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Swedish University of Agricultural Sciences
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Effects of a single omitted milking on one udder quarter in high cell count dairy cows in an AMR system with or without re-sorting for a second milking

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Effekter av en utelämnad mjölkning på en juverdel hos högcells kor i ett AMR-system med eller utan återsortering till en andra mjölkning

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Abstract

The milk production is developing towards fewer but larger herds with automatic milking systems. The most recent system for milking that has been developed for managing large herds is an automatic milking rotary (AMR), which was launched in 2010. Although several benefits with automated systems have been reported there is an increased risk for incomplete or omitted milking due to attachment failure or that the cow kicks off one or more teat cups after attachment. An incomplete milking of one or more udder quarters can lead to reduced milk yield, a lower quality of milk and milk leakage, which may lead to decreased udder health. Therefore, cows who are incompletely milked in an AMR are resorted to a second milking after the first milking occasion, but this resorting reduces the efficiency of the AMR and affects the cows' time budget. Somatic cell count (SCC) is the most common indicator for udder health and subclinical mastitis, increases in SCC is an important part of the cow's immune system. In Sweden, SCC above 200 000 cells / ml milk is classified as high cells in the milk and the cow probably has mastitis in one or more udder quarters. SCC can vary depending on various factors, such as stage of lactation and milk fraction. There is also a day to day variation in SCC and this is higher for cows with mastitis than for healthy cows.

The aim of the study was to evaluate if cows with a SCC above 200 000 cells/ml that have passed peak lactation really need to be re-milked after a single incomplete milking in an AMR. 16 cows were included in the study. In the morning day four of the experiment, milking was omitted on one udder quarter, the udder quarter with the highest SCC was selected to the treatment from all cows. Half of the cows were re-sorted into a second milking after the first milking occasion - to be milked on the fourth udder quarter. Milk samples were taken daily and were analyzed for SCC, milk composition and milk yield during three days before the treatment to ten days after the treatment. The results showed no significant effect on either the SCC, milk composition or milk yield due to the omitted milking, there were also no significant differences between the re-sorted group and the group that was not re-sorted. The study did not show any negative effects on SCC, milk composition or milk yield by an omitted milking on one udder quarter for cows with high SCC. However, the study needs to be repeated since the low number of cows included in the study allows limited conclusions of the data. Further studies success should have a higher number of cows to clarify if the results in this study are reliable.

Sammanfattning

Mjölkproduktionens utveckling går mot färre men större besättningar med automatiserade mjölkningssystem. Det nyaste mjölkningssystemet som utvecklats är en automatisk mjölkningskarusell (AMR), som lanserades år 2010. Även fast det finns flera fördelar med automatiska system, finns en risk för att kor kan bli ofullständigt mjölkade i en eller flera juverfjärdedelar, utifall kon sparkar av en spenkopp eller om robotarna misslyckat med påsättning av spenkopparna. En ofullständig mjölkning på en eller flera juverdelar kan leda till minskad mjölkavkastning, en sämre kvalitet på mjölken och mjölkläckage, vilket kan leda till försämrad juverhälsa. Därför återsorteras kor som mjölkat ofullständigt till en andra mjölkning i en AMR, men återsortering minskar effektiviteten av en AMR och kan påverka kornas tidsbudget. Somatiskt cell tal (SCC) är den vanligaste parametern för att mäta juverhälsa och subklinisk mastit, ökningar i SCC utgör en viktig del av kons immunförsvar. I Sverige klassificeras SCC över 200 000 celler/ml som höga celler i mjölken och kon har troligen mastit i en eller flera juverdelar. SCC kan variera beroende av olika faktorer, så som laktationsstadium och mjölkfraktion. Det finns även en dag till dag variation i SCC och denna är högre hos mastit drabbade kor än hos friska kor. För att undvika ytterligare ökningar i SCC hos kor som har mastit i en eller flera juverdelar kan en ökad mjölkningsfrekvens göra att bakterier sköljs ut ur juvret oftare.

Syftet med denna studie var att undersöka om kor med SCC över 200 000 celler / ml som har passerat topplaktation behöver återsorteras till en andra mjölkning efter en ofullständig mjölkning i en AMR. 16 kor ingick i studien. På morgonen den fjärde dagen av studien mjölkades endast tre juverdelar, den fjärde blev omjölkad från alla kor. Den juverdel med högst SCC från samtliga kor utsattes för behandlingen. Hälften av korna blev återsorterade till en andra mjölkning efter den första – för att bli mjölkad även på den fjärde juverdelen. Mjölkprover togs dagligen och analyserades för SCC, mjölkkomposition och mjölkmängd under tre dagar före behandlingen till tio dagar efter behandlingen. Resultaten visade ingen signifikant påverkan på varken SCC, mjölkkomposition eller mjölkmängd på grund av den överhoppade mjölkningen, det var inte heller några signifikanta skillnader mellan återsorteringsgruppen och gruppen som inte återsorterades. Studien visade inga negativa effekter på SCC, mjölksammansättning eller mjölkmängd av en utelämnad mjölkning på en juverdel för kor med högt SCC. Dock behöver studien upprepas, eftersom det låga antalet kor som ingick i studien ger begränsade slutsatser. Ytterligare studier bör använda sig av ett större antal kor för att klargöra om resultaten i denna studie är tillförlitliga.

Introduction

Dairy farming in many countries, including Sweden, is currently undergoing a size rationalization. Number of farms is decreasing, while the number of cows per herd is increasing. In parallel with this shift a marked increase in automatic milking systems (AMS) is seen (De Koning, 2010). The most recent automated system that has been developed in dairy production is an automatic milking rotary (AMR, DeLaval AB, Tumba, Sweden). DeLaval has developed the AMR for farms with > 300 cows, to make the milking more effective for the farmers.

