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on milk production and SCC in mid and late lactating cows with or without re-sorting for a second milking

Photo: Roger Nilsson

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Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, **556**
Uppsala 2016

Degree project / Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, **556**

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

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Effekter av en överhoppad mjölkning på en juverdel
på mjölkproduktion och celltal på kor i mitt- och sen laktation med eller utan återsortering i en AMR

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Examiner:
Omfattning: 30 hp
Extent:
Kurstitel: Examensarbete i Husdjursvetenskap/Degree project in Animal Science
Course title:
Kurskod: EX0551
Course code:
Program: Agronomprogrammet - Husdjur
Programme:
Nivå: Avancerad A2E
Level:
Utgivningsort: Uppsala
Place of publication:
Utgivningsår: 2016
Year of publication:
Serienamn, delnr: Examensarbete / Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, 556
Series name, part No:
On-line publicering: http://epsilon.slu.se
On-line published:
Nyckelord: Överhoppad mjölkning, mittlaktation, sen laktation, celler, juverhälsa, mjölkmängd, mjölkkomposition, laktos, protein, fett.
Key words: Omitted milking, mid lactation, late lactation, somatic cell count, udder health, milk yield, milk composition, lactose, protein, fat.

Innehållsförteckning

Abstract

The aim of this study was to investigate if one single omitted milking on one udder quarter with or without re-sorting affects milk yield and udder health in cows in mid or late lactation. The study was carried out in an automatic milking rotary (AMR). It is an identified problem in automatic milking that one or more udder quarters are incompletely milked due to failed teat cup attachment or cows kicking teatcups off. At present, cows that are registered as incompletely milked on one udder quarter or more in the AMR used in this study are resorted for a second milking since it has been shown that extended milking intervals in early- and mid-lactation can lead to a decrease in milk yield and also cause an elevated somatic cell count (SCC) which is an indicator of impaired udder health. Resorting reduces the capacity of the AMR and for the individual cow resorting leads to more time away from feed and cubicles which can impair cow welfare or milk production or both. 36 cows of Swedish Holstein and Swedish Red Breed in day 167 to 376 in lactation were used in the experiment and were divided into two groups, control and treatment. All cows were subjected to one udder quarter being omitted at one milking. After the omitted milking, control cows were resorted back for a second milking while treatment cows were directed to exit. Composite milk and udder quarter milk sampling was performed for three days before and ten days after the treatment day and used for determination of SCC, lactose, milk fat and milk protein. In addition, milk yield was registered throughout the experiment period. Milk yield was 3.7kg lower on the treatment day but no negative long-term effect on milk yield or SCC was found. Furthermore, milk yield from the omitted teat was low on resorting, indicating that a resorting likely does not lead to a complete emptying of the omitted quarter. Therefore, it was concluded that a cow in mid or late lactation has no beneficial effects of being resorted for a second milking in an AMR. A cow can instead the allowed to return to feeding and cubicles after a single incomplete milking, allowing a higher capacity of the AMR. Effects of incomplete milking in early and peak lactation and repeated incomplete milking in any stage of lactation remain to be studied.

Sammanfattning

Syftet med denna studie var att undersöka om och hur en överhoppad mjölkning på en juverdel med eller utan återsortering för ny mjölkning påverkar mjölkmängd och juverhälsa på kor i mitt- eller sen laktation i en automatiserad mjölkningskarusell. Ett identifierat problem i automatisk mjölkning är att en eller flera juverdelar blir ofullständigt mjölkande tack vare att spenkopparna sparkas av eller att påsättningen av spenkopparna misslyckas. När en ko registreras som ofullständigt mjölkad på en eller flera juverdelar blir hon i regel återsorterad för en andra mjölkning eftersom det har visats att förlängda mjölkintervall på kor i tidig och medellaktation påverkar mjölkproduktionen negativt och kan också orsaka en förhöjning av celltal vilket är en indikator på dålig juverhälsa. Återsortering sänker dock kapaciteten i robotkarusellen och en återsortering innebär att kon får spendera mer tid ifrån foder och liggbås vilket kan vara negativt för djurvälfärd och mjölkproduktion. 36 kor av Svensk Holstein och Svensk Röd Boskap i laktationsstadie från 167-376 dagar användes i experimentet och delades in i två grupper, kontroll och behandling. Efter den överhoppade mjölkningen på en juverdel som alla kor utsattes för, återsorterades kontrollkorna till en andra mjölkning medan behandlingskorna fick lämna karusellen och återvända till liggavdelning och foder. Helmjölk och juverfjärdedelsprover togs under tre dagar innan och tio dagar efter den överhoppade mjölkningen och analyserades med avseende på celltal och mjölkkomposition. Även mjölkmängd registrerades under hela försöksperioden. Mjölkmängden var 3.7kg lägre under

behandlingsdagen men inga långvariga negativa effekter på varken mjölkmängd eller celltal observerades. Den mjölkmängd som erhölls vid återsortering var också låg, vilket indikerar att en återsortering ändå inte leder till att juverdelen blir fullständigt mjölkad. Slutsatsen är därför att det inte finns någon anledning att återsortera en ko i mitt eller sen laktation till en andra mjölkning efter en enstaka ofullständig mjölkning. Om dessa kor istället får gå tillbaka till liggavdelning och foder upprätthålls kapaciteten i karusellen. Effekter av ofullständig mjölkning i tidig och topplaktation samt effekt av upprepade ofullständiga mjölkningar behöver utredas.

Introduction

The heavy and time consuming labor involved in milking dairy cows has led to the development of automatic milking systems (AMS). AMS has been shown to successfully decrease the need for labor with as much as 18% and also increase milk production with up to 12% (Jabobs and Siegford, 2012) mainly by an increased milking frequency (Wade *et al*., 2004). AMS has also changed the nature of labor, making it more flexible which in turn can increase the social life for the farmer (De Koning and Rodenburg, 2004). The newest AMS invention is the automatic milking rotary (AMR) which was developed to handle herd sizes with up to 800 cows. Although improving several aspects of milking, the automatic milking can lead to problems which would not occur in systems with manual milking. One problem that has been identified is the incomplete milking of one or several udder quarters due to failed attachment of teat cups or teat cups being kicked off during milking (Stefanowska *et al*., 2000). Management settings can be to direct cows for a second milking as soon as possible when milking is incomplete (DeLaval, 2015a). The two alternatives in the AMR consist of redirecting the cow for a second milking or to direct her to exit, for return to cubicles and feeding area. However, both of these alternatives can negatively affect the cow. This is partly explained by the limited time budget of today's high yielding dairy cow. Research on waiting time in AMS show that average waiting time per day upon entering the milking unit varies from 46 min (Helmreich *et al*., 2014) to 1.5 h (Munksgaard *et al*., 2011). However, for individual cows waiting time prior to milking could be as long as 3.5 h (Munksgaard *et al*., 2011). Waiting time in the AMR has not yet been researched and due to differences between regular AMS and the AMR it is difficult to estimate waiting time in a AMR. However, during this time, important activities such as resting and eating are unavailable which could compromise the cows time budget (O'Driscoll *et al*., 2009) and decrease welfare and milk yield and resorting may add further to this problem. On the other hand, for the individual cow incomplete milking is known to decrease milk yield and increase SCC (Caja *et al*., 2004). It has also previously been shown that losses in milk yield due to lower milking frequency are higher in early compared to late lactation cows (Stelwagen *et al*., 2013). This could be explained by that the lower milk yield in late lactating cows means lower udder fill and therefore less sensitivity to feed-back inhibition of milk secretion when milking is omitted or incomplete. Research has addressed omitted milkings on whole udder level (Stefanowska *et al*., 2000; Lakic *et al*., 2009) but often in AMS and AMR, only one udder quarter is incompletely milked. Knowledge about effects of one single omitted milking on one udder quarter on cows in mid and late lactation is needed.

