding (Prescott, 1996).

A problem related to low motivation to be milked in the AMS is the queue that can be formed in the waiting pen. By avoiding unnecessary waiting time in the waiting pen, milk leakages can be decreased and cows have more time to eat and rest, which increases the efficiency of the system (Ketelaar de Lauwere *et al.*, 2000). Results from different studies (Schukken *et al.*, 1990; Waage *et al.*, 1998) have shown that milk leakage was associated with an increased risk for mastitis. Stefanowska *et al.* (2000) found that milk leakage from the udder was seen in 60% of the cows following a missed milking.

Milking Frequency

Milking routines have changed along with milking frequency (MF) during the development of the AMS and the AMR. Cows may, within certain limits pre-set by the dairy farmer, choose when and how often they want to be milked in AMS or AMR.

An increased milking frequency (IMF) has been shown to have many benefits like increased milk yield and lower levels of milk SCC, which in turn results in better milk quality and udder health (Kelly *et al.,* 1998; Smith *et al.,* 2002). Studies have shown that MF can affect the persistency of the lactation curve, and may lead to prolonged lactation (Pettersson *et al.,* 2011; Österman & Bertilsson, 2003) and in the long run, increase the milk yield by 10-15% (Blowey & Edmondson, 2010).

Effect of Milking Frequency on Milk Composition

An IMF increases the milk yield and impacts the milk composition (protein, lactose and fat). Provided that the dairy cow is given the right amount and the right proportions of nutrients, it has been shown that milking more than twice a day will increase the milk yield, but decrease the percentages of fat and protein (Erdman & Varner, 1995; Smith *et al.,* 2002; Österman & Bertilsson, 2003).

Milk is an emulsion, which means that the fat fraction has a tendency to separate from the water-soluble components. This is because milk fat has a lower specific gravity than water, which causes the fat fraction to ascend within each alveolus. As a result, the fat concentration increases toward the end of milking. In addition, fat droplets during milking may move less rapidly than the aqueous phase (Ontsouka *et al.,* 2011). Studies have shown that there is a higher proportion of residual milk present in cows milked three or four times daily compared with two times daily. This may be a result of the decrease in fat associated with IMF (Rasmussen *et al.,* 1989). 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 milk fat and protein and an increase in free fatty acid levels has been reported (Klei *et al.,* 1997). 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 flavor of milk (Slaghuis *et al.,* 2004).

The structural changes of the protein may partly be due to the amount of time that the milk is stored in the udder. The shorter the time the milk remains in the udder, the shorter the time the enzyme plasmin has to break down the protein. This was observed by Klei *et al.* (1997) who studied the protein degradation in the udder of early, mid and late lactating Holstein cows where the cows were exposed to a 3 times/day milking routine versus 2 times/day. The results indicated that a 3 times/day milking routine improves milk protein quality since the plasmin has less time to degrade the casein. A higher level of casein in the milk is considered to be more valuable since the casein fraction determines the cheese yield. The milk price includes the protein content (Harding, 1999).

Several studies have reported different results regarding the effect of MF on SCC levels in milk and thereby on udder health. Some studies show that increased MF lowers the SCC levels (Klei *et al.,* 1997; Dahl *et al.,* 2004), while others report no such effect (Shields *et al.,* 2010). The lower concentrations of SCC due to increased MF may be a result of frequent removal of milk, making it more difficult for the bacteria to attach to the epithelial cells. Furthermore, frequent milking flushes the teat canal more often during removal of the milk and leaves shorter time for bacteria to grow inside the udder between milkings. However, more frequent milking also means more frequent opening of the teat canal, which increases the risk of bacteria entering the udder and causing mastitis (Rasmussen *et al.*, 2001).

Prolonged Milking Interval

A prolonged milking interval can occur during a technical stop in AMS and can cause an increased SCC level in the bulk tank. Lakic *et al.* (2011) investigated how the peak in SCC, which was a result of a prolonged milking interval of 24 h, affected the total milk quality and milk yield. The results showed that the milk yield was reduced by 0.75 kg/day for up to 10 days. A slight change in the milk composition was observed, but no major effects on the milk quality were noted. The SCC was three times higher during the 1-2 days after the prolonged milking interval compared to the level before.

A study by Clark *et al.* (2006) compared once-daily milking for Holstein-Friesian cows and Jersey cows. In both breeds the milk yield decreased when the MF changed from twice-daily milking to once-daily milking. However, the decrease in yield was greater for the Holstein-Friesian cows. Clark *et al.* (2006) also found an increase in SCC during once-daily milking compared with twice-daily milking, and a significant decrease in milk lactose content. Studies by Stelwagen & Lacy-Hulbert (1996) and Stelwagen *et al.* (1997) concluded that the milking interval must be less than 18 h to prevent unfavourable effects on milk yield and milk quality.

Udder Health

Optimal milk production requires good udder health. A disease that impairs udder health is mastitis.

Mastitis

Mastitis is an inflammation of the mammary gland and udder tissue. It is the most common disease among dairy cows and impairs the health and welfare of the cows (Dodd & Booth, 2000). Furthermore, it leads to increased risk of antibiotic residues both in dairy products for human consumption and in cow manure that is spread on fields (van Schaik *et al.*, 2002).

Mastitis can occur in two different forms: clinical and subclinical. The most prevalent form of mastitis is subclinical. In subclinical mastitis there are no visible signs. However milk production decreases and there is bacterial growth in the milk, which causes dramatic changes in milk composition (Harmon, 1994). In milk from cows that have mastitis, there is a very significant negative correlation between SCC and lactose content. Lactose content is reduced by about 10% due to mastitis (Korhonen & Kaartinen, 1995).

In clinical mastitis there are visible signs in the udder and/or in the milk. The principle visible signs are hot, swollen and painful udder quarters. The milk may also contain blood, clots and flakes. Clinical mastitis can be divided into mild (only changes in milk characteristics), moderate (also altered mammary tissue) or high grade (including fever, poor general condition). Mastitis can also be divided into acute and chronic, depending on how long the disease lasts.

The reason that mastitis has a negative impact on the economy of dairy production is because it affects milk quality and decreases the milk yield. During mastitis the synthesis of lactose, fat and protein is disturbed through damage to milk secretory cells in the mammary gland (Schallibaum, 2001). Elevated SCC does not only change the milk composition, it also causes a reduction in milk yield (Jones, 2009). Milk from cows with mastitis has an increased SCC, and the resulting impaired quality and composition leads to lower payment for the milk to the dairy farmer. Cell counts as well as fat content are included in the dairy companies' quality payment system. Different dairy companies deduct different amounts according to the cell count (Andersson *et al.,* 2011).

To improve udder health and to further understand the mastitis problem, Forsbäck *et al.* (2010) indicated that there is a need to follow the changes in milk composition at udder quarter level (UQL). Normally, only one udder quarter is affected if the cow is suffering from mastitis and a reduction in lactose content can be seen during early mastitis. Therefore, if samples are taken from the bulk tank or at UL and not at each individual UQL it can be difficult to detect mastitis at an early stage.

Somatic Cell Count

Measuring milk SCC is a major tool used to monitor udder health for management and selection purposes (Rodriguez *et al.,* 2000). The main important factor causing an increase in SCC is mammary inflammation usually caused by infection. See Table 1 for cells and their percentage found in normal bovine milk.

Table 1. Percentage of cells found in normal bovine milk

Cell type	% Cells (range)
Neutrophil (PMN)	0 - 11
Macrophage	66 - 88
Lymphocyte	10 - 27
Epithelial (ductal)	0 - 7

From Lee *et al.,* 1980

When leukocytes enter the mammary gland in response to invasion of the teat canal, mainly by bacteria, the udder becomes inflamed and swollen. The bacteria in the teat canal increase and produce toxins that cause damage to milk secretory tissue and the milk ducts in the mammary gland (Jones, 2009).

Milk SCC can vary from day to day in the udder quarters. In a healthy udder, the relative day-to-day variation can be as much as 10% (Sjaunja, 1986). For an UQ to be considered healthy, several studies have suggested a limit of 100,000 cells/ml (Hillerton, 1999; Hamann, 2005). However, milk SCC level over 200,000 cells/ml at udder level indicates mastitis (Hillerton, 1999).

The variation in SCC can be caused by changes in the cow's routines or other disturbances in the milking process (Klastrup *et al.*, 1987; Harmon, 1994). According to Klastrup *et al.* (1987), the variation is not always related to inflammation. SCC also varies according to the season (Schukken *et al.*, 1990). A study on sheep by Kukovics *et al.* (1996) concluded that there were significant differences in SCC between breeds and that SCC was higher in the afternoon than in the morning. Kukovics *et al.* (1996) also found that SCC increased with the age, year and number of lactations. Temporary increases of milk SCC can also be due to reasons such as physical stress (Yagi *et al.*, 2004).

Lactose

One milk component that gives milk and other dairy products valuable properties is lactose. Unlike fat and SCC that have an impact on the price of milk, lactose content does not have an impact on price in today's dairy market. Despite its contribution of valuable properties to milk and other dairy products, lactose doesn't have any nutritional properties that would result in greater economic value (Laben, 1963).

A rapid drop in lactose content may be associated with cell damage, supposedly due to mastitis. With a day-to-day variation of only 1% (Rook *et al.,* 1992, Forsbäck *et al.,* 2010), lactose content is the most stable milk component during the whole lactation period, due to its osmotic regulatory effect (Ling *et al.*, 1969). In this way, there is an increase in lactose content during early lactation, while the lactose content decreases during late lactation due to the involution of the gland (Auldist *et al.*, 1995; Lacy-Hulbert *et al.*, 1999). A few pathogens have the ability to ferment lactose, which also affects the lactose content in milk. Damage to the milk secretory cell may cause reduced synthesis of lactose and can cause leaking between the tight junctions between the milk secretory cells, which also leads to a decreased lactose content (Auldist *et al.*, 1995).