Automatic milking offers a reduction of manual work, but absence of manual handling during the milking process involves risks that do not occur in conventional milking. One possible problem is incomplete or omitted milking on one or more udder quarters because of unsuccessful attachment of the teat cups or if the cow kicks off the teat cups. If this happens in an AMR the cow is re-sorted to a second milking, to be completely milked. From an efficiency perspective, re-sorting has a negative effect, due to a reduced capacity of the number of cows per day in the AMR. For the individual cow re-sorting also results in a longer time in the waiting area, which means less time to eat and rest. On the other hand, milk production and milk quality may be affected if the cows are not re-sorted after an incomplete milking.

Leukocytes is a key factor in the defense against infection in the udder and therefore, elevated somatic cell count (SCC) in milk is a strong indication of mastitis. When SCC is elevated the quality of the milk and also the cows well-being deteriorates (Sordillo et al., 1997). For cows with mastitis in one or more udder quarters with SCC above 200 000 cells/ml milk it is important to empty the udder regularly to avoid further increases in SCC (Brolund, 1985). Omitted milkings in one or more udder quarters for cows with high SCC, might consequently have a negative effect on udder health. It has not been evaluated how one omitted milking on one quarter affect cows that have elevated SCC in an AMS. There is information about effects of a single omitted milking in cows with low SCC in conventional systems, Lakic et al. (2011) found that the milk yield and SCC are negative affected when cows with good udder health are subjected to one omitted milking on whole udder level.

Aim and Hypothesis

The aim of the study was to evaluate if cows with a milk SCC above 200 000 cells/ml that have passed peak lactation need to be re-milked after a single omitted milking on one udder quarter in an AMR. The hypothesis was that cows with SCC above 200 000 cells/ml at the last test milking should be re-milked when resorted for a second milking in the AMR.

Literature Review

Automatic milking rotary

The automated milking rotary (AMR, DeLaval, Tumba, Sweden) was first introduced in 2010 and was designed to suit dairy farms with 300-800 cows (figure 1). The AMR contains up to 24 milking places and cleaning, attachment of teat cups and teat dipping is performed by robot arms placed inside the rotary. It has a capacity to milk up to 90 cows per hour, depending on the number of robot arms (Jacobs & Siegford, 2012). Up to five robot arms can be installed, where two of them clean and stimulate two of the four teats each. Two other robot arms perform attachment of teat cups on each udder half (figure 2). The last robot arm is positioned at the end of the rotation, spraying the teats with disinfection after milking. Each robotic process takes 20-30 seconds and all robot arms has a camera with laser for teat which is used to detect the teat position of the cow. Each robot is positioned on stationary locations, while the platform that the cows are standing on is rotating (Hunter Nilsson, 2014).

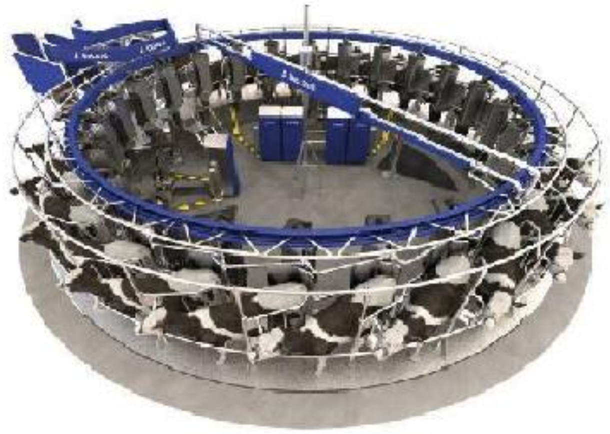

Figure 1. AMR. Modified from: Delaval.com.

Figure 2. Robotic arms in an AMR. Modified from: Delaval.com.

Anatomy of the udder and milk synthesis

A cow has four udder quarters, each quarter is to be regarded as a separate unit, even though there is some communication between the quarters through blood. Each udder quarter consists alveoli, milk ducts, gland cistern and teat cistern. The milk is produced in small gland vesicles, called alveoli, and the lumen of these alveoli is also where the main part of the milk is stored between milkings. The teat tip has a sphincter with smooth muscle which enables the teat canal to be open during milkings but closed between milkings. This part of the udder acts as an important protection that prevents foreign particles from entering the udder (Sandholm et al., 1995).

The alveoli are the functional units of the lactating mammary gland and it is the epithelial cells in the alveoli that produce the milk. Thousands of alveoli are needed to form one drop of milk. Milk synthesis gradually declines when alveoli are filled with milk. Precursors for milk are brought to the mammary tissue by the blood, some of them passing to the milk unchanged, while others are used for synthesis of specific milk components in the epithelial cells (Sjaastad et al., 2003; Swenson & Reece, 1993).

Milk composition is complex but the main milk constituents are water, milk protein, lactose and milk fat. Milk proteins include whey proteins and caseins. Caseins constitute around 80% of the proteins in milk from a cow. The whey proteins include immunoglobulins, lactalbumin, lactoglobulin and different enzymes. Milk protein synthesis is controlled by DNA and require that amino acids are provided to the mammary gland. The synthesized proteins are transported in vesicles to the Golgi apparatus, where they are packed into other vesicles and transported to the apical membrane and released into the alveolar lumen by exocytosis. Lactose is a disaccharide consisting of one glucose- and one galactose molecule. Lactose is formed by enzymes in the membrane of the Golgi apparatus, and out of the cell by exocytose on the same way as the proteins. On the way out to the lumen water is drawn into the vesicle by osmosis. Through this mechanism lactose synthesis largely controls milk yield. Milk fat consists of membrane-covered drops of fat. Approximately 98% of milk fat consists of triglycerides, with the remainder composed of phospholipids and cholesterol, mainly from the membrane around the fat droplet. Half of the fatty acids derived from the blood while the other half are formed de novo in the epithelial cells from acetate and β-hydroxybutyrate (Mather & Keenan, 1998; Sjaastad et al., 2003).