Aim and Hypothesis

The aim of the study was to evaluate the need for a second milking in cows in mid and late lactation with a milk somatic cell count (SCC) below 200 000 cells/ml milk, when one udder quarter is omitted during the first milking attempt.

The hypothesis of the study was that cows in mid or late lactation with a milk SCC below 200 000 cells/ml milk do not need to be re-milked when only one udder quarter has been omitted.

Literature review

Automatic milking

The practical work involved in milking cows is heavy and time consuming and the cost of labor increased during the 1980's in many countries. This led to a demand for increased labor efficiency in milk production (Rossing and Hogewerf, 1997) and one outcome was automatic milking systems (AMS). An AMS consists of one or more milking units (MU) equipped with a robotic arm that identifies and cleans the teats and attaches the cluster so the milking process can be carried out without any direct input of humans (De Koning and Van de Vorst, 2002). It has been found that an AMS can decrease the need for labor with as much as 18 % (Jacobs and Siegford, 2012). Even more importantly the AMS changes the nature of labor to duties less time bound which is appreciated especially in small, family-operated farms (De Koning and Rodenburg, 2004). Another advantage with the AMS is that it increases milk production up to 12 % when cows are milked more than twice daily (Jacobs and Siegford, 2012).

Automatic milking rotary

A more recent milking strategy based on AMS is the Automatic Milking Rotary (AMR), first introduced in 2010 by DeLaval AB, Tumba, Sweden (Jacobs and Siegford, 2012). The first commercial AMR was designed to handle herds in the size of 300-800 cows, with a capacity of 90 cows per hour. The platform had 24 milking stations and 5 robot arms to serve the increasing herd-sizes (Figure 1) (Jacobs and Siegford, 2012).

Figure 1. The AMR with 24 stations suitable for herd-sizes of 300-800 cows.
http://www.delaval.com/en/About-DeLaval/Innovation-at-DeLaval/AMR-System-Overview/(2015-11-24)

When entering the AMR, an electronic gate identifies each cow by id registration and the system is accessing the correct pre-stored data for the cow (Hunter Nilsson, 2015). Pre-stored data on milk yield enables the management system to calculate an expected milk yield on each teat. Further, pre-stored teat location enables the laser cameras to locate teats more quickly. Once inside the rotary, the cow is rotated from entry to exit through different stations in a start-stop operation, thus increasing the throughput compared to a single-box AMS (Kolbach *et al*., 2012). The AMR can be divided into three functional parts. 1) teat cleaning and stimulation, which is performed by the preparation module, consisting of two robots. 2) the automatic cup attaching unit, also consisting of two robots and 3) the teat spraying module, consisting of one robot, which disinfects the teats after milking (Ljunggren, 2015). Each robot need 20-30 s to finish its part of the process, therefore the preparation and cup attaching steps takes around one minute each while the teat spraying step only takes 30 s. When milking is finished, the cow is rotated to the teat spraying bail for disinfection. After completion, the cow is rotated to the exit lane and directed out of the AMR. An identified problem with the stationary robotic units is that they cannot reattach a failed or removed teat cup once the cow has left the attachment bail (Kolbach *et al*., 2012). If that happens, the cow is resorted back for a second milking instead of being directed to exit (DeLaval, 2015a).

Time budget

A high yielding cow has a very restricted time budget. Voluntarily, the cow spends her time in with activities such as resting, eating, socializing and drinking. Time spent on milking is the one factor that cows have little control over, since its mostly determined by management settings and barn design (Gomez and Cook, 2010). In loose housing systems cows often need to wait for more than one hour away from the resting and feeding area before milking starts (Dijkstra *et al*., 2012). This time could be regarded as stressful, since important resources like feed and rest are unavailable (O'Driscoll *et al.*, 2009). These factors are not only important for the animal welfare, but also for production (Helmreich *et al.*, 2014). A long waiting time before milking, especially in systems with two or three times daily milking could therefore lead to impaired production (Dijkstra *et al*., 2012). However, because the energy requirements decrease with a lower production, cows in late lactation have to spend less time feeding to support their nutritional demands (Norring *et al*., 2012).

Anatomy and physiology of milking

Udder anatomy

The udder consists of four functionally separate quarters: Right Front - RF, Left Front – LF, Left Rear – LR and Right Rear – RR, each with its own teat, connected to only that gland. The median suspensory ligament divides the udder in right and left udder halves and connective tissue divides the udder quarters into separate functional units (Swett *et al*., 1942). Usually, rear udder quarters are larger than front udder quarters, leading to a higher milk yield, longer milking time and higher peak flow in rear udder quarters (Tančin et al., 2006). All udder quarters, or glands, consist of secretory tissue where milk is produced and stored and cisternal cavities that offer further storage between milkings (Bruckmaier and Blum, 1998). The secretory tissue is organized in alveoli that are drained by milk ducts, leading towards the cisternal cavity which is connected to the teat. In cows the alveolar milk fraction is the largest, more than 80 %, while the gland cistern and the larger milk ducts store the remaining milk, less than 20%. However, in late lactating cows and close after milking nearly 100 % of the milk can be stored in the alveoli (Bruckmaier and Meyer, 2004). Milk stored in the gland cistern and the larger milk ducts is available immediately upon milking and can be removed by surmounting the teat sphincter barrier (Bruckmaier and Blum, 1998).

