Milk Removal

Effect on Milk Yield, Milk Composition and Milking Efficiency in Dairy Cows

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Cover: Teatcup attachment in an automatic milking system
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Abstract

Milk from dairy cows is a staple dietary component for humans all over the world. Regardless of whether milk is consumed in its purest, unaltered form or as high-end products such as fine cheese or ice cream, it needs to be of high quality when taken from the cow, produced at a low price and produced in a system that consider aspects such as animal health, animal welfare and sustainability.

This thesis investigated the role of milk removal and the importance of residual milk on milk yield, milk composition and milking efficiency in dairy cows. The specific aspects examined were whether residual milk retained in the udder, specifically its fat component, is involved in regulation of milk synthesis and secretion and whether removal of this residual milk influences milk yield, composition and quality, measured as milk fatty acid composition and free fatty acid content.

The results showed that milking efficiency could be increased by increasing the pulsation ratio or using a higher cluster or teat cup take-off threshold, without negative effects on milk yield or milk composition. Milking time in automatic milking systems could thereby be decreased by one minute per cow and milking or more.

Milk fatty acid composition was affected by several treatments tested, but the content of free fatty acids and the size distribution of milk fat globules were unaffected. Residual milk yield increased both due to repeated residual milk removal and to use of a higher cluster take-off level. Residual milk removal also increased the relative proportion of short-chain fatty acids in milk. The overall conclusion of this thesis is that residual milk removal in mid-lactation dairy cows does not affect milk yield and that milking efficiency in an automatic milking system can be increased with higher take-off levels without affecting milk yield or composition. No evidence of a regulatory mechanism in residual milk was found, but residual milk removal increased milk fat synthesis.

Keywords: Milk removal, milk yield, milk composition, milk fat, free fatty acids, dairy cow, automatic milking systems, milking management, udder emptying, milking frequency

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Dedication

To all of you who never let me give up. This is for you.

Only with laughter can you win
Rosie Thomas

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ferneborg, S., Kovac, L., Shingfield, K. & Agenäs, S. (2016). Effect of increased milking frequency and residual milk removal on milk production and milk fatty acid composition in lactating cows (manuscript).

II Ferneborg, S. & Svennersten-Sjaunja, K. (2015). The effect of pulsation ratios on teat condition, milk somatic cell count and productivity in dairy cows in automatic milking. *Journal of Dairy Research* 82(4), 453-459.

III Ferneborg, S., Stadtmüller, L., Pickova, J., Wiking, L. & Svennersten-Sjaunja, K. (2015). Effects of automatic cluster removal and prestimulation routines on milking efficiency, milk yield and milk fat quality. *Journal of Dairy Research* 83(2), 180-187.

IV Krawzcel, P., Ferneborg, S., Wiking, L., Dalsgaard, T.K., Gregersen, S.B., Black, R., Larsen, T., Agenäs, S., Svennersten-Sjaunja,K. & Ternman, E. Milking time and risk of over-milking can be decreased with early teat cup removal based on udder quarter milk flow without loss in milk yield (manuscript).

Papers II and III are reproduced with the permission of the publisher.

The contribution of Sabine Ferneborg to the papers included in this thesis was as follows:

I Was involved in planning of the practical study. Had the main responsibility for conducting the experiment. Analysed and summarised the results in collaboration with supervisors. Was responsible for completing the manuscript together with the co-authors.

II Planned the practical study. Was responsible for conducting the experiment. Analysed and summarised the results in collaboration with one supervisor. Was responsible for completing the manuscript with regular input from the co-authors.

III Developed the hypothesis together with a supervisor, was involved in the application for funding and planning of the practical study. Responsible for conducting the experiment. Analysed and summarised the results in collaboration with supervisors. Was responsible for completing the manuscript with regular input from the co-authors.