Milk fractions and Milk let-down

The milk fractions consist of cisternal milk, alveolar milk and residual milk. The main milk volume, > 80% is stored in the alveoli, where it is kept by capillary forces and requires release of the hormone oxytocin in order to get this milk available. A milk let-down is required in order to make the milk in the alveolar tissue available for milking. Milk stored in the larger milk ducts and the gland cistern is on the other hand available for milking without a preceding milk ejection (Bruckmaier & Blum, 1998). Alveolar milk becomes available when oxytocin initiates a contraction of the smooth muscle cells, which gives the result that the milk is released. This milk let-down reflex occurs by a stimulation of the teats and udder, which transmits a signal through the nervous system to release oxytocin from the pituitary gland to the udder. Nerve fibers in the teats are stimulated by the calf suckles or cleaning of the udder before milking and during milking of the milking cluster. Oxytocin also causes widening of the teat canal, which providing a better flow of milk (Svennersten-Sjaunja & Olsson, 2005). The milk-letdown reflex occurs within 40 seconds up to 3 minutes after the start of udder stimulation, partly due to the degree of udder fill. When udder fill is low it takes longer time because the muscle epithelial cells must contract more to squeeze out the milk when the alveoli are not entirely filled with milk. A fraction of milk is always left in the udder between milkings. This milk fraction is called residual milk and consists of 10-30% of the total milk volume (Bruckmaier & Blum, 1998).

Mastitis

Mastitis is an inflammation of the udder and this is a very common condition in dairy cows. The disease is multifactorial and therefore difficult to prevent, because there are many different causes. It can be caused by trauma, chemical irritation or invasion by foreign agents, for example bacteria. Bacteria from the cow’s close environment is the most common cause of mastitis. The main route of infection is through the teat canal. The purpose of the inflammatory reaction is to try to restore the normal functioning of the udder (Sandholm et al., 1995; Reneau, 1986; Cremonesi et al., 2006). Inflammation in the udder is induced when bacteria start to multiply in the mammary gland and produce toxins, which are harmful to the mammary gland. The mammary tissue becomes damaged and one effect is that the blood-milk barrier, that prevents substances leaking from the blood to the milk, is impaired. This results in leakage of blood components; serum proteins, enzymes, and salts into the milk. Synthesis of casein and lactose decreases and milk fat composition changes. The extent of the infection and the cow's immune system determine how large these changes will be (Harmon, 1994).

Mastitis is classified as clinical or subclinical, depending on the extent of inflammation in the udder. Clinical mastitis affects the general health of the cow and causes changes in color of the milk, reduced milk production, swelling of the udder and the general condition may also be impaired (Akers, 2002). Subclinical mastitis is the most common form of mastitis and stands without clinical symptoms, but elevated cell count – due to increased amount of leukocytes, and a decrease in lactose can be detected by milk sampling. SCC and milk composition has a significant role for the payment of the milk in several countries, including Sweden. Therefore, it is important to avoid both clinical and subclinical mastitis in a herd (Sandholm et al., 1995; Andersson et al., 2011). The prevalence of mastitis is strongly linked with milking routines and general hygiene. Other factors that affect the risk for mastitis are the characteristics of the individual cow, the stable design, litter and manure handling (Reneau, 1986).

Somatic cell count

Somatic cell count (SCC) is the most commonly used parameter for monitoring udder health and detecting subclinical mastitis. Increases in SCC constitutes an important part of the cow's immune response. Milk from a healthy udder has a low content of somatic cells and the cells are mainly macrophages, but also neutrophils, lymphocytes and a minor portion of epithelial cells. A cow in the middle of lactation with a healthy udder has SCC below 100 000 cells per milliliter (Schukken et al., 2003). If the SCC is above 200 000 cells per milliliter the cow likely has mastitis. There is not only an increase in the number of cells at a state of mastitis, there is also a change in the proportion of different cells. Cows with mastitis compared to healthy cows have mainly neutrophils in their milk. Neutrophils are key defense in the udder and phagocytose harmful agents, they may constitute more than 90% of the somatic cells in milk at a stage of acute mastitis. (Sordillo et al., 1997).

SCC can be determined on udder quarter level, at the individual level with the collection of samples from whole udder or at herd level with milk tank samples. When SCC is estimated, it is important to distinguish between SCC in collection of milk from all four quarters and the milk from the individual udder quarters. The reason is that it is not common that all udder quarters have the same status, especially at elevated cell count. Cows with low SCC in the milk collected monthly for the national milk recording scheme can have udder quarters with occasional elevated SCC, and cows with high SCC in the milk collection can have udder quarters with low numbers of cells (Forsbäck et al., 2009).

Variation in SCC

SCC is affected by several factors and the variation is not always related to infection. Disturbances in the cow's daily routines, such as the milking process can cause variations in the SCC (Harmon, 1994). It has also been demonstrated that SCC differs between breeds, where Swedish Holstein has generally higher SCC than the Swedish Red breed (Brolund, 1985). Furthermore, Kukovics et al. (1996) showed that there is an increase in SCC with age and parity. This is probably an effect of that the cow has been exposed to bacteria and a risk to get mastitis during a longer period of time (Kukovics et al., 1996).

Stage of lactation also influences variation in SCC. A higher SCC can be seen during the colostrum period and in late lactation. Cows with subclinical mastitis have a stronger increase in SCC towards the end of lactation (Sandholm et al., 1995). There is also a day to day variation in SCC of the udder quarters. This variation can be up to 10% for a healthy udder (Sjaunja, 1986). A mastitis affected udder quarter generally has a higher variability of SCC than a healthy udder quarter (Sandholm et al., 1995; Reneau, 1986).