Milk ejection

Milk ejection is a neuroendocrine reflex that shifts the alveolar milk fraction towards the gland cistern and thereby makes it available for milk removal (Bruckmaier *et al*., 1994). The first step in order to obtain a milk ejection is a tactile stimulation of the udder, for example by calf suckling or machine milking (Sjaastad *et al*., 2003). When stimulated, nerve impulses are sent from the udder via nerve fibers to the hypothalamus. This initiates release of the neuropeptide oxytocin from the pituitary into blood where it is transported to the udder (Bruckmaier *et al*., 1994). When it reaches the udder, oxytocin binds to myoepithelial cells surrounding the alveoli, inducing a contraction. This forces milk from the alveoli and small milk ducts to the cisternal cavities and large mammary ducts of the gland and teats (Bruckmaier *et al*., 1994). The time from stimulation to milk ejection is depending on fill grade and increases with a decreasing fill-grade from 40 s to >2 min (Bruckmaier and Hilger, 2001). Milk yield declines with stage of lactation post peak lactation and because of the lower yield udder fill is also lower. Thus, it takes longer before milk ejection occurs in late lactating cows and if teat cups are attached without pre-stimulation, it can lead to a high removal frequency of teat cups due to low milk flow (Rasmussen *et al.*, 1990). Oxytocin is rapidly broken down and its effect decrease quickly when release from the pituitary ceases. To maintain complete udder emptying it is therefore important to maintain stimulus throughout milking (Sjaastad *et al*., 2003). A decrease in secretion will quickly lead to less complete udder emptying (Bruckmaier *et al*., 1994). Caja *et al*. (2004) found drastic increases in the cisternal volume (98%) after an oxytocin injection and milk entering from the alveoli. The cisternal area peaked 3 min after OT injection and then slowly diminished in size, interpreted as milk reflux (cisternal recoil) to the alveolar compartment. Cisternal recoil is believed to be caused by the relaxation of the myoepithelial cells and smooth muscle fibers, causing the cisternal milk to be sucked back into the alveoli and milk ducts, becoming residual milk if no further milk ejection is induced (Caja *et al*., 2004). To milk an udder completely it is therefore important to milk cows a few minutes after stimulation without interruption (Sjaastad *et al*., 2003). A good understanding of the importance of teat stimulation before and during milking in cows, adjusted to their lactational stage, helps managing good milking routines for a successful milk ejection (Bruckmaier and Wellnitz, 2008).

Milk removal

Because of the short positive effects from oxytocin on milk ejection it is important to avoid delays between teat stimulation and milk removal (Bruckmaier and Blum, 1998). It has been observed that a prolonged interval between udder preparation and cluster attachment can affect milk yield and milking time negatively (Mayer *et* al., 1984). Milk removal itself is important for sustained milk production (Soberon *et al*., 2010) because of the negative feed-back presence of milk in the alveoli has on milk secretion (Peaker and Wilde, 1996). Exactly how the feedback mechanism works is not yet fully understood, but research points towards a protein that is found in milk (Peaker and Wilde, 1996) or the hormone serotonin (5-HT) (Hernandez *et al*., 2008) or both. The protein and the mechanism suggested to be the feedback inhibitor of lactation blocks constituent milk secretion from the mammary epithelial cells through an autocrine mechanism. The protein is also associated with a downregulation of prolactin receptors which in turn leads to decreased epithelial cell differentiation and thus affects milk production negatively in a long-term perspective. However, more research is needed before it can be determined if these long-term effects are due to action of the protein only, or by other factors (Peaker and Wilde, 1996). Studies regarding inhibition of milk secretion by serotonin suggest that it downregulates milk secretion by decreasing milk protein mRNA expression in bovine mammary epithelial cells (Hernandez *et al*., 2008). One of the affected proteins is α-lactalbumin which is essential for lactose synthesis and therefore milk volume, because it constitutes 50 % of the milks osmolarity (Sjaastad *et al*., 2003). Hernandez *et al* (2008) observed both a higher milk yield when 5-HT receptors were blocked and a decrease in milk yield when an infusion of 5-HT was performed in a lactogenic culture model. These findings indicate that serotonin serves as a feedback inhibitor of lactation, but more research is needed to fully determine how milk yield is altered by the serotonergic system locally in the mammary gland (Hernandez *et al*., 2008). Anyhow, the decrease in milk secretion is an immediate response to frequency and completeness of milk removal (Dahl *et al*., 2004). It is believed that the decreased milk secretion is an effect of the saturation of the milk holding capacity in the udder related to its cisternal capacity (Bach and Busto, 2005). It is also known that an increasing pressure in the alveolar compartment can lead to increased permeability in the epithelium through tight junctions. It has also been seen that milk leakage through tight junctions occur at the same time as milk secretion goes down. It is therefore believed that tight junctions could have a regulatory effect on milk secretion (Stelwagen, 2001). Therefore, the milk storage characteristics of the alveolar and cisternal compartment are important factors regarding milk loss during once daily milking, caused by omitted milking (Stelwagen *et al*., 2013). Another factor that tends to affect the yield loss is stage of lactation. During once daily milking Stelwagen and Knight (1997) found that the relative production loss was significantly greater in early lactation than in late. Even losses in mid lactation was higher than in late. These findings are supported by Davis *et al.* (2006) that investigated once daily milking for 7 days and found that production losses were greater in mid lactation (22%) compared with late lactation (13%). An explanation to this could be the greater cisternal size in proportion of total milk stored in the cistern, which increases as lactation advances (Knight and Dewhurst, 1994). Increased milking frequency and complete udder emptying avoids these negative effects and leads to higher milk yield (Dahl *et al*., 2004) while failed milk removal or decreasing milking frequencies, as with omitted milking, reduces milk yield (Clark *et al*., 2006).

Incomplete milking

Stefanowska *et al* (2000) highlighted cluster attachment failure problems in AMS, which varies from a few percent up to 15 %. Udder attachment failure varies between cows and occurs more frequently on cows with udder conformations less suitable for robotic milking. Because the AMR rotates during the milking session the robotic arm does not remain with the cow during the whole milking process (Ljunggren, 2015). Therefore, when a cow kicks off the teat cup(s) or an udder attachment failure occurs, on one or several udder quarters, the cow leaves the AMR with the respective udder quarters un-milked or incompletely milked. In general, when this occurs, cows are often sorted back for a second milking (Ljunggren, 2015). Caja *et al*. (2004) stated that interrupted or failed milking can decrease milking efficiency, especially in robotic milking, but also increase the residual milk and impair milk yield. Ljunggren (2015) investigated frequency of failed or incomplete milkings in an AMR and found a failure rate of 17.3 %. Further, Bach and Busto (2005) investigated udder attachment failure in an AMS and found a failure rate of 7.6 % of a total of 35,291 milkings. Due to udder attachment failure, milk yield decreased 26 % on the particular teats with a milking interval > 18 h. Also, during a failure of milk removal on one udder quarter, Bach and Busto (2005) found lower milk yields also on the unaffected quarters, suggesting that teat cup attachment failure on one teat can affect milk ejection on unaffected quarters. Finally, when an incomplete milking occurs on one or several udder quarters, a fast resorting for a second milking is believed to be in favor of short-term milk production and udder health (Kolbach *et al.*, 2012).