Berglund *et al.* (2007) investigated the difference between front and back quarters in milk production and lactose content. The back quarters had a higher milk production per hour and increased lactose content. The study also indicated that SCC had a statistically significant negative correlation with milk production per hour and with lactose content.

Conductivity

One detection method for subclinical mastitis is electrical conductivity. Electrical conductivity measures the concentration of anions and cations in the milk. If the cow has mastitis, the electrical conductivity increases because sodium and chloride concentrations increase and concentrations of lactose and potassium decrease in the milk. Changes in concentration are most likely due to damage in the udder tissue (Kitchen, 1981).

Pros and Cons of AMS and AMR

It is a major investment for the dairy farmer to install an AMS (Castro *et al.,* 2012; Priekulis & Laurs, 2012) or an AMR. The AMS and the AMR have several similar functions and both have advantages and disadvantages.

Pros

The main and most crucial reasons for investing in an AMS or AMR are, as mentioned above, the reduced manual labour required for milking and the possibility to increase MF. MF and the entire lactation yield are correlated; when increasing the MF from twice to three times per day, the entire lactation yield was increased from 6 to 25% (Erdman & Varner, 1995). A further advantage is that the herdsman can also adapt the system to the lactation stage. Studies have shown that increased MF during early lactation increased total milk production significantly (Svennersten-Sjaunja & Pettersson, 2005). In short, the benefits can be expressed in three aspects: profitability, farm management and flexibility.

Profitability is a result of reduced milking labour, increased milk yield, improved milk quality and lower milk harvesting costs. Smoother and more efficient farm management makes the workplace more attractive for farm employees. In addition, the herdsman gets more time to focus on each animal's welfare (DeLaval International AB, 2014).

AMS and AMR suit many types of farming operations and are very flexible. The herdsman can collect and view data and follow up historical data on each cow via the management computer that controls the whole system (DeLaval International AB, 2014). With an AMS or AMR, the farmer can choose how often the cows should be milked and adapt this to the different lactation stages. As mentioned above, several studies have shown that MF of two or three times per day increases the entire lactation yield (Klei *et al.,* 1997; Österman & Bertilsson, 2003; Wagner-Storch & Palmer, 2003; Speroni *et al.,* 2006). Furthermore, the dairy farmer can adapt the AMS or AMR to the relevant management system.

Cons

A main disadvantage of AMS and AMR is the high investment cost. The initial cost for a given herd is often two to three times greater than for a traditional milking parlour (Rotz *et al.,* 2003). Another disadvantage related to AMS and AMR is the waiting time for the cows before milking in the waiting area. This is most problematic for the cows that are low in the herd hierarchy. Instead, cows could maximize their time in the barn and have more time to eat and rest. In this way the cows would have less risk of developing lameness and disease (Ketelaar de Lauwere *et al.,* 2000).

Studies have been done on whether the use of AMS affects the milk SCC. After AMS was established it was observed that milk SCC increased for cows milked in an AMS (Klungel *et al.,* 2000; Rasmussen *et al.,* 2001). However, other studies have proved that if both cow health status and herd management are good from the beginning, milk SCC does not increase (Zecconi *et al.,* 2003). Reports have shown that higher milk SCC is the result of cows not being able to enter the milking unit due to technical stoppages, and this causes the cows to start leaking milk. It has been found that increased milk leakage increases the risk of mastitis. It has been indicated that there is greater leakage of milk in AM compared to conventional milking probably because the cows have to queue up to enter the robotic unit (Persson Waller *et al.,* 2003). During stoppages in a conventional milking system, all cows can be milked rather quickly, compared to an AMS or AMR where some cows during stoppages are forced to experience extremely long intervals between milkings. Stoppages up to four hours showed an increase in bulk milk SCC from 50,000 to 250,000 cells/ml. Repeated stoppages also increased the bacteria count, which highlights the importance of service and spare parts being available on short notice to minimize downtime of the AMS (Pettersson *et al.,* 2002) and the AMR.

Differences between AMR and AMS

Both the AMR and the AMS are technically advanced processes. The AMS is a system running 24 hours per day. With the AMR, the herdsman can choose how often the system should run. In the AMS, only one cow at a time can be milked and the cows are usually fed with concentrate while being milked, which generates a high motivation for visiting the milking unit. In the AMR, the cows are usually not fed with concentrate while being milked.

Incomplete Milking

The term incomplete milking implies that an unacceptable amount of milk is left in the udder after teat cups are detached. Since the AMR is a system that rotates during the milking occasion, the robotic arm does not remain with the cow during the entire milking process. This means that cows who kick off the teat cup(s) or if the robot fails to attach the teat cups on one or more udder quarters, the cow will leave the AMR with one or more udder quarters incompletely milked or un-milked. In the AMR, when a cow is identified as incompletely milked she is directed to a new milking permission in a 2nd milking attempt shortly after the 1st milking attempt.

Few studies have been done relating to incomplete milking. However, a study by Wheelock *et al.* (1965) investigated the effect on milk yield and milk composition with an incompletely milking or an extended milking interval. During the 1st milking after treatment, the milk yield increased due to a carry-over effect, but through the 2nd milking the yield was unchangeably low and then increased until the original yield, or marginally less, was improved in five days. Overall, the concentrations of lactose and potassium decreased, while sodium, chloride, casein, whey proteins and fat increased. In studies where the cows are milked in conventional systems it has been observed that a single prolonged milking interval of 24 hours has a negative effect on milk yield and milk SCC at udder level (Lakic *et al.,* 2009, 2011).

Studies by Stefanowska *et al.* (2000), reported that after an omitted milking (OM), cows urinated or stood more in the cubicle after leaving AMS instead of lying down. These behaviours are signs of discomfort. Metcalf *et al.,* (1992) demonstrated that there was 25% higher blood flow to the mammary gland when the cows were lying down instead of standing. A study by Österman and Redbo (2001) reported that cows milked twice daily displayed signs of discomfort by standing up for long periods while ruminating, but in contrast, cows milked three times daily displayed signs of comfort by lying down more often for periods of 90 minutes or more.

Bach and Busto (2005) did a study with the AMS where they investigated the effect of teat cup attachment failures and MI regularity on milk production. The results showed that the average attachment failure rate was 7.6% of total milkings (35,291 milkings). Attachment failures caused a 26% decrease in milk production with an extended milking interval compared to the regular milk yields. The study also showed that milk production recovered its level prior to the attachment failure within seven milkings.

Kolbach *et al.* (2012) did a study in a prototype robotic rotary where failed attachment during the 1st milking was referred to as incomplete. The percentage of incomplete milkings during the 1st milking was 19%. During the 2nd milking attempt (when the cows were directed to a new milking) the success of attachment was 48%. In the study Kolbach *et al.* (2012) also found a correlation between milk yield, attachment success and complete milking at the 2nd milking attempt. A higher production level resulted in a higher success of attachment and complete milking, and Kolbach *et al.* (2012) made the assumption that this probably was due to that a fuller udder makes it easier for the automatic attachment device to locate the teats.

Material and Method

The study was carried out at Swedish Livestock Research Center, Lövsta, Swedish University of Agricultural Sciences, Uppsala during autumn 2013 and spring 2014. The study was divided into Experiment 1 and Experiment 2. Experiment 1 was a survey where the frequency of unsuccessful milkings (including incomplete milkings) was recorded. In Experiment 2 the effect of OMs on one udder quarter was studied. The experiments were approved by the Uppsala Ethical Committee.

In the present study, the term *unsuccessful milking* refers to a milking occasion where some sort of failure occurs during the milking process, for example failed teat cup attachment, kick off and/or tramp on tube. Included in the concept of unsuccessful milking is the term *incomplete milking*, which in the present study refers to a milking occasion where the AMR selects the cow for a 2^{nd} milking attempt. It is important to note that a cow could be registered manually as an unsuccessful milking in the Experiment 1 template even though the AMR did not select the cow for a 2^{nd} attempt.

Housing and Management of Cows, Experiment 1 and 2

The dairy cows in both experiments were of the breeds Holstein and Swedish Red and they were kept in a loose housing system. The average production in the herd from which the cows were chosen for both experiments was 10,100 kg energy corrected milk (ECM) and the average SCC was 193,000 cells/ml in the year 2013. All cows had free access to grass silage and had access to concentrate from concentrate feeders according to their milk production. The concentrate allowances were chosen with the intention to obtain a proportion of silage/concentrate of 70/30 in total ration on dry matter basis.

The cows were milked in an AMR provided with 24 milking stations, with stationary robotic arms for teat preparation and attachment of teat cups before milking as well as for disinfection spraying teats after milking. The AMR milking started at 05:15 am and 03:45 pm.

Milk Samples, Experiment 1 and 2

All milk samples were collected from each cow directly after the teat cleaning procedure and before the teat cup attachment. Approximately 20-30 ml milk from each udder quarter was collected. Note that during the milking occasions when the OM occurred, milk samples were only taken from LF, LB and RB udder quarter since the RF was the udder quarter to undergo the OM treatment, as per the following:

Milk samples taken at UQL during regular milking occasion

LF	RF
LB	RB

Milk samples taken at UQL during milking occasion with omitted milking (OM)

LF	**OM**
LB	RB

An antibacterial agent, Bronopol (2-bromo-2-nitropropane-1,3-diol), was added to the samples intended for analysis of SCC and lactose, and milk samples were stored at +8 °C until the analysis was performed.