When an udder quarter is colonized by bacteria there is first an increase in bacteria, and then an increase in SCC through the recruitment of neutrophils from the blood to the udder. When the neutrophils begin to reduce bacteria, the number of bacteria decreases, which in turn leads to that the number of neutrophils later drops. The drop in neutrophils gives room for the bacteria to increase in number again, which again requires higher neutrophil numbers. This results in fluctuations in bacteria and SCC during the inflammatory process. The fluctuations of bacteria are not always fully synchronized with the fluctuations of the SCC because the process is also influenced by many other immunological factors (Daley et al., 1991).

Furthermore, SCC varies in the different milk fractions. Investigations of the milk fractions from a cow indicate that the cistern- or foremilk has a relatively high SCC, and that SCC is lowest in the early alveolar fraction, higher in the late alveolar fraction and highest in the residual milk. This applies both cows with healthy udders and cows with mastitis, but for an udder with mastitis the increase is more pronounced (Sandholm et al., 1995; Sarikaya & Bruckmaier, 2006).

Interaction between SCC and milk composition

Milk composition varies between different breeds, stage in lactation, milk yield and type of feed. SCC also affects milk composition. At elevated SCC in a stage of subclinical mastitis there is an increase in milk components that have a negative effect on milk quality like proteolytic enzymes, sodium and chloride, while the nutritional components; fat, casein and lactose decreases (Hamann, 2001).

At elevated SCC the concentrations of sodium and chloride in milk increase as a result of damage on tight junctions, which constitutes the blood-milk barrier. Lactose leak out from the alveoli, so the content of lactose decline. The damage of the secretory cells and the tight junction also leads to a reduced capacity to synthesize lactose. In milk from an udder with mastitis the lactose decreases with about 10%. Thus, the relation between lactose and SCC is negatively significant (Sandholm et al., 1995; Berglund, 2003). Therefore, lactose can be used as an indicator of mastitis, by comparing the lactose content between udder parts of the cow (Berglund, 2003).

Milking intervals in relation to SCC

The milking interval has a strong effect on SCC. Too short or too long milking intervals may influence the udder negatively and the most favorable is to spread the milkings evenly over the day. The milking interval should not be less than six hours and not exceed 12 hours. With higher milking frequency bacteria is flushed out of the tissue more often and this is a likely explanation for the beneficial effect on SCC by increased milking frequency and there is an association between increased milking frequency and lower incidence of mastitis. On the other hand, a shorter milking interval also leads to that the teat becomes more worn and the teat canal is open and exposed to pathogens for longer time per day (Hovinen & Pyörälä, 2011). Studies have shown different results regarding the effect of milking frequency at the SCC in milk. Dahl et al (2004) found that increased milking frequency lower SCC levels for cows with both low and high output values of SCC, while others present no effect on SCC (Shields et al., 2010).

A single extended milking interval or an incomplete milking of an udder quarter may arise at the milking in an AMR. It may be caused by failure of the robotic arm to attach one or several teat cups or if the cow kicks the teat cup off after the attaching. A study by Kolbach et al. (2012) did an experiment in a prototype robotic rotary and found that the robotic arm had the highest failure to attach the teat cup on the left rear udder quarter. It also appears that the teat cup attachment is more frequently successful on the front udder quarters. Kolbach et al. (2012) also found that the percentage of incomplete milking during the first milking occasion was 19%, because of failed attachment. During the second milking, when the cows were re-sorted, the attachment success was 48%.

Lakic et al. (2009) demonstrated an elevation in SCC after a single prolonged milking interval on whole udder level. The study included 29 cows of Swedish Red and all of the cows had SCC below 100 000 cells/ml and were at the middle of lactation. Milk samples were taken twice a day during 7 days before the omitted milking and 5 days after. The highest SCC peak was shown at the second milking after the omitted milking. The milk composition was changed one day after the omitted milking, protein and fat was increased while lactose and FFA content was decreased. An older study by Fox & Schultz (1985) showed that infected udder quarters with high SCC had pronounced SCC increases after one omitted milking, while no increase in SCC was seen in uninfected udder quarters.

Several studies have investigated continuously extended milking intervals as milking one time per day. Studies over short periods (a couple of weeks) with milking one time per day indicate low elevated SCC, while studies over a full lactation demonstrates higher increased SCC. In these full lactation studies cows with good udder health had a tendency to get milder SCC increases, while cows with subclinical mastitis had more pronounced increases (Davis et al., 1999). A study in New Zealand investigated the effect of milking once a day compared to twice on Holstein and Jersey cows. They found that milking only once a day gave an increase in SCC and lowered the level of lactose in the milk. These negative effects were greater for the Holstein cows than for the Jersey cows, which was due to the Holstein cows had a greater reduction in the amount of milk, which gave a higher concentration SCC in the milk (Clark et al., 2006). Another study investigated the response of omitting one milking weekly during five weeks. It turned out to be no effect on SCC for cows that had good udder health, while cows with poorer udder health (> 324 000 cells per milliliter) showed a tendency for increased SCC, even though there were no significant effects. The milk composition did not change significantly due to the experiment (Ayadi et al., 2003).

Re-sorting in an AMR

The time budget for a cow indicates how much time she is using on various basic behaviors, such as eating, drinking, resting, ruminating and socializing with other cows. The behaviors that require the most time of the day are eating and resting. A study that analyzed time budgets for dairy cows present that the mean value of eating was 4.3 hours per day and lying was 11.9 hours per day (Gomez & Cook, 2010) Intensive dairy production can lead to time constraints for the cows. The duration of different behaviors is affected by the environment. Such adaptions do not have to lead to poor animal welfare, but when important behaviors are affected, such as eating, drinking and resting, it can lead to negative consequences, like poorer health and lower production (Munksgaard et al., 2005).

Cows who are incompletely milked in an AMR are often re-sorted for a second milking. This re-sorting give extra time spent in the AMR and also in the waiting area, before the second milking. The re-sorted cows get an increased time away from cubicles and feed, which interfere with feed intake and rest. A study conducted by Cooper et al. (2008) analyzed the effect of not allowing cows to eat or lie down for 2 hours. The results showed that the cows compensate by eating more during 24 hours after the deprivation. The treatment had no negative effects on the milk production.