Somatic cell count

A general indicator of udder health is the somatic cell count (SCC) (Dahl *et al.,* 2004). The SCC is also used as an indirect measure of milk quality, because an elevated SCC in both quarter and composite milk and bulk tank milk has been shown to affect milk composition (Wickström *et al*., 2009). A high SCC in bulk tank milk will reduce payment which is one of the reasons for farmers to keep SCC at a minimum. The SCC limit for milk accepted for processing and manufacturing varies internationally, for example from European legislation (400 000) to US legislation (750 000) (More, *et al*., 2013). Further, an udder with SCC < 100 000 is not likely to have an infection (Brolund, 1985). SCC can vary between lactations, stage of lactation, milking frequency and breed (Wickström *et al*., 2009). The milking frequency in particular has a strong influence on the SCC. When having a low milking frequency, as when milking once per day, SCC is elevated (Stelwagen and Lacy-Hulbert, 1996). It is also known that SCC increases with increasing parity (Nyman *et* al., 2014). Recent findings indicate that SCC is elevated on both whole udder (Lakic *et al*., 2011) and on udder quarter level (Ljunggren, 2015) after one omitted milking. Frequent milk removal with milkings 3 times per day might cause a reduction in SCC (Smith *et al*., 2002). Another factor that is known to affect udder health negatively is milk leakage. When this happens, pathogens are provided access to the mammary gland through the teat barrier (Stelwagen *et al*., 2013). Leakage occurs when an udder is stimulated but no milk removal takes place (Stefanowska *et al*., 2000) or due to less frequent milk removal, which increases the pressure on the teat sphincter (Gleeson *et al*., 2007). After one omitted milking, Stefanowska *et al* (2000) found that 60 % of the cows had milk leakage, which likely will increase the SCC and also the risk for mastitis. Further, complete emptying of the udder is important because residual milk works as a transcendent substrate for microorganisms in the udder (Elbers *et al.,* 1998).

Milk composition

Lactose

When milking once daily, lactose is known to decrease in yield along with milk yield (Clark *et al*., 2006). When tight junctions open, possibly due to an increased pressure on the mammary epithelium, solubles such as lactose is allowed to pass over the epithelium into blood, leading to lower concentrations of lactose in milk. However, at the same time sodium ions are allowed into milk where they contribute to the osmolality (Shennan and Peaker, 2000). It has also been observed that enzymes involved in lactose synthesis have a lower activity in cows milked once per day. Decreased conversion of glucose into lactose has also been observed when milking intervals increase which could indicate that lactose synthesis is limited early in the metabolic pathway (Delamaire and Guinard-Flament, 2006). During a study with one prolonged milking interval Lakic *et al* (2009) found that the only constituent in milk affected for a longer period was lactose. An increase in lactose was seen day 1 after the treatment and the concentration then remained elevated throughout the study.

Protein

The protein content in milk tends to increase when milking once daily (Davis *et al*., 1998). Studies on protein with one prolonged milking interval has shown significantly elevated concentrations during both milkings the day after the prolonged milking interval but then rapidly went back to normal day two (Lakic *et al*., 2009). For what is known, changes in protein are similar both in long- and short-term studies (Lakic *et al*., 2009).

Fat

Milk fat, which mainly consists of triglycerides, is synthesized in the alveolar compartment of the udder. Half of the fatty acids found in milk are produced through *de novo* synthesis in the mammary epithelial cells. The other half of the milk fat is made up by pre-formed milk fatty acids that are brought to the udder in very low density lipoproteins or chylomicrons. These are taken up into blood after they have been degraded to free fatty acids and glycerol (Sjaastad *et al*., 2003). The fatty acid hydrolysis is performed by the enzyme lipoprotein lipase. This enzyme occurs naturally in milk but is mostly not active. Lipase activators exists in blood and when tight junctions become impaired these will enter into milk and the enzyme lipase is activated (Thomson *et al*., 2005). When activated, the triglyceride concentration in milk decreases (Sjaastad *et al*., 2003). The milk fat content is known to increase during longer milking intervals as when milking once daily instead of two (Delamaire and Guinard-Flament, 2006). This is supported by Lakic *et al* (2009) who observed an increased fat content up to 3 milkings after a prolonged milking interval of 24 h. The increase in fat is explained by a concentration effect caused by a lower milk yield when milking less frequently (Delamaire and Guinard-Flament, 2006).

Effects of SCC on milk composition

A factor that is known to change due to alterations in SCC is the milk composition (Auldist *et al*., 1998). If milk composition is altered by elevated SCC, shelf life and quality can be negatively affected which is why dairies often reduce the pay to farmers when SCC is high (Lakic *et al*., 2009). To avoid unfavorable effects, the milking interval should not be longer than 18 h (Stelwagen *et al*., 1997). Changes in composition is most pronounced during clinical mastitis, but alterations have been studied even with a small and short-term increase of SCC (Auldist *et al*., 1998). However, not much research has been performed on how one single prolonged milking interval affects milk composition (Lakic *et al*., 2009).

Materials and Methods

The study was conducted at the Lövsta Research Center, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, during September 2015. All animal handling was ethically evaluated and approved by the regional animal ethics committee, Uppsala.

Animals and management

The study included 21 Swedish red (SRB) and 15 Swedish Holstein (SH) dairy cows. Both primiparous (n=3) and multiparous (n=33) cows with lactation number varying from one to six were included. To be selected for this study, cows had to have passed peak lactation which was set at 105 days, have a SCC<200 000 cells/ml on whole udder and have a milk yield of at least 12 kg per day. Cow selection was based on data from test-milking's June-august and SCC were determined on whole udder level. Lactation stage varied from 167 to 376 days in milk (DIM), with 8 cows in mid (weeks 24-29) and 28 cows in late (weeks 30-54) lactation. All cows were fed grass silage *ad libitum* and concentrate in feeding stations according to milk yield (Spörndly, 2003).

Cows were grouped into one control- and one effects treatment group with 18 animals in each. Grouping was based on DIM, milk yield, parity and SCC on whole udder level. Cell count analyses from day -2 to -3 on udder quarter level were used to select the treatment teat. Teats were selected based on similar SCC on same udder quarter. If SCC variation within same udder quarter exceeded variation from the other udder half (divided in back and front) the other udder half was selected instead, e.g. left front together with right front. Udder quarter spread on left back (LB), left front (LF), right back (RB) and right front (RF) were 13, 7, 8 and 8 respectively.

Average milk yield per milking during start of the experiment was 16.1 kg and 15.6 kg for control and treatment group respectively. Furthermore, max yields per milking were 22 kg and 24 kg while min yields were 11 kg and 8 kg for control and treatment group respectively. Average DIM at experiment start was for control and treatment group 272 and 255 respectively. Average SCC on treatment teat at experiment start is shown in table 1.