IV Developed the hypothesis together with a supervisor, was involved in the application for funding and planning the practical study. Did not perform the practical study or analyse the results. Provided input on the manuscript as a co-author

Abbreviations

AMS	Automatic milking system
FA	Fatty acid
FFA	Free fatty acids
MF	Milking frequency
MFG	Milk fat globule
MU	Milking unit
RMR	Residual milk removal
SCC	Somatic cell count
VMS	Voluntary milking system

1 Introduction

Milk from dairy cows is a staple dietary component for humans all over the world. Regardless of whether it is consumed in its purest, unaltered form or processed into delicate products, the raw material, the milk that is harvested, needs to be safe, nutritious, tasty and produced at a reasonable cost. Moreover, the animals used must be treated well and the sustainability of both the animals and the system needs to be considered. To achieve sustainable dairy production, several factors directly linked to the milking process are important, such as milking efficiency and milk removal.

There are several different milking systems for cows, but they all share the same working principle, which is udder emptying using vacuum. The latest development in milking systems is automatic milking systems (AMS). In an AMS, the cows can choose when they want to eat, rest and be milked. The milking is performed in an automatic milking unit, which milks each teat individually. Often, a small amount of concentrate is provided during the milking. The concentrate is provided both to increase the cows' motivation to go to the milking unit and to facilitate milk ejection, and is often referred to as a teaser feed. During milk ejection, milk is shifted from the upper parts of the udder (alveoli), where it is mainly produced, to the lower parts of the udder (cistern), where it is mainly stored, and onwards out through the teat, from which it can be harvested. Without ejection, only around 20% of the milk present in the udder can be milked out, so ejection is an important process to maximise milk yield. After efficient milk ejection, around 80-90% of the milk can be harvested and the remaining fraction of milk in the udder is referred to as residual milk. This residual milk has a very high fat content, and its importance for milk quality and its possible involvement in regulation of milk secretion has not been fully investigated.

Milk flow has four phases: the increase phase, the plateau phase, the decline phase and the over-milking phase. The over-milking phase starts when milk

flow from the alveoli to the cistern is lower than milk flow from the cistern through the teat, and during this phase a lot of strain is put on the teat tissue. This strain can cause damage to the teat tissue and increase the susceptibility to intramammary infection. It is therefore important that this phase is as short as possible, or completely avoided. Milking at whole udder level, where all teat cups of the milking machine are attached to one cluster and attached and detached at the same time, increases the risk of over- or under-milking one or several of the teats. The reason for this is that the udder quarters can have very different milking times, due to differences in milk yield and flow between the individual quarters. In recent decades, technology for milking each udder quarter separately has become available, and this is referred to as quarter milking. In these milking systems, the teat cups are removed separately for each udder quarter and the degree of emptying is therefore adjusted to the milk yield and flow of the individual quarters, which reduces the risk of uneven milking and especially over- and under-milking.

Milk ejection is sustained throughout milking by stimulation of the teat by a liner, which alternates between massaging the teat and opening to draw out the milk by the pressure difference created by the pulsator. The pulsator regulates when there is vacuum outside the liner, which causes the liner to open and collapse at a set pace. The pace, or pulsation rate and ratio, are both adaptable. The rate determines the number of pulsation cycles per minute, and the ratio determines the proportion of time spent in the massage phase and in the milking phase. The timing of teat cup or cluster removal is based on milk flow and is often referred to as the take-off level. Much research on both pulsation ratio and take-off level has been conducted with cluster milking systems. Since the occurrence of over- and under-milking is lower in quarter milking systems, the information from cluster milking studies cannot be directly applied to quarter milking systems. There is therefore a need for new research in areas that may already have been sufficiently researched as regards cluster milking, but are under-researched as regards quarter milking.

2 Background

2.1 Anatomy of the Bovine Udder and Milk Ejection

The bovine mammary gland consists of four glands, each of which is connected to one teat. The two hind quarters generally have higher milk production than the two front quarters within the same cow (Tančin *et al.*, 2006; Weiss *et al.*, 2004). The glands are separated from each other and milk is not transferred between the glands in healthy cows.