When a cow is re-sorted the degree of udder filling is lower than at the first milking occasion, since some udder quarters already have been milked. Low udder filling requires a longer pre-stimulation in order to obtain a milk let-down. It may occur three minutes after the start of tactile teat stimulation, compared with about one minute at high udder fill. Stimulation of the teats is therefore especially important for cows that are directed to a second milking (Bruckmaier & Wellnitz, 2008). Also, the re-sorting routine is different from the usual milking routine due to a different entry route for the cow and it is known that inhibition of oxytocin release can occur at milking in unfamiliar conditions, due to increased plasma levels of cortisol which further adds to the problem with re-sorting. To achieve a continuous oxytocin release and thereby a complete milking it is important to have a stress-free- and familiar environment. Feeding during milking can lower cortisol levels and thereby the response of oxytocin release becomes stronger (Svennersten-Sjaunja et al., 2004; Bruckmaier et al., 1996).

If re-sorting of incompletely milked cows in an AMR is not done it may lead to impaired animal welfare and lower production. A study by Stefanowska et al. (2000) found that cows who had one omitted milking on whole udder level in an AMS showed signals of discomfort by urinating more and also standing in the cubicles, instead of lying. The same study also showed milk leakage from 60% of the cows after the omitted milking. This milk leakage that can occur after one omitted milking or after teat cup attachment failure during robotic milking is a risk factor for mastitis. This because bacteria can enter the teat easier, due to the open teat canal and milk spots in the cubicles is a good substrate for bacteria to grow on (Stefanowska et al., 2000). Österman & Redbo (2001) indicated that a reduction of pressure in the udder seems to improve the cows' ability to move and suggested that this is an improvement of the animal welfare.

Material and Method

The study was done in collaboration with DeLaval AB and conducted at the Swedish Livestock Research Center at Lövsta, Swedish University of Agricultural Sciences, Uppsala. The experiment was carried out during two weeks from September 14 to September 28. Animal handling was approved by the Local Ethics Committee in Uppsala.

Animals and housing

The study involved 16 dairy cows, nine Swedish Holstein (SH) and seven Swedish Red (SR). Inclusion criteria were SCC and stage of lactation when the cows were chosen to the experiment. They had SCC above 200,000 cells per milliliter milk from the last milk recording scheme and had passed peak lactation. The lactation stage for the cows were 180-379 days in milk. Three of the cows were primiparous and 13 were multiparous. Age, breed, days in milk, parity, next calving and milk yield for each cow is shown in Table 1. All cows were kept in a loose housing system with cubicles and were fed with grass silage *ad libitum* and concentrate according to milk yield (Spörndly, 2003). Water was available *ad libitum* through water bowls.

Well-being of the cows was checked every day during the study. This was done by going around the cows and looking at their general condition and how they spent their time in the stable. The food consumption for each cow was checked every day in DeLaval DelPro™ management program.

Table 1. Information for each cow in the experiment

Cow ID	Age (m)	Breed	DIM[1)]	Parity	Yield[2)]	Next calving
25	50	SR	290	2	24,9	2015-12-28
40	49	SH	328	2	25,9	2015-12-15
47	49	SH	317	2	28,9	2016-01-25
50	48	SH	339	2	36,2	2016-01-01
56	48	SH	189	2	33,4	2016-03-28
116	44	SR	186	2	32,8	2016-04-08
218	35	SH	247	1	28,4	2016-02-26
224	34	SH	249	1	21,9	2016-02-17
250	33	SR	180	1	31,9	2016-05-25
1575	71	SR	211	4	32,7	2016-03-22
1588	69	SR	242	4	25,3	not pregnant
1619	64	SR	333	3	17,3	2016-01-03
1651	59	SR	243	3	19,7	2016-02-09
5391	62	SH	252	3	27,7	2016-02-03
6517	63	SH	228	3	25,1	2016-05-18
6542	54	SH	379	2	21,1	2015-12-15

[1)] Days in milk (DIM) at start of experiment

[2)] Milk yield kg/day from 2015-09-03

Treatment

All cows were milked in an AMR two times per day, at 6.00 am and 5.00 pm. The cows were exposed to one omitted milking on one udder quarter. The 16 cows were divided into two groups, one resorted group and one non-resorted group. The cows were grouped in these groups as evenly as possible regarding SCC, lactation stage, lactation number and breed. There were five Swedish Holstein cows and three Swedish Red cows in the resorted group, while in the non-resorted group there were four Swedish Holstein cows and four Swedish Red cows. During the morning milking September 17, the treatment was conducted. Milking was omitted on one udder quarter for all cows, for each cow the udder quarter with the highest SCC the first two days of the experimental period was chosen to be omitted. Teat cup attachment was done manually during the treatment milking´.

After exiting the rotary cows in the non-resorted group were directed to exit and back to their resting and feeding area, while cows in the resorted group were directed by a selection gate to a second milking. The unmilked udder quarter was milked at the second visit to the rotary for the resorted group when sorted back. The cows in the non-resorted group was programmed to be milked on three udder quarters, not on the treatment teat, before milking was started. This was done so that they could be registered as completely milked and not re-sorted after the first milking moment. The treatment resulted in an extended milking interval on one selected udder quarter.

Milk sampling

Two types of milk samples were collected during the study; udder quarter milk samples for SCC determination and composite milk samples for fat, FFA, protein, lactose but also SCC determination on whole udder level. The baseline days were the first three days of the study, thus before the treatment. The scheme of the samplings is shown in Table 2.