Experiment 1

Experimental Design

Experiment 1 was carried out on all healthy cows milked in the AMR system. The experiment was carried out over five days and eight milkings were included (day one, two, four and five). On day three the teat and udder assessment was done. The average total number of cows registered in the template was 171 cows. The number of total milkings was 1496.

The design of the experiment was a so-called "observation study". The registration template was used to record the frequency of unsuccessful milkings of cows in the AMR, the reason for the disturbance during the milking and at which udder quarter(s) the disturbance occurred (Appendix 1). Teat assessment was done on all the cows and udder conformation was noted.

Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
March 31	April 01	April 03	April 22	April 23
2 milkings	2 milkings	Teat Assessment and notation of Udder Conformation	2 milkings	2 milkings

Animals

Since all cows milked in the AMR participated in Experiment 1 the status of the cows varied with regard to age, parity and lactation stage. During the first four months in the year 2014 the average milk production in the AMR herd was 29 kg ECM/cow and average milk SCC was 194,000 cells/ml milk.

Staff Routines

The staff routines for milking were changed in one aspect. Normally, in the case of failed teat cup attachment, kick off or tramp, the staff would immediately attach the teat cup. For the purpose of this study the staff did not re-attach the teat cups during the 1st milking occasion. If the cluster attachment failed, or the cow kicked off the cluster or tramped on the hose during the 2nd milking the staff attached the teat cups manually.

AMR Settings

The AMR settings for each cow in this study were the settings that were pre-programmed, in other words no changes in the AMR settings were made for this study. The AMR was set to the expected amount of milk (%) for each individual cow so that the AMR could identify cows that were completely milked and subsequently direct them to the resting and feeding area. When the AMR identified a cow as incompletely milked, the cow was automatically directed into the waiting pen for a new milking permission, in order words, the cow was directed to a 2nd milking attempt. These incomplete milkings were also registered as unsuccessful milkings in the template. An udder quarter was considered incompletely milked when the milk yield was < 50% of expected yield, provided that the expected yield was > 1 kg or the total milk yield at that specific udder quarter was < 3 kg. If a cow had milked more than her individual pre-programmed limit, the AMR could designate her as completely milked even though she had kicked off a teat cup. If a cow tramped on the milking tube, the teat cup(s) would often fall off.

The control unit (Figure 6) registered every cow that entered the AMR. The individual milk yield for each udder quarter was displayed on the screen. An "X" appeared in the individual cow's position on the screen if the robot missed attachment of the teat cup(s) or if the cow kicked off the teat cup(s) or tramped on the milking tube so that the teat cup(s) fell off.

Figure 6. DeLaval control unit in the AMR.
https://www.flickr.com/photos/118643446@N08/12900486195 (2014-05-20)

Recordings and Milk Sampling

A registration template (Appendix 1) was filled in during the milking over a period of four days (8 milkings) to identify the teat(s) affected and to document the frequency of:

- Unsuccessful milking
    - Failed attachment of one or more teat cups
    - Kick off of one or more teat cups
    - Tramps on the milking tube resulting in a teat cup falling off
    - Incomplete milking (AMR selects the cow for 2nd milking attempt)

In the registration template, "Kick off" is divided into three categories: Kick off at automatic cup attacher position, Kick off during the milking process and Kick off during the detachment of the teat cups. "Tramp" refers to hoof on the milking tube causing teat cup(s) to fall off.

In the registration template, a cow was registered under "2nd attempt" if she was automatically directed by the AMR to the waiting pen for a new milking permission.

For those cows that had kicked off the milking unit during morning milking, milk samples were collected the same day during evening milking per UQL for analysis of milk SCC to check if the reason for kicking off the unit could be due to pain caused by inflammation.

Teat Assessment

Teat assessment (Appendix 2) was done on all the cows. The assessment included udder conformation, teat position, teat ends and warts/sores on teats. To identify the condition of RF, LF, LB and RB teat ends, a scoring system was used where the number 0 refers to the desirable teat and number 3 refers to the undesirable teat (Figure 7). The teat position was also assessed and scored, where the number 0 refers to teats that are crossing over each other, number 2 refers to the desirable position (teats suspended perpendicular to the ground) and number 4 refers to teats splayed outwards. Number 1 refers to the position between number 0 and number 2. Number 3 refers to the position between number 2 and number 4. See Figure 8 for teat position 1, 2 and 4.

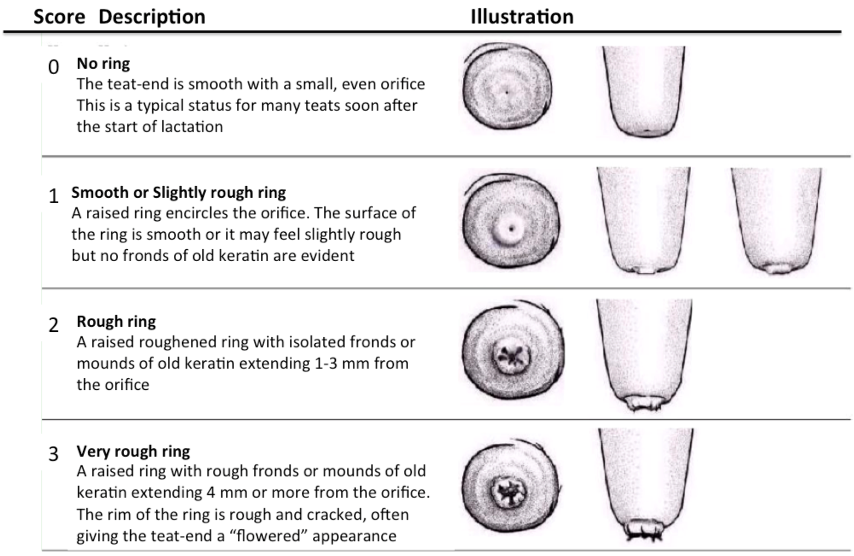


Figure 7. A modified scoring system for teat-end condition (Mein et al., 2001).

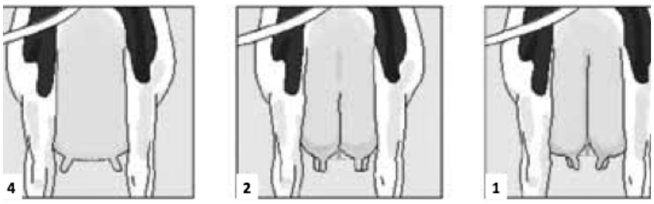


Figure 8. A modified scoring system for teat position. From the left a cow with teat position 4. In the middle a cow with teat position 2 and on the right a cow with teat position 1. (http://www.theorganicfarmer.org/how-to-select-a-good-cow/ (2014-05-05)).

Milk Analysis

Milk samples were analyzed for milk SCC using fluorescence-based electronic cell counting (Fossomatic 5000, A/S N. Foss Electric, Hilleröd, Denmark).

Statistical Analysis

The data are presented as descriptive data for the frequency of the different registrations. The calculations for mean and SD were done with Microsoft® Excel program.

Experiment 2

Experiment 2 consisted of two different experimental treatments, A and B with eleven cows in each treatment. Treatment A and Treatment B both started on 14 October 2013. Treatment A ended on 27 October and Treatment B ended on 31 October 2013.

The treatment was OM, where one udder quarter was not milked, i.e. the teat cup was not attached. Treatment A consisted of one OM in a total of 28 milkings. Treatment B consisted of three OMs in a total of 36 milkings. The OM was carried out on the RF quarter in both Treatment A and Treatment B. In Treatment A the OM occurred at the pm milking on day 4. In Treatment B the OM occurred at the pm milking on day 4, 6 and 8. The period before the first OM is referred to as *Baseline* and the period after the last OM is referred to as *Post-OM*. For both Treatment A and Treatment B the baseline consisted of seven milkings and the Post-OM continued for ten days and consisted of twenty milkings (Table 2).

Table 2. *Time-line for Experiment 2.*
Treatment A = 14 days (28 milkings in total, one omitted milking at right front udder quarter at afternoon milking day 4)
Treatment B = 18 days (36 milkings in total, three omitted milking at right front udder quarter at afternoon milking day 4,6 and 8)
Note that in the Milking *row, odd numbers represent* am *milkings and even numbers represent* pm *milkings*

Treatment A

Period	**Baseline**							**OM**	**Post-OM**																			
Milking	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Day	1		2		3		4		5		6		7		8		9		10		11		12		13		14	

Treatment B

Period	**Baseline**							**OM**									**Post-OM**																			
								OM				**OM**				**OM**																				
Milking	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Day	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18	

Animals

Experiment 2 included 22 clinically healthy cows (Appendix 3), with no clinical signs either in the milk or at UL. The cows were selected with regard to SCC level, lactose content and milk yield. From the total herd twenty-two cows fulfilled the selection criteria that were as follows: lactose content was equal between the udder quarters (≥ 4.5%) and SCC ≤ 150,000 cells/ml per udder quarter. The 22 cows were divided into two equal groups, Treatment A and Treatment B, with 11 cows per treatment, see Table 3.