Table 1. Average somatic cell count (cells/ml milk) on udder quarters in the two experimental groups at the start of the experiment.

Group	Teat			
	LF	**LB**	**RB**	**RF**
Control	34250	37200	31000	7750
Treatment	12000	32000	68333	38500

Due to missed resorting in the control group, two cows had to be regrouped to the experiment group, changing animal frequency to 16 and 20, respectively. Because of failure during removal of pre-selected udder quarter on the treatment day, one cow in the treatment group got corrected, changing udder quarter spread to 14, 6, 8 and 8 respectively. 4 days after the omitted milking, missing registrations due to communication failure between the AMR™ and the DeLaval Delpro Farm Manager™ management system led to a decrease in milk yield to 27.9 compared with the yield of 31.7 kg/day during control days, $P < 0.05$. Since this effect had no relation to the omitted milking, significant effects caused by this artefact were ignored.

All cows were housed indoors in a free housing environment and milking was performed twice daily at 06:00 and 17:00 in an automatic milking rotary (AMR™, DeLaval International AB, Tumba, Sweden). Milking intervals were 13 and 11 hours before morning and evening milking respectively. Animals had free access to silage and water throughout the experimental period. Milk yield, milk flow and milking time was automatically collected in the AMR. Age, lactation, breed and DIM were gathered from DeLaval Delpro Farm Manager™ management system. Notes regarding feed and water availability and general health in the stable were recorded manually by daily visits from the research team.

Study Design

An overview of the study design, dates when sampling for composite-milk and udder-quarter-milk samples occurred is shown in table 2. The experiment started on Monday 14/9 and ended on Monday 28/9. The day when milking was omitted, Thursday 17/9 is referred as day 0.

Table 2. Experimental design and periods used in the statistical analyses with the timing of collection of milk samples at udder quarter level and cow composite level indicated (√). Udder quarter milk samples were taken during evening milking and composite milk samples were taken first at evening milking and then the following morning milking.

Period		**Control**			**Treatment**	**1**	**2**	**3**	**4**	**5**	**6**	**7**				
Experimental day		-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
Date in September 2015		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Milk sample	**Udder quarter**	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
	Composite		√	√		√	√				√	√			√	√

Treatment

All cows in the study were subjected to a single omitted milking which was executed during morning milking Thursday 17/9. Teat cup attachment was performed manually to ensure correct treatment teat. Therefore, robots for teat cup attachment were disabled during milking. Cows in the treatment group were made 3-teated prior to treatment to avoid resorting and directed to exit, while the control group was redirected for a second milking. During re-sorting one person made sure that cows entered the queue to re-enter the AMR.

Milk sampling, Analysis and Recording

Samples of composite-milk and udder-quarter-milk were collected and analyzed for milk quality. Sampling was carried out on all cows in the experiment. Milk recordings was performed in the DeLaval Delpro Farm Manager™ management system.

Composite-milk sampling

One composite milk sampling was performed on all cows each sampling occasion which occurred 6 times, 3 during evening- and 3 during morning milking. Sampling was performed to cover a 24-hour interval where the first sampling occurred during evening milking and the second sampling during morning milking the following day. Tubes for milk sampling were labeled with serial number and all samples were treated with Bronopol, 2-bromo-2-nitropropane-1.3-diol before sampling. Milk was first collected during milking in a sampling vessel located at the milking means. Sampling into test-tubes was performed after milking. Prior to test-tube sampling, milk was mixed for a few seconds in the sampling vessel to ensure that milk was mixed before sampling. Approximately 25ml was sampled per test and samples were stored at 8ºC. Samples were sent to the Department of Animal Nutrition and Management for determination of whole udder SCC and gross milk composition regarding fat, protein and lactose with mid-infrared spectroscopy (Fourier Transform Instrument, FT 120, Foss, Hillerød, Denmark).

Udder quarter-milk sampling

Udder quarter-milk sampling was performed during evening milking throughout the experiment period, in total at 14 occasions and comprised 4 tests per cow each occasion. Tubes were labeled with serial number and teat. Sampling was performed manually before teat cup attachment, therefore the robots for teat cup attachment were switched off during evening milking and teat cups was attached manually after sampling. Approximately 25ml was sampled per test and samples were run in a DeLaval cell counter (DCC™, DeLaval International AB, Tumba, Sweden) either directly or after one night's storage at 8ºC. The DCC is battery operated and small, with a good portability which makes it suitable for on-farm use (DeLaval, 2015b). The device counts cells optically and each sample takes less than 1 min (approximately 40 s). Milk samples is taken in cassettes which contains small amounts of the fluorescent stain propidium iodide (PI). The cassette is then placed into the DCC at the counting window and exposed to a LED light source. Cells is then counted by fluorescent signals from the cell nuclei that is recorded in an image, thus making it possible to determine the SCC in raw bovine milk (Gonzalo *et al*., 2006).

Data handling and statistical analyses

Day in the study was expressed as "period", distinguishing between the three days before the treatment day (Period -1), the treatment day (Period 0) and the first 1-6 days after the treatment day (Period 1-6) and then the last five days as Period 7. SCC values were converted to $\log_{10}$ values in order to obtain a normal distribution of the data.

The model included the fixed effects of breed, parity, treatment and period. Repeated measures on cow nested within treatment with autoregressive covariance structure was included as a random effect using the statement 'repeated order/subject=COW(treatment) type=AR(1)' (SAS 9.4, SAS Inst. Inc., Cary, NC, USA). For the fixed effects, least-squares means (LSMEAN) were calculated and differences between them were tested for significance using t-tests adjusted with Tukey's test when comparing three or more LSMEANS. Normality and equality of variance were checked by visual inspection of the residuals. Values given are mean(SD) or LSMEAN±SEM.

```
PROC MIXED;
CLASS COW BREED PARITY REAL_GROUP period;
MODEL FFA=REAL_GROUP BREED PARITY period;
REPEATED/SUBJECT=COW(REAL_GROUP) TYPE=ar(1);
lsmeans REAL_GROUP period breed parity/pdiff adjust=Tukey;
run;
```

4 Results

4.1 Milk yield on whole udder and udder quarter level

4.1.1 Milk yield on whole udder per day and per milking

Milk yield per day was 11% lower during the day when milking was omitted than previous days (28.0±1.1 and 31.7±1.0 kg/day respectively, $P < 0.001$) (Figure 1). After the day when milking was omitted, no significant effect on milk yield was found and there was no difference between the treatment groups.