At the evening milking the first 14 days of the experiment udder quarter milk sampling for SCC was performed from all cows. The milk samples were collected manually from each cow after the procedure with the robot that cleans the teats and before the teat cup attachment. The two robots in the AMR who did the teat cup attachment was turned off during the evening milkings, the teat cup attachment was done manually. One milk sample was taken from each udder quarter from all cows. The amount of milk collected to each tube was approximately 20 ml. Milk samples were stored at temperature and for a maximum of 16 hours until SCC determinations were done using a portable cell counter from DeLaval (DCC; DeLaval, Tumba, Sweden). 20 microliters of the milk sample was automatically drawn up in a disposable cassette where it was mixed with propidium iodide, which is a fluorescent DNA-specific color. The cassette was then placed in the DCC which scanned fluorscenscence from the stained cell nucleus and thereby counted each cell.

The composite milk samples were collected eight times during the experimental period, at four evening milkings and four following morning milkings. The sample was collected by using the equipment used in the AMR for obtaining milk samples for the national milk recording scheme (TruTester^{TM}), milk was gently mixed in the sampling tubes before samples of approximately 20 ml was transferred to test tubes. The test tubes were prepared with

Bronopol, 2-bromo-2-nitropropane-1,3-diol (VWR, International AB, Stockholm Sweden), which is an antibacterial agent, and then stored in a fridge for one day before the milk was analyzed for composition of fat, FFA, protein and lactose. Each tube used for composite milk was labeled with a serial number and details about samples were stored in a separate protocol. Milk composition determinations were conducted at the Department of Animal Nutrition and Management, University of Agricultural Sciences in Uppsala by mid-infrared spectroscopy technique (MilkoScan FT 120 from FOSS Electric, Hilleröd, Denmark). Analysis for SCC on whole udder level was made the same as for udder quarter level, by using a DCC.

Table 2. Experimental design for the samplings and the treatment. 0 = udder quarter milk sampling for SCC. X = composite milk sampling. * = Treatment

	Morning	Evening
14-sep		o
15-sep		o x
16-sep	x	o
17-sep	*	o
18-sep		o x
19-sep	x	o
20-sep		o
21-sep		o
22-sep		o
23-sep		o x
24-sep	x	o
25-sep		o
26-sep		o
27-sep		o x
28-sep	x	

Information from the management system

Records of background information from each cow, such as age, lactation number and stage of lactation and milk yield, milk flow and milking time during the experimental period was collected from DeLaval DelPro^{TM} management program.

Statistical analysis

For the statistical analysis used program SAS (Statistical Analysis System, version 9.3). The procedure "Mixed" in SAS was applied for the analyzes. To designate significant results in this report significance levels $P < 0.05$, $P < 0.01$ and $P < 0.001$ was used. Due to SCC values have a high variation, these were converted to $\log_{10}$ values to obtain a normal distribution of the data.

Days of the study is redesigned to periods in SAS. The three baseline days before the treatment is combined to period -1, the treatment day is period 0, one day after the treatment is period 1, and so on to the period 7 that standing for the last five days of the study. Effects of breed, period and treatment on SCC, milk yield, lactose, protein, fat and FFA were tested.

A calculation was made of the difference in SCC between the treatment teat and the corresponding teat of the other udder half. This difference between two teats on each cow was analyzed to ensure that the omitted milking did not affected the SCC.

Results

SCC on udder quarter level

There was a significant difference in SCC between the treatment teat and the other three teats in both the resorted and non-resorted group, both before and after the omitted milking, the difference was unchanged by treatment. A significant difference could also be seen between the breeds; Swedish Red had a higher SCC than the Swedish Holstein cows ($P<0.05$). However, omitted milking at one udder quarter did not affect SCC, there was no significant effect on the treatment teat between the resorted group and non-resorted group ($P=0.49$), neither between any of the periods ($P=0.55$) and the least squares means (LSmeans) for SCC can be seen in table 5 with 10logarithmic values. A day-to-day variation in SCC was observed during the baseline days. In table 3 shows SCC from four of the 16 cows during the three first days of the experiment.

Table 3. SCC (cells per milliliter milk) on udder quarter level for four of the cows in the experiment during the baseline days

Cow 40	LF	RF	LB	RB
14-sep	10000	46000	74000	612000
15-sep	6000	28000	163000	41000
16-sep	9000	53000	1581000	83000

Cow 5391	LF	RF	LB	RB
14-sep	112000	5000	255000	14000
15-sep	254000	17000	717000	30000
16-sep	73000	15000	171000	20000

Cow 6517	LF	RF	LB	RB
14-sep	4000	32000	6000	940000
15-sep	45000	44000	5000	888000
16-sep	3000	28000	9000	240000

Cow 1619	LF	RF	LB	RB
14-sep	36000	68000	5676000	104000
15-sep	101000	36000	2028000	510000
16-sep	26000	8000	1895000	348000

In table 4 the average, maximum and minimum SCC for the treatment teat is shown for both the resorted- and non-resorted group. Average SCC for the resorted group increased three days after the treatment day compared to the three baseline days before the treatment. For the non-resorted group the average has instead decreased after the treatment, the same applies to the value of maximum. The minimum value has increased more after the treatment for the non-resorted group, than for the resorted group.

Table 4. SCC (cells per milliliter milk) on treatment teat before and after treatment for both groups

SCC of treatment teat on resorted group	SCC of treatment teat on non-resorted group
• Three days before treatment Average: 499 000 Max: 1338 000 Min: 131 000	• Three days before treatment Average: 1332 000 Max: 4885 000 Min: 128 000

• Treatment day and three following days Average 641 000 Max: 1882 000 Min: 137 000	• Treatment day and three following days Average: 1108 000 Max: 3011 000 Min: 291 000

The difference in SCC between the treatment teat and the corresponding teat of the other udder half for each cow did not differ between the periods or between the groups. The data analysis also showed that Swedish Red had a significantly higher difference in SCC between the treatment teat and corresponding teat of the other udder half, with a LSmeans at 532 386 cells/ml milk, while LSmeans for Swedish Holstein was only 70110 cells.