Table 3. The status of the cows in treatment A and treatment B, mean and SD, at start of the experiment presented as milk yield ECM (kg/day), lactation number and lactation week (n=11 cows per treatment). Treatment A= One omitted milking, treatment B= Three omitted milkings

	Treatment A Mean ± SD	Treatment B Mean ± SD
Holstein cows	5	4
Swedish Red cows	6	7
Milk yield (kg/day)	40.2 ± 9.17	40 ± 7.31
Lactation number	1.9 ± 0.94	1.9 ± 1.04
Lactation week	17.7 ± 5.87	18 ± 7.95

Staff Routines

The staff routines were unchanged in Experiment 2, i.e. if the cluster attachment failed then teat cups were attached manually.

Recordings and Milk Sampling

For each milking, the AMR recorded the cow ID, the date, the total milk yield per cow, and the milk yield per udder quarter. This data was stored in the database BASREG from which it could be retrieved for use in calculations.

For cows in both Treatment A and Treatment B, milk samples were taken at every milking at UQL during the experimental period. Note that no milk samples were taken from RF quarter at the time of OM.

Milk Sample Analysis

Milk samples were analysed for lactose content and SCC at Kungsängen Research Center at the Swedish University of Agricultural Sciences, Uppsala. Lactose content was analysed using the mid-infrared spectroscopy technique (Fourier Transform Instrument, FT120 Foss, Hilleröd, Denmark). Milk SCC was analysed as described above under Experiment 1.

Statistical Analysis

The data for milk yield from BASREG and the data for lactose content and milk SCC from the laboratory in Kungsängen were entered into SAS 9.3 (Statistical Analysis System, Cary USA) and calculations were carried out at UQL. Milk SCC values were converted to $\log_{10}$ values to obtain a normal distribution of the data.

The averages of the values prior to OM are presented as baseline values. For Treatment B the values between OMs were compared as exact values to the baseline values. For Treatment A and Treatment B, after final OM, the exact values for the first six milkings were compared individually against the baseline values. For the 14 final milkings in both Treatment A and Treatment B, average values were calculated and compared against the baseline values.

The effect of OM on one udder quarter was tested as an effect on differences between two adjacent udder quarters. If there is no effect of treatment the difference between the two quarters is expected to be zero, while a significant difference from zero indicates a difference due to treatment of one of the udder quarters. In this experiment RF quarter was exposed to OM whereby the differences in milk yield, lactose content and milk SCC between RF and LF quarter was calculated and it was tested if the difference between the two quarters significantly differed from zero. The calculations were carried out by the use of the statistical program SAS 9.3. T-test was done to identify if the differences between udder quarters differed significantly from zero.

Depending on what values are presented in the tables, each baseline value for milk yield, lactose content and SCC level was calculated as an average value of the initial three pm milkings and as an average value of the initial four am milkings before the OM. The post-OM values were also calculated separately for am and pm milkings.

Results

Experiment 1

The results for Experiment 1 are as follows:

- Average number of cows milked/milking occasion in the AMR and recorded manually in the template was *171 cows* ***comprising 5472 quarters to be attached for milking with a successful attachment rate on quarter level at first attempt of 95 % (5200 quarters)***
- Total numer of milkings *1496 milkings* (including 2nd milkings)
- 261 milkings (201 milkings in 1st attempt + 60 milkings in 2nd attempt) noted in the template as unsuccessful milkings (17.4% of 1496 milkings)
    - Directed by AMR to Exit - 43 milkings (16.5% of 261 milkings)
    - Successful 2nd attempt - 70 milkings (27% of 261 milkings)
    - Unsuccessful 1st and 2nd attempt - 60 milkings x 2 attempts = 120 milkings (46% of 261 milkings)
- 60 milkings (4% of 1496 milkings) were unsuccessful after the 2nd milking attempt, which means that 96% of all milkings were successful according to AMR registrations
- However, if the 43 unsuccessful milkings where some disturbance were observed but the cows were directed to Exit are added to the 60 milkings that were unsuccessful in the 2nd attempt, the total is 103 unsuccessful milkings (6.8% of 1496 milkings), a success rate of 93.1%
- Regarding the 130 milkings selected by the AMR for a 2nd attempt, 90 of the these were due to failed attachment at UL, i.e. 6% of the total milkings (1496 milkings)

Unsuccessful milking could be due to failed teat cup attachment, kick off or tramp on tube. One cow can experience one, two or all three disturbances during one milking occasion. Failed teat cup attachment was the main reason for unsuccessful milkings noted in the template. Descriptive data of the unsuccessful milkings is presented in Tables 4a, 4b and 5.

Table 4a. Unsuccessful milkings per milking occasion (261 milkings noted in the template) presented as mean and SD, and as % of all milkings (1496 milkings)

	Unsuccessful milkings
Mean and SD	33 ± 7.17
% of total milkings	17.4%

Table 4b. Disturbances per milking occasion (261 milkings noted in the template) presented as mean and SD, and as % of 261

	Failed teat cup attachment	**Kick off**	**Tramp tube**
Mean and SD	23.6 ± 6.99	9 ± 5.57	1.1 ± 1.4
% of unsuccessful milkings	72.4%	27.6%	3.4%

Table 5. Incomplete milkings selected by the AMR for a second milking attempt and successful milkings during the second milking attempt presented as mean and SD, and as % of unsuccessful milkings (261 milkings)

	Incomplete milkings	**Successful second milkings**
Mean and SD	16.3 ± 5.93	8.8 ± 4.0
% of unsuccessful milkings/incomplete milkings	49.8% (130/261)	53.8%[1] (70/130)

[1] Percentage of the cows that had a 2^{nd} milking (n=130)

As seen in Figure 9 below, 52% of all failed attachments noted in the template (365 total failed attachments at UL) were at one udder quarter. The frequency of failed attachment was 20% for two udder quarters, 11% for three udder quarters, and 17% for all four udder quarters at the same time.

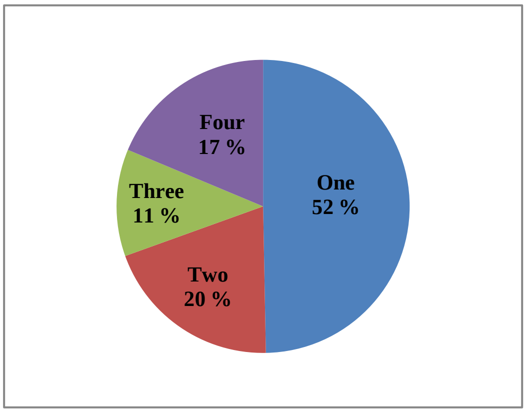


Figure 9. Number of udder quarters due to failed attachment (%)

Most frequent failed attachment (365 total failed attachments at UL) noted in the template occurred most frequent at RB udder quarter, i.e. 28% of the cases. LB represented 26%, LF 26% and RF 20% of the failed attachments (Figure 10).

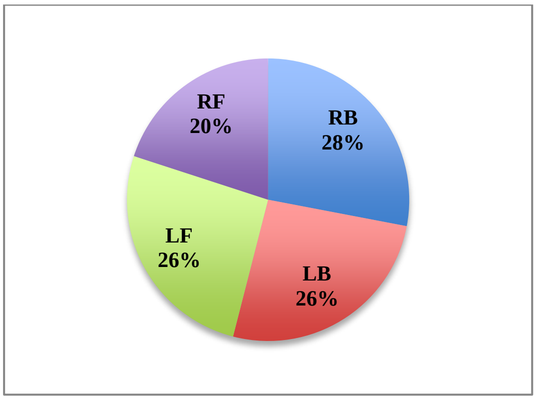


Figure 10. Udder quarters due to failed attachment (%)

In the template a cow could be registered as an unsuccessful milking two times during one milking occasion. Therefore a cow could theoretically be registered up to 16 times over the eight milkings at UL and up to 64 registrations at UQL (4 teats x 2 attempts x 8 milkings).

- During the eight milking occasions, 92 cows were registered as unsuccessfully milked in the template (which means that they experienced milking disturbances during 1st and/or 2nd milking) at one or more milking occasions, irrespective of whether the AMR identified the milking as incomplete or not.
- The number of unsuccessful milkings registered per cow in the template varied between 1 and 15. The frequency of cows registered for unsuccessful milkings (261 milkings of 1496 total milkings) in the template is presented in Table 6.

Table 6. Number of unsuccessful milkings per cow that were noted in the template

No. of notations/cow	1	2	3	4	5	6	7	8	9	10	15
No. of cows noted	40	16	13	5	4	4	4	3	1	1	1

There were 23 cows noted for unsuccessful milking in the template 25% or more (i.e. four or more registrations for unsuccessful milking during the 16 milking occasions over eight days). Of these 23 cows, 15 cows (65%) had a round udder shape and three cows had udder shape heavy backwards and one cow had udder shape that was heavy forward. Fifteen cows (65%) had a front teat position scored 2 i.e. pointing downwards, while only five (22%) had rear teats that were scored 2. In the whole herd 85% of the front teat position had score 2 and rear teats 37%. The rest of the cows had either rear teats pointing outwards or rear teats that were placed very narrow. It was only two (8.7%) of the 23 cows who had a teat score of 1 or below i.e. a teat end with no ring or a smooth or slightly rough ring. For the whole herd, teat score of 1 or below was 15-19% for rear teats and 30% for back teats.

The results for the milk samples taken from 24 cows that had kicked off the teat cups during morning milking in the 1st milking attempt were as follows:

- SCC was analyzed and the average milk SCC was 28,090 cells/ml (antilog value)
- The range was between 5,000 – 3,766,000 cells/ml/udder quarter
- Three cows had one or two udder quarters with a milk SCC above 1,000,000 cells/ml

Experiment 2

The results in Experiment 2 are presented separately for Treatment A and Treatment B.