Each gland is divided into two major compartments, the cisternal and the alveolar compartments. Milk is synthesised by epithelial cells in the alveoli, while the cisternal compartments, which include the gland cistern and large milk ducts, mainly serve as storage. Each alveolus is built up from a single layer of epithelial cells, which is sealed by tight junctions between the cells. These tight junctions prevent transfer of components from blood to milk and *vice versa*, forming the blood:milk barrier. Between milkings, the majority of the milk is stored in the alveoli and the remaining fraction is in the cisternal compartments. The proportion of milk stored in the alveoli ranges from 62 to 96% during a milking interval of 1 to 12 h, and differs depending on lactation stage (Knight *et al.*, 1994). Alveolar milk needs to be shifted from the alveolar compartments through the milk ducts towards the cisternal compartments and made available for milk removal. This process is referred to as milk ejection or milk letdown (Schams *et al.*, 1984; Ely & Petersen, 1941). The cisternal milk is readily available to be harvested without milk ejection, and the only barrier to overcome is the teat sphincter. Each teat is connected to the gland cistern and it is only through the teat that the gland can be drained. As milk ejection occurs, intramammary pressure increases, the teat cistern is filled with milk and the teat sphincter, which is the only barrier between the inside and outside of the udder, relaxes. The teat sphincter acts to open or close the teat canal for milk to be harvested and to protect the udder from infections between milkings.

2.2 Milk Synthesis, Composition and Quality

The main components of milk are water, lactose, fat, protein, vitamins and minerals. Milk composition varies between species, breeds, individuals, lactation stages, diets and degree of milk removal. The typical milk composition for dairy herds is around 5% lactose, 4% fat and 3% protein, and the water content is around 87%. In individual cows, the lactose concentration is stable, with a daily variation of only 0.9% within an individual udder quarter, while fat content and milk yield show large day-to-day variation, of 7.7% and 7% respectively. The content of protein and of different protein fractions varies by 1.4-1.8% daily, while somatic cell count (SCC) can vary by 2% (Forsbäck *et al.*, 2010).

Lactose is a disaccharide composed of glucose and galactose and is the major carbohydrate in milk. Glucose is converted to galactose in the mammary gland, so two glucose molecules are required for the synthesis of one lactose molecule. Lactose is synthesised in the golgi and requires the proteins galactosyltransferase and α-lactalbumin for its synthesis (Linzell & Peaker, 1971). It acts as an osmotic regulator, drawing water into the milk. Since lactose is unique to milk, lactose in blood and urine is a sign of leakage across the blood:milk barrier (Stelwagen *et al.*, 1997).

There are two main classes of proteins in milk, casein and whey proteins. The majority of proteins are caseins, which account for around 80% of the protein fraction and are present in milk in the form of micelles. α-lactalbumin is one of the main whey proteins in cow milk, together with β-lactoglobulin. Milk proteins are synthesised in the rough endoplastic reticulum and further processed in the golgi (Linzell & Peaker, 1971).

Milk fat is present in milk in the form of fat globules that comprise a triglyceride core and a lipid bilayer membrane consisting of mainly phospholipids, sterols and proteins. Triglycerides account for more than 95% of total milk lipid. Triglycerides consist of three fatty acids (FA) linked to one glycerol molecule. Milk fatty acids that are not bound to glycerol are referred to as free fatty acids (FFA). FFA are a product of lipolysis of milk fat and can cause rancid flavour in milk. Bovine milk lipid contains at least 400 different fatty acids, of which only a few is present in proportions over 1% (Jensen, 2002). Fatty acid composition is expressed in relative proportions of the total amount of fatty acids, which affects the interpretation of analyses and results. The fatty acid composition of milk can vary due to a number of factors such as breed, individual, feeding, parity and lactation stage (Samková *et al.*, 2012). Around 50% of the fatty acids present in cow milk are preformed, and originate from feed or mobilisation of body tissue, while the remaining fraction comprising the so called *de novo* fatty acids is synthesised in the mammary

gland. The *de novo* fatty acids are shorter than the fatty acids originating from feed or body tissue. Fatty acids 4:0-12:0 are synthesised exclusively *de novo*, and also the majority of 14:0 and around 50% of 16:0. The most important precursors for synthesis of fatty acids in the secretory cells of the udder are acetate and β-hydroxybutyrate, which originate from fermentation of carbohydrates in the rumen. Lipid droplets are formed at the surface of the endoplastic reticulum, co-elute and exit the epithelial cell through exocytosis (Mather & Keenan, 1998).