SCC on whole udder level

As for SCC on udder quarter level, there were no significant differences in SCC on whole udder level between the periods, see table 5. No significant differences were seen between the resorted- and the non-resorted group in SCC on whole udder level. Difference in SCC between the breeds could also be seen in this analysis, Swedish Red had slightly higher SCC than Swedish Holstein.

Table 5. LSmeans of logSCC on udder quarter and on whole udder level during the periods

Period	-1	0	1	2	3	4	5	6	7
SCC A[1)]	4.8350	4.8484	4.8905	4.9076	4.8671	4.8654	4.8935	4.8413	4.9521
SCC B[1)]	5.27		5.47	5.36				5.27	5.33

A[1)] Log10 values of SCC on udder quarter
B[1)] Log10 values of SCC on whole udder

Milk yield

The omitted milking did not affect milk yield, neither for the resorted group or the non-resorted group. The amount of milk did not give significant differences between the days during the experiment, see table 6. A small decrease in milk yield without significant effect was seen on the treatment day, due to the omitted milking. A significant difference was seen on milk yield between the groups, but this was because the resorted group had an average higher milk production at the beginning of the study. The resorted group had an average of 12.52 kg, while the non-resorted group had an average of 10.85 kg milk per milking. Milk yield between the breeds was found without significant differences (P=0.05), LSmeans per milking for the Swedish Holstein cows were 12.41 kg and 10.96 kg for the Swedish red cows.

Table 6. Least squares means of milk yield (kg) per milking during the periods

Period	-1	0	1	2	3	4	5	6	7
Milk yield	12.36	10.01	11.02	11.76	12.37	12.35	12.75	10.97	11.56

It was also made an analysis of the difference in milk yield between the treatment teat and the corresponding teat of the other udder half. There was no significant effect of the omitted milking in milk yield for this analysis either.

Composite milk

The milk composition did not change during the experiment, thus the contents of lactose, protein, fat and FFA were unaffected by the treatment. The amount of these components did not give any significant differences between the periods for either the resorted group or the non-resorted group. A significant difference was seen between the breeds in lactose and protein, the Swedish Red cows had a higher protein content in their milk ($P<0.01$) while Swedish Holstein cows had a higher content of lactose in their milk ($P<0.01$). There was also a significant difference between the groups in lactose and protein from the beginning of the study and the same difference throughout the experiment period.

Re-sorting

The eight cows in the resorted group had an average of 31 minutes longer time in waiting area and milking due to the second milking occasion, compared to the non-resorted group who milked once. The extra time spent at the AMR ranged between the resorted cows, the shortest extra time was 22 minutes and the longest time was 38 minutes.

Three of the resorted cows were not milked with expected milk yield from the omitted teat after re-sorting, they milked on average only 0,5 kg milk compared with their average expected milk yield of 3 kg from the omitted teat.

Discussion

The hypothesis of the study was that the cows needs to be re-sorted after an incomplete milking to avoid increases in SCC and changes in the milk composite. The results did not prove the hypothesis, because there was no significant effect on the treatment teat between the resorted group and the non-resorted group or between the periods. Therefore, the results indicate that there is no reason to re-sort incomplete cows in an AMR regarding to SCC, milk yield and milk composition.

The study did not present elevations in SCC after the single prolonged milking interval which differs from the results reported by Lakic et al. (2009) in cows with SCC<100 000, where an increase in SCC was found after a single prolonged milking interval on whole udder level. An explanation for this may be that the prolonged milking interval was on whole udder level in their study, while only one teat was exposed to the prolonged milking interval in the present study. It may be that stronger effects appear when the whole udder is exposed to an omitted milking, compared to only one udder quarter and it may be suggested that the udder has a capacity to handle one unmilked udder quarter. Another difference between the present study and Lakic et al. (2009) was pre-treatment SCC. Lakic et al. (2009) used cows with SCC<100 000 at the onset of the study while the threshold was SCC>200 000 for the recent study. It would have been more likely that the cows in the present study would show a higher elevation in SCC. However, because of the large day to day variation in SCC in cows with high SCC the experimental group of 16 cows may have been insufficient to show effects. It is known that cows with subclinical mastitis are more affected and have a significantly higher variation in SCC compared with cows that have good udder health (Sandholm et al., 1995; Fox & Schultz, 1985). Fox & Schultz (1985) describes that cows with subclinical mastitis are more affected by an omitted milking since there is a high accumulation of bacteria due to the prolonged milking interval, which gives a greater influx of leukocytes in to the udder. The number of cows in the study by Lakic et al. (2009) was 29, which gives stronger results, comparatively with only 16 cows that were included in the recent study. It is possible that effects would be found in the present study if more cows were included. That was unfortunately not possible at the time of the study since no more cows with high SCC were available in the herd. A further study with the same purpose as the recent study should therefore use a higher number of cows to ensure if there are effects of the treatment.

The milk yield and lactose content did not change because of the treatment. The relationship between lactose and SCC is negatively significant, when SCC increases lactose decreases. (Sandholm et al., 1995; Berglund, 2003). Since the results shown that SCC was not affected by the omitted milking, this also explains why no significant differences were observed in lactose concentration and thus milk yield between the treatments. At elevated SCC nutritional components like fat and protein decreases (Hamann, 2001), but if SCC was unaffected by treatment it is not surprising that fat, FFA and protein also did not change.

It was unfortunate, there were only 16 cows available for the study, since this is a small number. This may be the most plausible explanation that no significant effects of the treatment occurred. At the same time, significant differences in SCC were seen between the treatment teats and the other three teats both before and after treatment, but the difference was unchanged by treatment. Significant difference in SCC, protein and lactose was also found between the breeds. In milk yield a significant difference was seen between the groups, this was because the resorted group had an average higher milk production at the beginning of the study. Although these significant differences are not of great interest for this study, it shows nonetheless that there were significant differences which strengthens the statistical analysis.