Treatment A. Differences between Front Udder Quarters

The effect of one OM on one udder quarter was studied at UQL. The OM was done on the RF udder quarter and comparisons were made with the LF udder quarter.

The baseline values for milk yield, lactose content and milk SCC during am and pm milkings in the RF and LF udder quarters are presented in Table 7.

Table 7. The baseline values for milk yield (kg), lactose content (%) and milk SCC (cells/ml) during am and pm milkings for right front and left front udder quarters for cows that were exposed to one omitted milking, mean and SD, n=11

	RF quarter		**LF quarter**	
	Baseline[1)]	SD	Baseline	SD
am milking				
Milk yield (kg)	4.89	1.45	4.84	1.28
Lactose (%)	4.94	0.16	4.94	0.13
SCC (Cells/ml)[2)]	4.09	0.41	4.19	0.47
(anti-log value)	(12 300)		(15 500)	
pm milking				
Milk yield (kg)	3.86	1.21	3.70	1.06
Lactose (%)	4.84	0.20	4.91	0.17
SCC (Cells/ml)[2)]	4.28	0.33	4.33	0.47
(anti-log value)	(19 100)		(21 300)	

[1)] Baseline value = Baseline, average of 4 am milkings and 3 pm milkings, respectively, before the omitted milking day 4 during pm milking
[2)] $\log_{10}$- values

When the RF udder quarter was exposed to one OM, the difference in milk yield showed a significant increase on day 5 during am milking (Table 8). Thereafter the difference in milk yield decreased during the pm milking (Table 9). After day 5 there was no statistically significant difference in milk yield between the RF and LF quarters during am or pm milking.

During am milking the difference in lactose content (Table 8) showed a statistically significant decrease on day 5 and 6. However, at day 7 the difference dropped and stayed at the same level during am milkings for the remaining days of the experiment. The baseline-values for lactose content during the pm milking showed a significant negative difference between the RF and LF quarter (Table 9) with initially lower lactose content in the RF quarter. The statistically significant negative difference became more pronounced after the OM on day 5. Day 6 and thereafter the difference were not statistically significant.

During am milking there was a significant difference in milk SCC on day 5 and 6, with an increase in milk SCC in RF udder quarter (Table 8). During the pm milking (Table 9), when the RF udder quarter was exposed to OM, the difference in milk SCC showed a significant increase on day 5, 6 and 7. This difference then dropped and on day 8-14 it was not statistically significant.

Table 8. Milk yield (kg), lactose (%) and milk SCC (cells/ml) during am milking, results presented as differences between front quarters in cows exposed to one omitted milking at right front quarter during pm milking day 4, n=11

	Days				
	BL[1)]	5	6	7	8-14
	RF-LF[2)]	Diff.[3)]	Diff.	Diff.	Diff.
Milk yield (kg)	0.05	1.03**[4)]	-0.66	-0.21	-0.25
Lactose (%)	0.00	-0.13**	-0.21*	-0.07	-0.07
SCC (cells/ml)[5)]	-0.10	0.47*	0.45*	0.26	0.16

[1)] BL= Baseline, average of 4 am milkings before omitted milking
[2)] RF-LF = Differences between right front (RF) quarter baseline and left front (LF) quarter baseline
[3)] Diff. = Differences between right front and left front during am milkings day 5, 6, 7 and average am milkings day 8-14
[4)] Statistically significant differences between RF and LF quarters, * = p≤ 0.05; **= p≤ 0.01
[5)] log_{10}-values

Table 9. Milk yield (kg), lactose (%) and SCC (cells/ml) during pm milkings, results presented as differences between front quarters in cows exposed to one omitted milking at right front udder quarter during pm milking day 4, n=11

	Days				
	BL[1)]	5	6	7	8-14
	RF-LF[2)]	Diff. [3)]	Diff.	Diff.	Diff.
Milk yield (kg)	0.19	-0.57*	-0.49	-0.26	-0.29
Lactose (%)	-0.07**[4)]	-0.5***	-0.21	-0.13	-0.02
SCC (cells/ml)[5)]	-0.04	1.00***	0.68**	0.44**	0.08

[1)] BL= Baseline, average of 3 pm milkings before omitted milking
[2)] RF-LF = Differences between right front (RF) quarter baseline and left front (LF) quarter baseline
[3)] Diff. = Differences between right front and left front during pm milkings day 5, 6 and 7 and average of evening pm day 8-14
[4)] Statistically significant differences between right front and left front quarters, * = p≤0.05; **= p≤0.01; *** = p≤0.001
[5)] log_{10}-values

As can be observed from Figure 11, 12, and 13 the effect of one OM on the milk yield, lactose content and milk SCC was most pronounced in the udder quarter that was exposed to the OM.

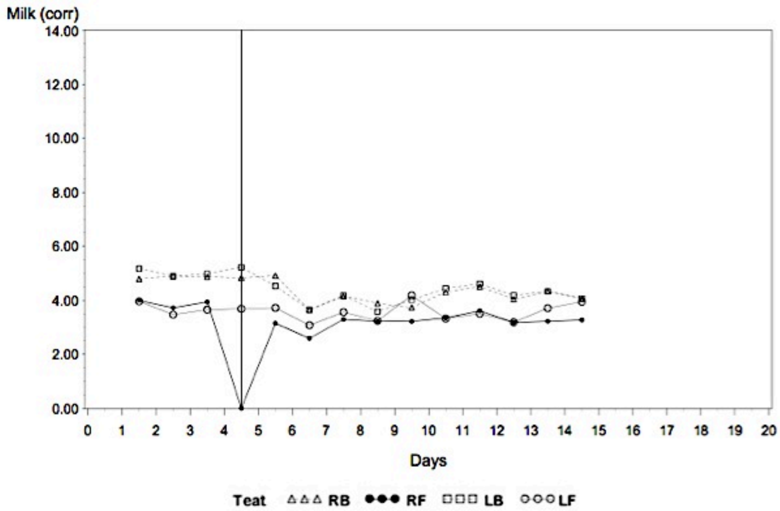


Figure 11. Milk yield (kg) at udder quarter level for cows exposed to one omitted milking at right front udder quarter during pm milking day 4, n=11

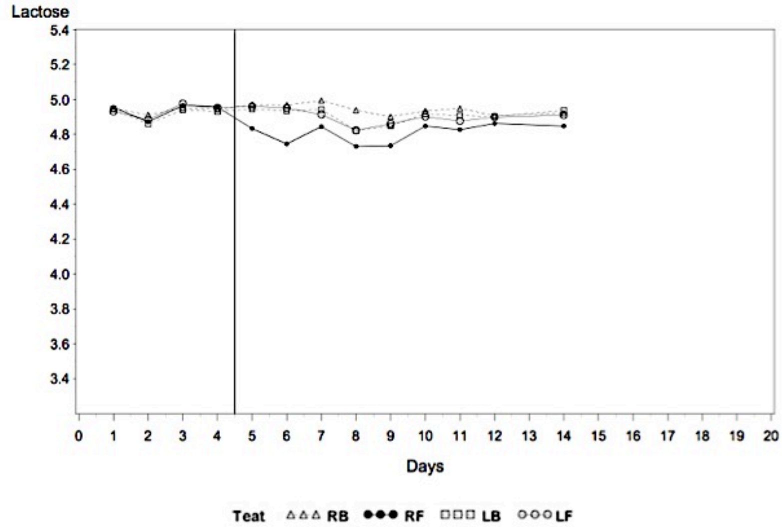


Figure 12. Lactose content (%) at udder quarter level for cows exposed to one omitted milking at right front udder quarter at <u>pm milking</u> day 4, n=11

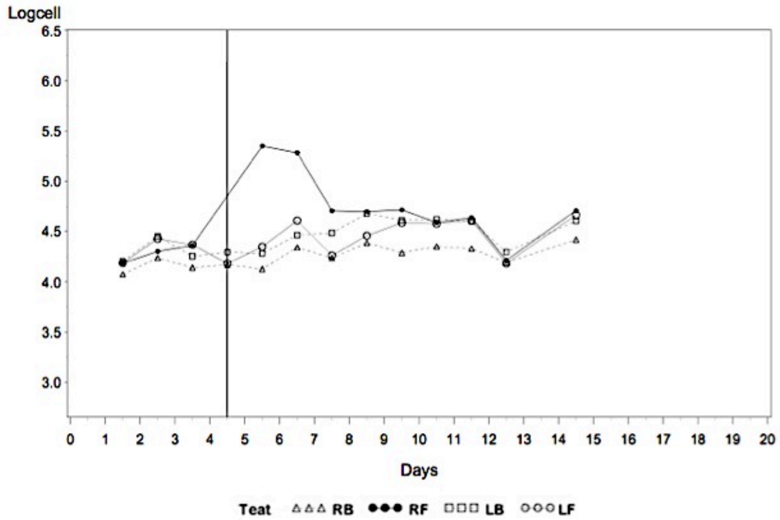


Figure 13. SCC (cells/ml), presented as log_{10}-values, at udder quarter level for cows exposed to one omitted milking at right front udder quarter during <u>pm milking</u> day 4, n=11

Treatment B - Three OMs

Baseline values for milk yield (kg), lactose content (%) and milk SCC (cells/ml) are the average values for the samples taken during the first seven milkings (am and pm milkings respectively, on days 1, 2, 3 plus am milking on day 4. Cows were exposed to a total of three OMs during the pm milking, on day 4, 6 and 8, on the RF udder quarter.