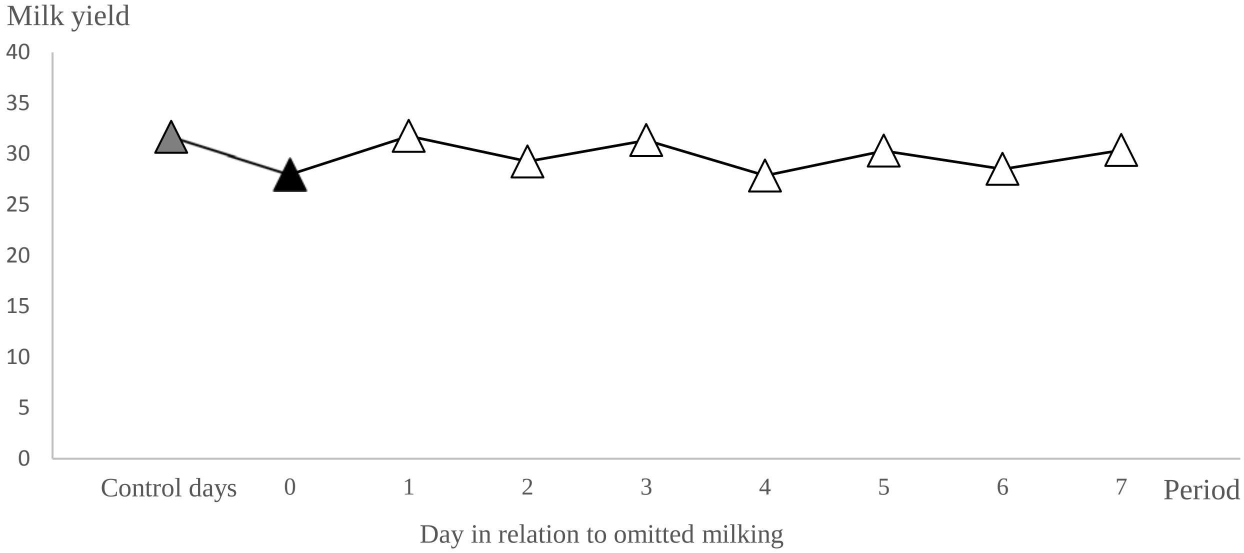

Figure 1. Milk yield per day on whole udder level in cows exposed to one omitted milking on one teat. Day 0 was the day of the single omitted milking. Values were compared with the mean value over the three days prior to day 0 (▲) and values that differed significantly from the control days are indicated with filled black symbols (▲).

Milk yield per milking was 23% lower on the day when the omitted milking was conducted, compared with previous days (11.7±0.6 and 15.2±0.4 kg/milking respectively, P <0.001) (Figure 2). In addition, a difference in milk yield was found between control and treatment group (14.7±0.3 and 13.8±0.3 kg/milking respectively, P < 0.05).

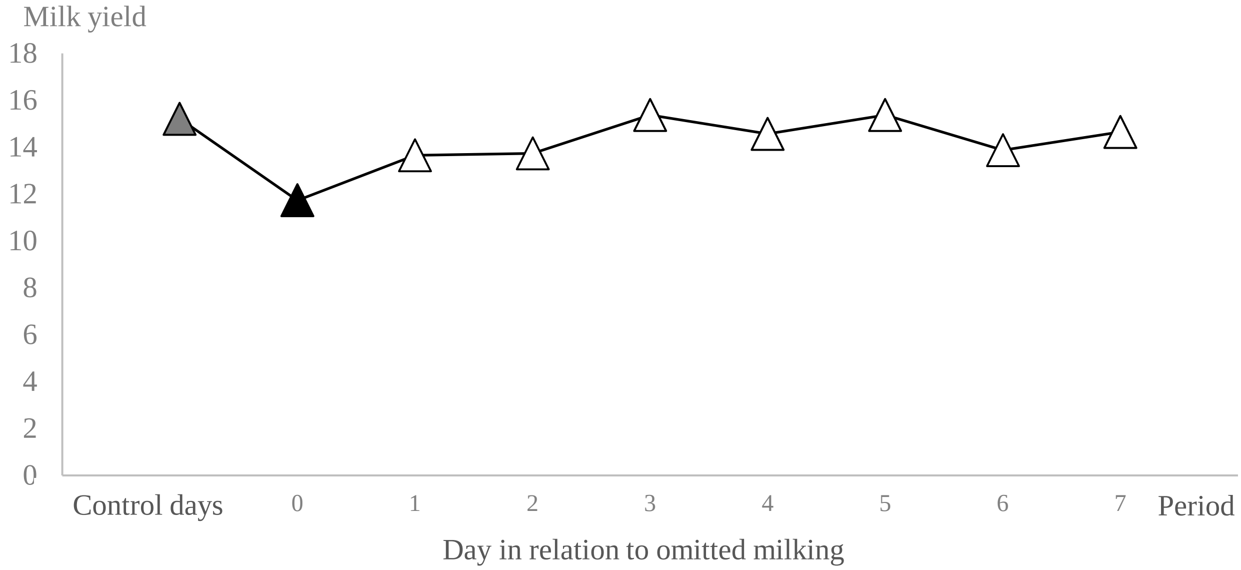

Figure 2. Milk yield per milking on whole udder level in cows exposed to one omitted milking on one teat. Day 0 was the day of the single omitted milking. Values were compared with the mean value over the three days prior to day 0 (▲) and values that differed significantly from the control days are indicated with filled black symbols (▲).

4.1.2 Milk yield per day on treatment teat

Milk yield was 27% lower on the day when the omitted milking was conducted compared with previous days (5.8±0.6 and 8±0.6 kg milk/day respectively, P < 0.001) (Figure 3). No differences between control and treatment group was observed.