The significant difference in SCC between breeds demonstrated that Swedish Red had a higher SCC than Swedish Holstein. This was unexpected as earlier literature has shown that Swedish Holstein generally has higher SCC (Brolund, 1985). The contradiction is probably due to the low number of cows and because of a coincidence the Swedish Red cows in the study had higher SCC at the onset of the experiment.

The day to day variation in SCC for each cow was high during the experimental period (as shown in table 3) and therefore SCC data was challenging and it was not surprising that the statistical analyses showed that the treatment did not cause any significant differences in SCC. The high day to day variation in SCC was seen especially on udder quarters with very high SCC at onset of the study. In an udder quarter with clinical or subclinical mastitis, the inflammatory process causing fluctuations in SCC, which can be high (Daley et al., 1991). The current routine in the official milk recording in Sweden is to analyze SCC and composition in the milk one or two milkings once a month. This can provide reliable results for cows with good udder health, because the variation from day to day in SCC is reasonably low. However, as demonstrated in the data on high SCC cows in this study, for cows with udder quarters that have elevated SCC, sampling only on one or two milkings per month can give misleading results on individual cow level, due to the high day to day variation. This clearly is a weakness since SCC on individual cows from the milk recording often is used for grouping cows at farms. It is not unlikely that cows are placed in wrong groups.

A significant difference between the treatment teat and the other three teats of each cow were seen from the beginning of the study. This is explained by that the teat with the highest SCC was selected to be the treatment teat. Interestingly, several of the cows (seven of 16) in the study had the highest SCC from the left rear udder quarter. A study in a prototype robotic rotary performed by Kolbach et al. (2012) found that the robotic arm had the highest failure to attach the teat cup on the left rear udder quarter, probably because the rear teats have a worse position to be found for the attaching robot, than the front teats. Left rear teat is also the furthest away from the robot arm. This may be one reason that many cows in the recent study had higher SCC in the left rear udder quarter, in case this quarter has been incomplete milked repeatedly due to attachment failures. Probably the cleaning robot arm also has the highest failure to clean the left rear teat compared to the other three teats, which give a poorer hygiene for the left rear quarter and therefore a higher risk for elevated SCC. The data from Kolbach et al. (2012) and the present study demonstrates that the AMR is inserting a systemic risk for impaired udder health on one specific udder quarter. However, this has not been investigated in other milking management systems.

Not re-sort cows that are omitted or incompletely milked is a question of cow-wellbeing. Stefanowska et al. (2000) demonstrated that cows urinated more and laid down shorter time after one omitted milking. The non-resorted cows for the present study did not show any signals of discomfort after the omitted milking of what could be seen, their general condition was the same as during the other days of the experiment. This can be due to that the cows only were omitted on one udder quarter, which probably did not give the same discomfort in comparison to omitted on whole udder. Milk leakage from the omitted teat was observed from several of the non-resorted cows after the treatment, it may increase the risk for bacterial growth on the cubicles. Bacteria can enter the open teat canal and therefore there is a higher risk for mastitis when the cows lie down and have a milk leakage, as Stefanowska et al. (2000) pointed out.

On the other hand, re-sorting cows for a second milking after omitted or incomplete milking can interfere with the time budgets for the cows, depending on how long extra time that is spent in the waiting area and in the AMR for the second milking. The re-sorting probably does not affect the time budget for the cows if they are entering the second milking occasion just a couple of minutes after they have gone out from the first milking occasion. At the treatment day of the recent study the extra time spent at the AMR for the resorted cows ranged from 22 minutes to 38 minutes. This time is probably shorter than normally, because the cows were pushed forward manually and did therefore not stay in the waiting area long time before the second milking. The prevalence for re-sorting of cows is also important for the time budget, if the same cow is re-sorted once a month the time budget perhaps would not be as affected, compared if the cow is re-sorted almost every day. In the study by Cooper et al. (2008) cows compensated by eating more after a 2 hours' deprivation of feed and lie down. The experiment was done only once time, if the deprivation of feed and lie down would be repeated continuously the cows probably would become stressed and the milk production would be affected.

On the treatment day, when half of all cows in the experiment were re-sorted, it was found that three of the cows were not milked completely from the fourth teat during the second milking occasion. The amount of milk obtained from the treatment teat after re-sorting was lower than normal for these three cows. There may be several explanations for this. One explanation may be that the re-sorting means an unfamiliar environment, since the cows are not used to the re-sorting alley the separate waiting area for re-sorted cows. This can create stress for the cows, which gives an inferior oxytocin release when they enter the AMR the second time (Svennersten-Sjaunja et al., 2004; Bruckmaier et al., 1996). Another explanation is that the degree of total udder filling at re-sorting is lower than at the first milking occasion. The need for pre-stimulation when only one udder quarter is unmilked has not been studied, but it is well known that lower udder fill requires a longer pre-stimulation to get a milk let-down (Bruckmaier & Wellnitz, 2008). The teat preparation robots in the AMR are working equal time for each cow. Cows with a low degree of udder filling do not get a longer pre-stimulation of the teats, it is therefore possible that some cows do not receive oxytocin release and therefore not milked completely. Kolbach et al. (2012) found in their study that there were higher percentage of failed attachments at the second milking occasion, compared with the first occasion. This can be described by that the attachment is easier on a more filled udder.

Conclusion

Based on this study, it is not possible to draw reliable conclusions about the effect of the omitted milking on one udder quarter, although the results indicate that there are no negative effects. Further studies with a larger number of cows are needed to make it possible to show whether the re-sorting in an AMR of cows with SCC above 200,000 cells/ml makes any difference or not.

The results of this study suggests that cows with SCC of 200,000 cells / ml do not need to be re-sorted with respect to SCC and milk composition. There are other aspects that give pros and cons with re-sorting and not re-sorting cows. Studies to examine how various aspects, as the time budgets for cows, efficiency of the AMR and animal welfare, are affected by re-sorting in the AMR need to be done.

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