Front Udder Quarters

Table 10 shows the baseline values for milk yield, lactose content and milk SCC during am and pm milking in the front udder quarters when cows were exposed to the treatment three OMs in RF udder quarter.

Table 10. Baseline milk yield (kg), lactose content (%) and milk SCC (cells/ml) during am and pm milking for right front and left front udder quarters for cows exposed to three omitted milkings, mean and SD, n=11

	RF quarter		**LF quarter**	
	Baseline[1)]	SD	Baseline	SD
am milking				
Milk yield (kg)	4.52	1.15	4.57	1.20
Lactose (%)	4.88	0.11	4.92	0.12
SCC (Cells/ml)[2)]	4.06	0.48	4.07	0.35
(anti-log value)	(11 500)		(11 700)	
pm milking				
Milk yield (kg)	3.75	0.86	3.57	0.89
Lactose (%)	4.79	0.17	4.86	0.13
SCC (Cells/ml)	4.24	0.40	4.26	0.31
(anti-log value)	(17 400)		(18 200)	

[1)]BL= Baseline, average of 4 am milkings and 3 pm milkings, respectively, before the omitted milking day 4 during pm milking
[2)] $\log_{10}$-values

Differences between Front Quarters

When the effect of three OM on the RF udder quarter was tested at UQL a significantly increased difference in milk yield during am milking day 5 and day 9 was observed (Table 11). After one OM, more milk is stored in the gland and will be harvested the following milking, which actually occurs day 5, 7 and 9 during am milking. No significant effect was observed at the end of the treatment period. During pm milking (Table 12), however, there was a significant difference between udder quarters in milk yield due to treatment up to day 9 i.e. one day after the third and last OM. A decreased milk yield in RF quarter was observed those days, thereafter the difference dropped.

The lactose content showed a significant decrease due to the OM treatment during am milking (Table 11), but the effect disappeared day 11 and subsequently. During pm milking (Table 12), the lactose content decreased in RF quarter up to day 11 i.e. three days after third and last OM, thereafter the difference dropped.

During am milking (Table 11), milk SCC increased due to the OM treatment in RF quarter compared with LF quarter. However, day 11 and onwards there was no statistical difference between the udder quarters (Table 11). During pm milking, there was an effect on milk SCC the days directly after the OM. Though already day 10, there was no statistical difference between front udder quarters (Table 12).

Table 11. Differences between right front and left front udder quarters in milk yield (kg), and lactose (%) and SCC (cells/ml) during am milking in cows exposed to three omitted milkings day 4, 6 and 8 at right front udder quarter during pm milking with three milkings in between, n=11

	Days								
	BL[1)]	5	6	7	8	9	10	11	12-18
	RF-LF[2)]	Diff.[3)]	Diff.	Diff.	Diff.	Diff.	Diff.	Diff.	Diff.
Milk yield (kg)	-0.05	1.04**[4)]	-0.39	0.69	0.13	1.21**	-0.46	-0.49	-0.10
Lactose (%)	-0.04	-0.08*	-0.25*	-0.08	-0.21**	-0.01	-0.18**	-0.08	-0.03
SCC (cells/ml)[5)]	-0.02	0.32	0.5*	0.20	0.39*	-0.14	0.28**	-0.06	-0.01

[1)] BL= Baseline, average of 4 am milkings before omitted milking
[2)] RF-LF = Differences between right front (RF) quarter baseline and left front (LF) quarter baseline
[3)] Diff. = Differences between right front and left front quarters
[4)] Statistically significant differences between right front and left front udder quarter * = p≤0.05; ** = p≤0.01
[5)] Log_{10}-values

Table 12. Difference between RF and LF udder quarters in milk yield (kg), lactose (%) and SCC (cells/ml) during pm milking in cows exposed to three omitted milkings day 4, 6 and 8 at right front udder quarter during pm milking with three milkings in between, n=11

	Days								
	BL[1)]	5	6	7	8	9	10	11	12-18
	RF-LF[2)]	Diff[3)].	Diff.	Diff.	Diff.	Diff.	Diff.	Diff.	Diff.
Milk yield (kg)	0.18	-0.59***[4)]	-2.61***	-0.29**	-3.08***	-0.50**	-0.63	-0.28	0.07
Lactose (%)	-0.06	-0.32**		-0.23**		-0.35**	-0.15*	-0.17**	-0.05
SCC (cells/ml)[5)]	-0.02	0.63*		0.37*		0.39*	0.14	0.18	-0.03

[1)] BL= Baseline, average of 3 pm milkings before omitted milking
[2)] RF-LF = Differences between right front (RF) udder quarter baseline and left front (LF) udder quarter baseline
[3)] Diff. = Differences between right front and left front udder quarters
[4)] Statistically significant differences between right front and left front udder quarter * = p≤0.05; ** = p≤0.01; *** = p≤0.001
[5)] Log_{10}-values

The effect of three OMs on all four udder quarters is shown in the figures below, where it is noted that the most marked effect of treatment occurs on the RF quarter (Figure 14-16).

Relation between RF Quarter and LF Quarter for Milk Yield

RF quarter and LF quarter have nearly identical baseline values (Figure 17). However, during the OM-period, the RF increased its milk yield immediately after each OM. In the post-OM period the values for RF and LF milk yield quickly recovered and the values became nearly identical again by the end of the experimental period.

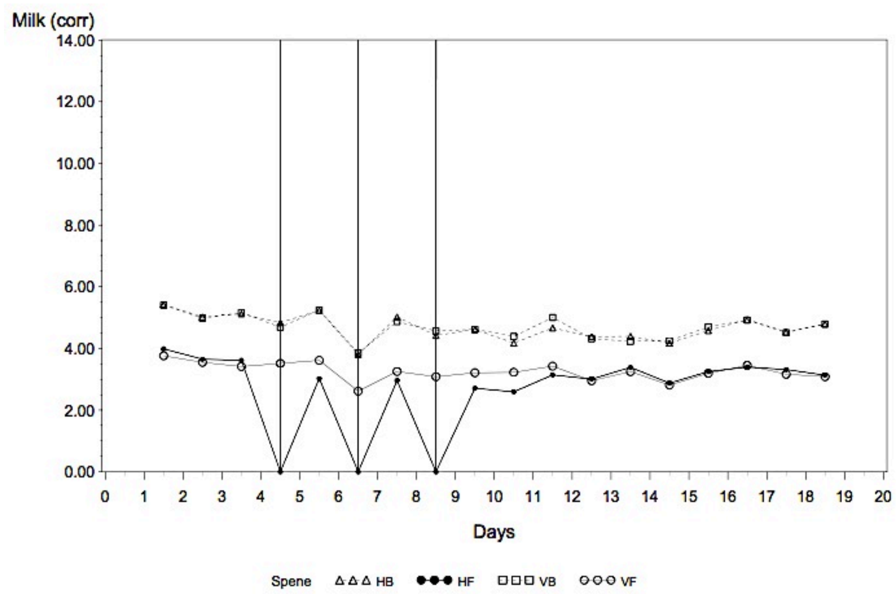


Figure 14. Milk yield (kg) at udder quarter level during <u>pm milking</u> for cows exposed to three omitted milkings at right front udder quarter day 4, 6 and 8, n=11

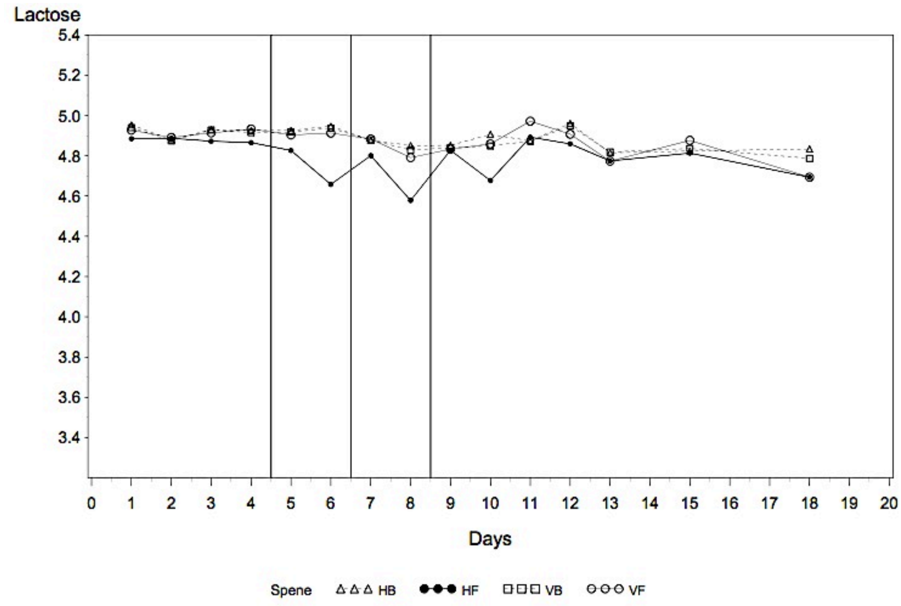


Figure 15. Lactose (%) at udder quarter level during <u>am milking</u> for cows exposed to three omitted milkings at right front quarter at pm milking day 4, 6 and 8, n=11