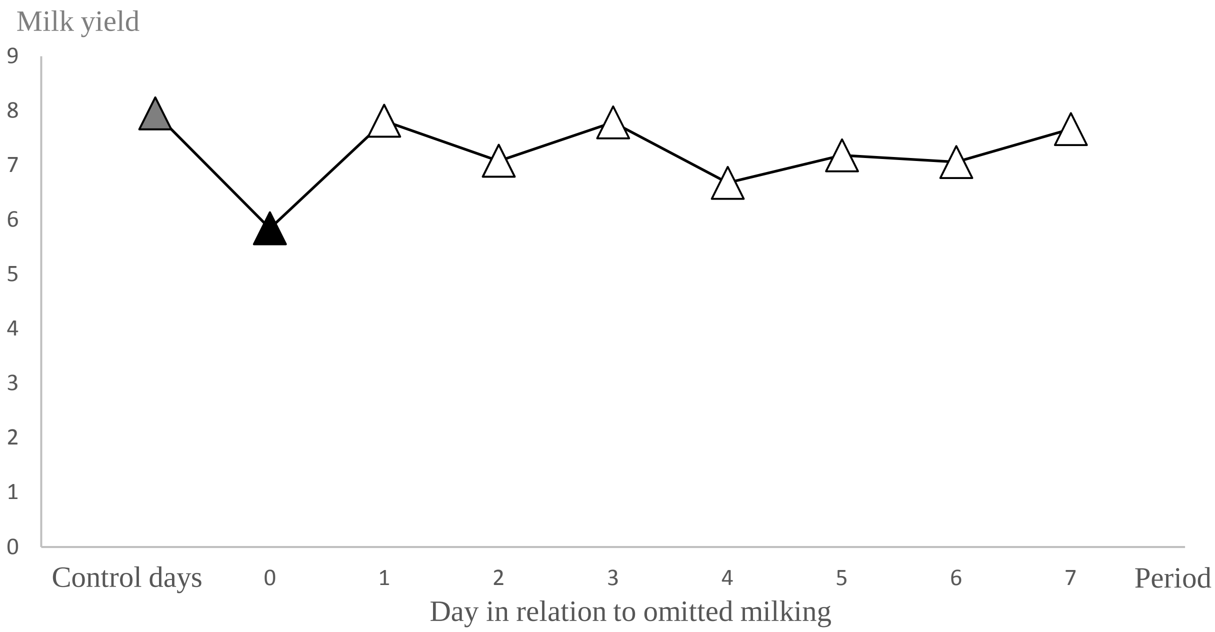

Figure 3. Milk yield per day on treatment teat in cows exposed to one omitted milking on one teat. Day 0 was the day of the single omitted milking. Values were compared with the mean value over the three

days prior to day 0 (▲) and values that differed significantly from the control days are indicated with filled black symbols (▲).

4.2 Effects of resorting on milk yield

Milk yield per milking on treatment teat was 4.4kg on average during morning milking in the control group. Due to resorting, milk yield on treatment teat was lower, on average 1.72kg. In addition, 7 out of 16 control cows milked less than 1kg on resorting. A source of error during this study was false registration of milk yield in the AMR on udder quarter level. This was detected by that the registered milk yield on one teat was 200-300% over the expected yield while other teats had no registered milk yield at all. Resorting time varied from 20 min to 50 min.

4.3 Somatic cell count on whole udder level and on treatment teat

SCC on whole udder level was not affected by the single OM (Table 3) and no significant differences were found on SCC between control and treatment group on whole udder. Parity 5 cows showed a higher SCC on whole udder than cows in parity 2 ($P < 0.01$) and 3 ($P < 0.05$) with lgSCC = 4.9±0.1, lgSCC = 4.4±0.03 and lgSCC = 4.5±0.1 respectively. SCC on treatment teat was not affected by the single omitted milking (Table 3). A difference was found between parity 5 and parity 2 cows on treatment teat ($P < 0.001$), with lgSCC = 4.8±0.1 and lgSCC = 4.2±0.05 respectively. Furthermore, SH had a higher SCC than SR on treatment teat ($P < 0.01$) with lgSCC = 4.5±0.1 and lgSCC = 4.3±0.1 respectively.

Table 3. LSMeans for LogSCC variation on whole udder and teats during the control period (=Day -3 to -1), Period 0 (=treatment day) and during the following 11 days divided into Period 1-7 in cows subjected to one single omitted milking in Period 0 in 36 dairy cows in mid and late lactation.

	Period								
	Control	0	1	2	3	4	5	6	7
Whole udder	4.67		4.7	4.6				4.64	4.61
Treated teat	4.34	4.28	4.53	4.43	4.42	4.44	4.37	4.26	4.44

4.4 Milk composition changes on whole udder

Lactose was higher during the control days than from alveoli to the gland cistern 1, 6 and 7 ($P < 0.001$). Protein varied between control days and period 7 ($P < 0.05$). Fat content increased during period 1 ($P < 0.01$) and 6 ($P < 0.001$) respectively compared with control days (Table 4).

Table 4. LSMeans for milk composition (%) in 36 cows in mid and late lactation subjected to one omitted milking on one udder quarter. Data is shown for the control period (=Day -3 to 1), Period 1 (=one day after the treatment), Period 2 (=2 days after the treatment). Period 6 (=6 days after the treatment) and Period 7 (Days 7-11 after the treatment).

	Period				
	Control	1	2	6	7
Lactose	4.75	**4.70**	4.72	**4.65**	4.68
Protein	3.43	3.44	3.43	3.45	**3.45**
Fat	4.18	**4.60**	4.06	**4.68**	4.28

Values indicated in **bold** were significantly different ($P< 0.05$) from the control values.

Discussion

In the present study there was no difference in milk yield between cows that were resorted or not resorted to a second milking after having one udder quarter omitted in the first milking. However, on the treatment day a lower whole udder milk yield was observed in the non-resorted group. Because the drop in milk yield was equivalent to the amount of milk that the omitted teat should have contributed with, the excluded teat was the probable cause. Other than that there were no differences between the groups in milk yield and milk composition.

The low milk yield on resorting was most likely an effect of failed milk ejection. Stress is a factor that can inhibit milk let down (Sjaunja *et al*., 2004), an explanation to the failed milk ejection could therefore be that the cows that entered the queue became stressed due to the unfamiliar environment entering the AMR via a different route than they were used to. Another possible explanation could be that the second milking attempt failed due to the lower fill-grade in the udder on the second milking. With a lower fill grade, milk ejection takes more time and it is likely that the teat cups may have been automatically removed before milk ejection occured. If that was the case, only cisternal milk was removed on the second milking attempt. No negative effects were found after the omitted milking. This was in line with the findings of Ljunggren (2015) who also observed effects of one omitted milking on one udder quarter in late lactating cows. The results however contradict the findings of Lakic *et al*. (2009) who found a negative effect up to ten days on afternoon milking, also on one omitted milking but in mid lactating cows and on whole udder level. Furthermore, this study showed different results compared with those presented by O'Brien *et al*. (2000) who observed significant differences in milk yield when comparing ODM and twice daily milking for ten weeks. However, the results observed by O'Brien *et al*. (2002) could be explained by a regularly higher intramammary pressure and a long-term effect due to less frequent milking. A possible explanation to the differences between this study and the findings of Lakic *et al*. (2009) could be the stage of lactation, where mid lactation cows (Lakic *et al*., 2009) most likely experienced a higher intramammary pressure and therefore received a small, but still negative effect on the secretory cells, compared with the cows in late lactation included in the present study. However, it has been found that late lactating cows, compared to late lactating cows milked twice daily, had a negative response on milk yield when milking once per day (Stelwagen *et al*., 2013). The explanation for not finding negative long-term effects on milk yield in this study could be that cows had milk let down during the first milking, when one udder quarter was omitted, which may have decreased pressure in the alveolar compartment by shifting milk from alveoli to the gland cistern. This differs from the study when the whole udder was omitted (Lakic, *et al*., 2009). In addition, because no effects on milk yield were observed in either this or the study by Ljunggren (2015) it could be suggested that udder fill due to one longer milking interval in late lactation is not enough to cause a significant udder distension and therefore no pronounced downregulation of milk synthesis. This was likely explained by a larger udder capacity compared with the milk production over 24 hours when cows are in peak lactation. Most of the cows in this study were in late or very late lactation which probably made them less sensitive to the omitted milking. It could be so that the results on milk yield would have been different if the majority of cows would be very close after peak lactation. Also, this study was performed with strict milking intervals of 11 and 13 h throughout the study. In commercial AM systems, when milking frequency often is above two per day, one omitted milking would likely have even less importance with regard to udder health and production. Moreover, because milk yield did not decrease in the control group after treatment, it could be hypothesized that late lactating cows with a lower energy requirement is not negatively affected by a longer time away from feed and cubicles caused by resorting.