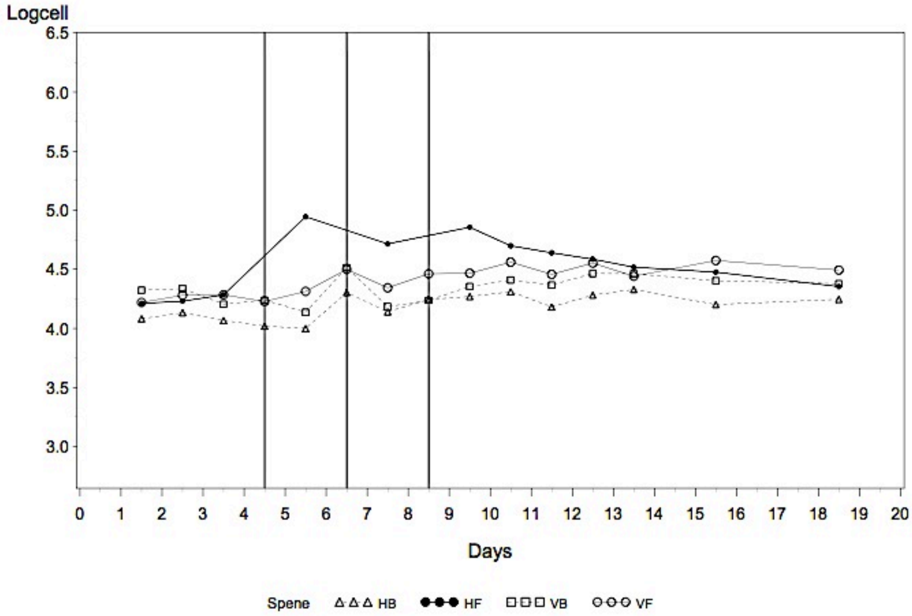


Figure 16. SCC (cells/ml) log10-transformed values at udder quarter level during <u>pm milking</u> for cows exposed to three omitted milkings at right front udder quarter day 4, 6 and 8, n=11

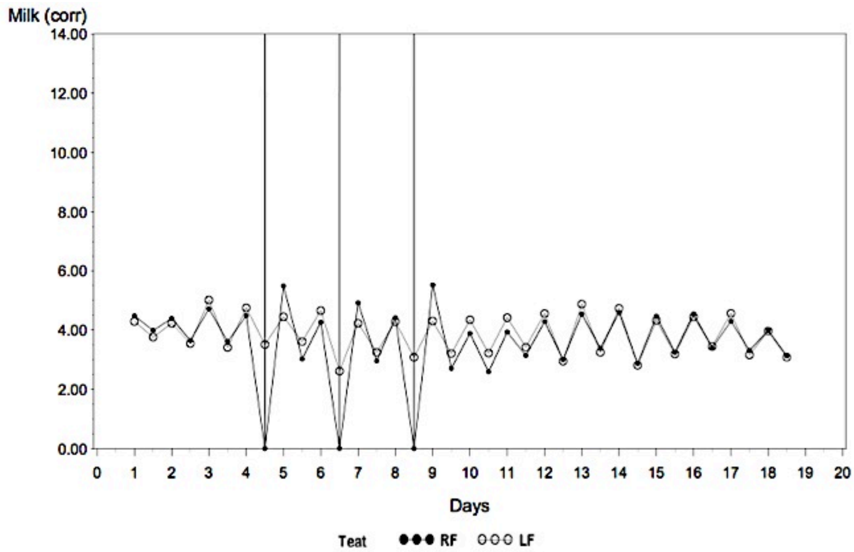


Figure 17. Results for both <u>am and pm milking</u>, n=11. Milk yield (kg) differences between right front and left front quarters for cows exposed to three omitted milkings (omitted milkings at right front quarter in pm milking on Days 4, 6, and 8).

Discussion

Experiment 1

In Experiment 1 the number of milkings was 1496 milkings. Of these, 261 milkings (17.4%) were unsuccessful and could be due to failed attachment (72.4%), tramp (3.4%) or kick off (27.6%) of 261 milkings. It is important to note that all three disturbances could occur during the same milking occasion for one cow. The present study, in comparison with Kolbach *et al.* (2012), showed a slightly lower percentage of failed attachment during both 1st and 2nd milking occasion. This could be due to that the equipment used in the study by Kolbach *et al.*, (2012) was a 16 bail prototype automated milking rotary (DeLaval HBR) with only one automatic cup attacher compared to the 24 bail AMR with two cup attachers used in the present study.

Of the 261 milkings that were unsuccessful, the AMR selected 43 milkings to Exit. It is noteworthy that only seven of these 43 milkings were tramp on tube or kick off at detachment. This means that 36 milkings were directed to Exit despite that the robot had failed attachment on one or more udder quarters or the cow kicked off the teat cup(s) either immediately at attachment or during the milking. When these 43 milkings are taken into consideration, the success rate for the AMR is between 93.1% and 96%.

The most common reason for unsuccessful milking was failed teat cup attachments (72.4%). If a cow is not directed to a new milking after an unsuccessful milking it can lead to production losses and problems in cow wellbeing. For cows that leave the milking unit with an udder quarter full of milk, a decrease milk production may occur in that specific quarter later and/or the cow may experience milk leakage, causing a risk for mammary gland infection (Waage *et al.,* 1998). Stefanowska *et al.* (2000) found that milk leakage was seen in 60% of the cows following a missed milking. In addition to production losses, it is also a question of cow wellbeing. The same study by Stefanowska *et al.* (2000) showed that cows that had OMs showed obvious signs of discomfort, e.g. standing instead of lying down. A study by Metcalf *et al.* (1992) showed that when cows are lying down at rest, the blood flow increases about 25% to the mammary gland and as mentioned above, the main substances in the milk are transported form the blood and there is a high correlation between the blood flow to the mammary gland and milk yield (Davis & Collier, 1985). A study by Österman and Redbo (2001) reported that cows milked three times daily showed more comfort signs, for example lying down, than cows milked two times daily.

Regarding the 130 milkings selected by the AMR for a 2nd milking attempt, 90 of these were due to failed attachment at UL, i.e. 6% of the total milkings (1496 milkings). This result could be considered as demonstrating the improvement in automated milking equipment since 2005. The results of the study by Bach and Busto (2005) showed that failed attachment occurred in 7.2% of all milkings (35,291 milkings).

Kick off occurred mostly during the pm milking 22 April. This may be due to the hoof trimming that occurred during the day 22 April. Kick off did not seem to be due to increased milk SCC since the cows that had kicked off one or more teat cups had an average milk SCC of 28,090 cells/ml and therefore not a high SCC. The reasons can be many, e.g. pain, restlessness (long waiting time), stress, the teat cup vacuum level, first time in the AMR. It is relevant to note the frequency of kick off because it provides a measure of cow wellbeing. Further studies are required to determine the reasons for kick off.

Studies by Capelletti *et al.* (2004) and Hamann *et al.* (2004) concluded that back udder quarters had more failed attachments than front udder quarters for a robot provided with automatic teat cup attachment. The results of the present study are in agreement with these results, as the present study showed failed attachment occurring most frequently at RB udder quarter.

The study by Kolbach *et al.* (2012) compared and found a correlation between failed attachment and lower milk yield (< 11 kg). The shape of the udder could be a factor since a higher amount of milk in the udder leads to a more rounded shape, which possibly makes it easier for the automatic cup attacher to locate the teats. Kolbach *et al.* (2012) also concluded that there was a significant difference in attachment success between individual quarters. In comparison, the present study investigated not only udder conformation but also looked more closely at teat status. In the present study it was observed that some of those cows that had unsuccessful milking, (≥ 25% of the milkings) had an unfavourable udder conformation such as round udder shape heavy forward or heavy backwards, and that the back teats were splayed outwards. Those cows also had teat ends with rough or very rough rings. These results indicate that the teat position and status could have an impact on the success rate of attachment. One caution in this case is that the teat assessment and notation of udder conformation in the present study was done directly after the milking event. In future studies, teat assessment and notation of udder conformation should be done immediately prior to a milking event. The present study confirms the findings of Jacobs and Siegford (2012) regarding the critical importance of udder and teat conformation in optimizing both production and cow wellbeing in the AMR system.

Experiment 2

The aim of Experiment 2 was to test the hypothesis that one or several OMs at UQL have a negative influence on milk yield, lactose content and milk SCC at UQL. This was proved, although the effects were not as negative as anticipated. This might be due to that the group of 22 cows tested in this experiment was clinically healthy.

The three parameters investigated in this experiment (milk yield, lactose content, milk SCC) are parameters that can be used to indicate effects on udder health in daily dairy production. In this experiment the effect of OM on milk yield was rather short term. In treatment A the difference between udder quarters disappeared already day 6, i.e. the second day after OM. The difference between udder quarters almost disappeared at the end of the treatment period also for Treatment B. This contradicts to the study by Lakic et al (2009) where the milk yield was affected for up to ten days. Future research is required to confirm these results and to draw more detailed conclusions from them.

Lactose has normally a low day-to-day variation in milk (Rook *et al.*, 1992), but has been observed to decrease when milk SCC is increasing (Forsbäck *et al.*, 2010). This relation was also indicated between milk SCC and lactose content in the present study. When milk SCC increased the lactose content decreased. This is also in agreement with several studies that found a highly negative correlation between milk SCC and lactose content (Auldist *et al.*, 1995; Berglund *et al.*, 2007). However, the effect of treatment in the present study was short term also for lactose and SCC since the difference between udder quarters had disappeared both for lactose and SCC at the end of the study. It has to be considered that the present study was done with cows with a low milk SCC. How cows with high or moderately high levels of milk SCC are affected was not evaluated and this should be investigated in future research.

The physiology of the cow ensures that milk synthesis takes place in each udder quarter independently of the other three udder quarters. However, the blood circulation is shared among all udder quarters. In the present study, data from the RF quarter was compared with the data from the LF quarter regarding milk yield, lactose content and milk SCC. This comparison was done to determine what would happen to one udder quarter if the other udder quarter in a pair (RF - LF) was exposed to an omitted milking treatment. In the present study the baseline values showed that the two quarters were almost equal in milk yield, lactose content and milk SCC prior to the treatment. The effect of the treatment resulted in immediate significant differences between the two quarters. These differences slowly decreased during the subsequent 14 milkings and at the end of the study the difference had almost returned to the baseline values.

Conclusions

The present study has shown the following:

- The AMR had a successful attachment rate on quarter level at first attempt of 95 %.

- The AMR showed a complete milking of all four quarters during the 1^{st} milking attempt of 85.5%. This proves the hypothesis of Experiment 1 in the present study.

- The individual conformation of the udder and teats has an impact on milking efficiency in automatic milking processes. More research is required to confirm and further develop these findings.

- Kick off did not seem to be due to increased milk SCC since the cows that had kicked off one or more teat cups had an average milk SCC of 28,090 cells/ml. Further studies are required to determine the reasons for kick off.

- When the number of successful 2^{nd} milking attempts is included, the milking efficiency for AMR is 96%. However, it can be concluded from the results in Experiment 1 that efficiency is actually somewhere between 93.1% and 96%. More research is necessary regarding the occurrence of disturbances in milking and their impact on milking efficiency, udder health and cow wellbeing.

- In Experiment 2, the results for both treatments showed that milk yield and lactose content decreased and milk SCC increased after the OM treatment(s) in the UQ exposed to OM. These effects were immediate and had disappeared at the end of the study.

Further studies

The AMR system is a relatively new system and is still under development. Access to studies on AMR is therefore spare and many references used in the literature review are based on other AMS. The present study is an important step in starting to understand the AMR and its efficiency. The results from the present study show that further development of the AMR is needed to optimize the automatic cup attacher robot in order to reduce the occurrence of OMs. Failed or incomplete milking in the AMR can cause an immediate production loss and an increase in milk SCC. Further studies would provide better insight and should include a greater number of cows and a wider range in udder health. The present study is based on two optimized groups of cows, however the study still provides noteworthy results and offers a platform for further research.

Acknowledgements

The present study was the result of collaboration between the Swedish University of Agriculture Sciences, Department of Animal Nutrition and Management, and DeLaval International, Tumba, Sweden. First and foremost I would like to thank my supervisor Kerstin Svennersten-Sjaunja for great support and for all her help and positive attitude toward this thesis. I would also like to give a special thanks to Ingemar Olsson for all statistical help and for teaching me the basis of the SAS program, and to Dietrich von Rosen who was main statistical adviser. I wish to express my appreciation to the staff at Lövsta Research Center, Uppsala, and the laboratory staff at Kungsängen, Uppsala, for helping me with sample collection and analysis. I would like to express my gratitude and give a big hug to Louise Henry for invaluable support regarding the English language. Last, but not least, I would like to thank my fiancé Lucas Andersson for practical help and support as well as wonderful pep talks when I really needed them!

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Appendices

Appendix 1. Experiment 1: Example template of raw data for types of disturbances in 1st and 2nd milking attempts in AMR.

Appendix 2. Experiment 1: Udder and teat assessment of cows that experienced disturbances during milking

Appendix 3. Experiment 2: Specifics of the cows

Appendix 1. Experiment 1: Example template of raw data for types of disturbances in 1st and 2nd milking attempts in AMR.

Recorded on March 31 – April 1 (4 milkings) and April 22 – April 23 (4 milkings) = 8 milkings in total

NB: Previously planned hoof trimming was carried out on April 22 before pm milking

Line	Cow ID	Date	Milking am/pm	Udder quarter				Disturbance during 1st milking attempt FA Failed attachment KoA Kick off at attachment KoM Kick off during milking KoD Kick off at detachment TT Tramp on tube	Directed by AMR to Exit or 2nd attempt (incomplete)	Disturbance during 2nd milking attempt FA Failed attachment KoA Kick off at attachment KoM Kick off during milking KoD Kick off at detachment TT Tramp on tube
				RF	LF	LB	RB			
1.	961	Apr 01	am				KoA	KoA	2nd attempt	
2.	961	Apr 23	pm				KoM	KoM	Exit	
3.	972	Mar 31	pm				KoD	KoD	Exit	
4.	972	Apr 01	am				TT	TT	2nd attempt	
5.	972	Apr 22	pm				KoM	KoM	2nd attempt	
6.	972	Apr 23	pm			FA		FA	2nd attempt	
7.	973	Mar 31	am			KoA	KoA	KoA	2nd attempt	
8.	973	Mar 31	pm				FA	FA	Exit	
9.	973	Apr 01	pm	FA FA	FA FA	FA FA	FA FA	FA	2nd attempt	FA
10.	973	Apr 22	am		FA	FA	FA	FA	Exit	
11.	973	Apr 22	pm				FA	FA	Exit	
12.	973	Apr 23	am	FA	FA	FA	FA	FA	Exit	
13.	982	Mar 31	am				FA	FA	2nd attempt	
14.	982	Mar 31	pm	KoA KoA	KoA KoA	– KoA	KoA KoA	KoA	2nd attempt	KoA
15.	982	Apr 22	am	FA FA	FA FA	FA FA	FA FA	FA	2nd attempt	FA

<table>
<tr><td rowspan="2">Total</td><td>FA
189</td><td>KoA
19</td><td>KoM
48</td><td>KoD
5</td><td>TT
9</td><td rowspan="2">261 milkings*</td></tr>
<tr><td colspan="5">Udder level
Number of disturbances <u>at udder level</u> per type of disturbance in 261 milkings = 270
Nb: Two or more <u>types</u> of disturbances could occur per milking</td></tr>
<tr><td colspan="7"></td></tr>
</table>

Appendix 2. Experiment 1: Udder and teat assessment of the whole herd

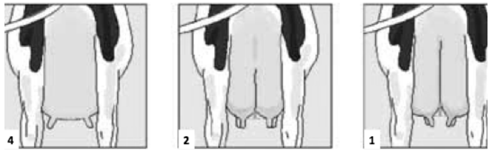
Recorded on March 31 – April 1 (4 milkings) and April 22 – April 23 (4 milkings) = 8 milkings in total

Cow ID	Number and type of disturbances* during 1st and 2nd milking attempts at udder quarter level (UQL)					Udder shape	Udder bulk	Teat position**		Teat end condition***			
	Failed attach-ment	**Kick off at attach-ment**	**Kick off during milking**	**Kick off at detach-ment**	**Tramp on tube**	**Round Trough R / T**	**Front / Back**	**Front pair**	**Back pair**	**RF**	**LF**	**LB**	**RB**
12	6	-	-	-	-	R		2	0	SR	SR	S	S
13	-	1		2		T		2	1	S	S	S	S
18	2		1			T		1	0	R	R	R	R
21	1		3			T		1	0	SR	SR	SR	SR
27	2		11		3	R		2	1	VR	VR	R	R
30	1					T		2	0	SR	SR	SR	SR
33	6				1	R		2	0	SR	SR	S	S
48	1		1			T		2	2	VR	VR	VR	VR
52	2					R		2	2	SR	SR	S	S
56	-		2			T		2	1	SR	SR	SR	SR
57	-			1		T		2	1	N	N	N	N
61	-		1	4		T		2	0	SR	R	R	R
62	4					T		2	0	SR	SR	SR	SR
65	1					T		2	1	R	R	SR	SR
70	-		1			T		2	1	VR	R	R	SR
76	-		4			R		2	1	SR	SR	S	S
82	6	2		1		T		2	2	S	SR	S	S
83	-		3			T		2	2	SR	SR	SR	SR
85	2	1				R		2	1	S	S	S	S

*** Disturbances**

Note that the five different types of disturbances are specified at udder quarter level. This means that one disturbance could occur at more than just one udder quarter per milking and two or more disturbances could occur during one milking. The total also includes disturbances that occurred in 2nd milking attempts if the cow was directed by the AMR into a 2nd milking attempt during the period of this study.

**** Teat Position Assessment**



A modified scoring system for teat position. From the left a cow with teat position 4. In the middle a cow with teat position 2 and on the right a cow with teat position 1. (http://www.theorganicfarmer.org/how-to-select-a-good-cow/ (2014-05-05)).

***** Teat end condition**
Scoring system for teat end condition
(Mein et al 2001)

N = No ring
S = Smooth ring
SR = Slightly rough ring
R = Rough ring
VR = Very rough ring

Appendix 3. Specifics of the cows used in Experiment 2

The classification was done 28th September

Treatment A				
Cow ID	Breed	Lactation number	Lactation week	Milk yield ECM (kg)
973	2	2	18	45
1007	2	1	17	45
1512	1	3	27	28
1560	1	3	13	42
1620	1	1	24	35
1674	1	1	14	30
1682	1	1	24	35
5403	1	1	20	33
6460	2	3	10	60
6471	2	3	9	46
6510	2	2	19	43

Treatment B				
Cow ID	Breed	Lactation number	Lactation week	Milk yield ECM (kg)
992	2	2	10	41
1464	1	4	15	48
1530	1	3	13	46
1573	1	2	17	49
1603	1	2	22	43
1669	1	1	24	35
1685	1	1	22	32
1688	1	1	12	36
5362	2	3	26	43
5397	2	1	5	25
5401	2	1	32	42

Breed 1 = Swedish Holstein
Breed 2 = Swedish Red

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