Cows with higher parity were found to have a higher SCC than younger cows which was in line with literature (Nyman *et al*., 2014). An explanation to the higher SCC in SH compared with the SR breed could be that SH had a slightly higher daily milk yield (2.58kg). Furthermore, no long-term effect on SCC was observed in this study which corresponds to the findings of Ljunggren (2015). However, an elevated SCC on treatment teat the first day after the treatment (period 1) was seen in both control and treatment group. The rise in SCC on treatment teat could be explained by increased permeability in the udder due to an increased intramammary pressure (Stelwagen and Lacy-Hulbert, 1996). There is also a possibility that the omitted milking led to high intramammary pressure in the omitted udder quarter which could have led to milk leakage and an allowance of pathogens to the udder (Stelwagen *et al*., 2013). Occurrence of milk leakage in the AMR and after exit was unfortunately not included in the study design. An explanation to the increase in SCC in the control group could be the low milk yield on resorting. The low milk yield could have led to an accumulation of milk in the udder to an extent where it caused impaired tight junctions or milk leakage. It has earlier been observed by Mayer *et al*. (1984) that the obtained milk yield was lower when milk removal was delayed in relation to milk ejection. If milk ejection did not occur during the second entry in the AMR, the obtained milk was from the first ejection. Another explanation could be cisternal recoil which transfers some of the milk back to the alveoli and milk ducts when stimulation ends, leaving small amounts of milk in the cistern (Caja *et al*., 2004).

The error observed on milk registration on udder quarter level could have affected the average milk yield per teat used to calculate the expected milk yield on resorting. Secondly the results of milk yield on resorting could be wrong due to milk yield registration on the wrong teat. However, no indications were observed that total milk yield on whole udder level was incorrect, meaning that only results regarding a specific teat could be misleading.

The effects on milk composition presented here were small except for fat. However, these changes probably did not affect production or milk quality characteristics. An explanation for the small effects could be that the treatment only was applied on one teat, while milk composition samples were taken on whole udder. It is likely that if milk composition sampling would have been performed on treatment teats only, the observed effects would have been larger, this was however not possible in the AMR used in the study. Still, it is the quality of milk from the whole udder that is important on farm level.

Lakic *et al*. (2009) found that the concentration of lactose in milk decreased and remained decreased throughout the study, both in morning milk and evening milk after one prolonged milking interval. Because lactose is a major osmole in milk, a decrease in lactose content should also lead to a decrease in milk yield. In this study however, no changes in milk yield was observed. It is therefore not likely that the lower milk lactose content observed in the present study was due to a lower lactose synthesis, since that should have resulted in lower milk yield but not a change in lactose content of milk. The decreased levels of lactose observed here could instead be explained by a daily lactose variation between morning and evening milkings. Both samples that show significant differences compared to control value were taken during evening milking. At the same time the control value consists of one morning and one evening milking. Lakic *et al* (2009) observed a lower lactose content of 10 percentage units in evening milk compared with morning milk. If the daily lactose variation in this study was of similar proportion, it could explain the significant differences observed here. It was not likely that the very small increase in milk protein content (0.018 percentage units) found in period 7 compared to control days in this experiment was related to the single omitted milking. Milk fat content was higher in period 1 compared with before treatment. It is not likely that this increase was explained by a concentration effect from the lower milk yield after the treatment, because milk sampling occurred 3 milkings after the omitted milking. In general, with the design used in the present study it was not possible to determine if the changes observed in this study were because of the omitted milking.

The resorting time varied greatly between cows, this could be explained by the rank order in a herd, where a low-ranked cow is pushed away and therefore have to stay in the waiting area for a longer time. A generally short time from first to second milking in the present study was achieved by the person guiding the cows and helping them to get back into the queue to re-enter the AMR. If no one would have been there pushing other cows back in the line as under practical conditions, resorting time would most likely have been longer. Even though production did not suffer from resorting, adding the time spent during milking and resorting could mean several hours away from feed and cubicles which could affect the cow negatively due to compromised time available for eating and for lying down. It is also possible that if several cows per hour are incompletely milked and resorted back, it would have a negative effect on the AMR capacity. These factors were however not measured in this study and remain to be investigated.

Conclusions

No differences were found between control and treatment groups. This indicates that resorting these cows after one udder quarter is omitted on cows in mid and late lactation with SCC < 200 000 was not necessary.

The cow's used in this study did not have a significant negative long-term effect on SCC due to one omitted milking on one udder quarter.

Milk composition was altered due to one omitted milking on one udder quarter. However, the changes in composition observed were mainly small. This indicates that it is possible to omit one milking on one quarter without negative effects on milk quality.

These cows in mid or late lactation who were incompletely milked on one udder quarter in this study did not have any beneficial effects of being resorted for a second milking.

The majority of cows used in this study were in late or even in very late lactation. It is possible that this treatment would have had a greater impact on udder health and milk composition if they were to be selected more strictly after peak lactation.

Further Studies

It was indicated in the current study that the udder and milk composition may have been affected, although not so pronounced, by one single omitted milking on one udder quarter. Earlier research has focused on what happens with udder permeability and composition of milk with increased milking frequencies or longer milking intervals. It would therefore be a future interest to determine which components there are that play key roles in the udder when a single omitted milking on one udder quarter occurs. It would also be of interest to see if other udder quarters than the one omitted are affected and how that affects milk composition. Because cows used in this study mainly were in late or very late lactation it would also be of interest to target how the udder, yield and composition is affected with cows selected more strictly on peak lactation. Finally, if future research will find a need for resorting cows with one quarter incompletely milked, it would also be of interest to investigate how the capacity of the AMR is affected.

Acknowledgments

This study was made possible through the collaboration between the Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, and DeLaval international, Tumba, Sweden. First I would like to thank my supervisors Sigrid Agenäs and Emma Ternman for the great support with planning and execution of this study, but also for the great knowledge and input regarding the biology of lactation. I would also like to give Sigrid Agenäs a special thanks for doing the statistical analysis in the SAS program, enabling me to focus on the processes of milking. I would also like to show my appreciation to the staff at Lövsta Research Center, Uppsala and the laboratory staff at the Centre for Veterinary Medicine and Animal Science, for assisting at milking and milk analyses.

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