Milk naturally separates into two main fractions, the lipid phase and the aqueous phase. When milk is stored in the mammary gland, the same separation occurs, causing fat content to be higher in alveolar milk (Ontsouka *et al.*, 2003; Kernohan & Lepherd, 1969). The cause for this separation is that the hydrophobic milk fat globules (MFG) separates from water and the hydrophilic compounds in milk. Due to this, most of the large MFG are stored in the alveolar compartment of the udder, while smaller MFG are found in the cisternal fraction of the milk (Kernohan & Lepherd, 1969).

Milk production is both systemically and locally regulated. The systemic regulation is exerted through hormones such as prolactin, while the local regulation is exerted by specific factors in the milk. The local regulation uses a negative feedback mechanism, where milk present in the gland inhibits further secretion. Therefore, more frequent or more complete removal of milk has been shown to increase milk production (Lollivier *et al.*, 2002).

The first local regulatory factor in milk to be discussed in literature was the Feedback Inhibitor of Lactation (FIL), an unidentified protein that was shown to decrease milk synthesis (Peaker & Wilde, 1996; Wilde *et al.*, 1995). It has since been shown that the mammary epithelial cells produce serotonin and that serotonin inhibits milk secretion. Serotonin has therefore also been suggested to act locally in the gland to decrease milk synthesis (Collier *et al.*, 2012; Hernandez *et al.*, 2011; Hernandez *et al.*, 2008). Research to date on local regulatory factors in milk has focused on milk proteins, but throughout the years, theories have arisen about the importance of the lipid fraction in milk for milk secretion (Brandsma, 1978). The mammary gland is never completely emptied, and the residual milk that is left in the udder after milking or suckling has a high fat content and a high proportion of large MFG (Kernohan & Lepherd, 1969). Possible effects of MFG in the lumen of the alveoli on the apical membrane have not been studied and effects of this lipid fraction on the continued secretion of milk are not known.

2.3 Milk Ejection

For the majority of the milk stored in the udder to be available for removal, a milk ejection is required. Milk ejection is a neuroendocrine reflex in response to tactile stimulation of the teats, and it causes milk to be expelled from the udder (Ely & Petersen, 1941). Other types of conditioned stimuli such as the sight or sound of calves or feed, have also been suggested to be able to induce milk ejection (Ely & Petersen, 1941). When the teat is stimulated by tactile stimulation, oxytocin is released from the pituitary gland. This oxytocin then binds to oxytocin receptors in the mammary gland which causes the myoepithelial cells that surrounds each alveolus to contract and physically eject milk from the alveoli. Bruckmaier *et al.* (1994) showed that a continuously elevated concentration of oxytocin is required for complete milk ejection, and stimulation therefore needs to continue throughout milking. This is achieved through suckling but also by the milking machine, which alternates between massage of the teat and opening of the teat canal throughout the milking process.

Milk ejection is quite easily disturbed, and can be inhibited both centrally and peripherally (Wellnitz & Bruckmaier, 2001). Central inhibition occurs when the release of oxytocin from the pituitary gland is inhibited, and arises for instance when cows are milked in unfamiliar surroundings. Peripheral inhibition has been suggested to occur when oxytocin is released into blood but is unable to bind to receptors in the mammary gland (Wellnitz & Bruckmaier, 2001).

2.4 Milk Removal and Milking Routines

There are four phases of the milk flow curve; the increase, plateau, decline and over-milking phases (Tančin *et al.*, 2006; Weiss *et al.*, 2004). When milking starts, in the increase phase, the intramammary pressure increases, which causes milk flow to start and increase rapidly (Bruckmaier & Blum, 1996). During this phase, there can be a bimodal pattern of milk flow, depending on the quality of the milk ejection (Bruckmaier & Blum, 1996; Phillips, 1978), udder fill (Bruckmaier & Hilger, 2001) and milking system (Bava *et al.*, 2005). The plateau phase is reached when milk flow has stabilised and milk yield is increasing steadily at a constant rate. When milk flow rate starts to decline together with milk yield increase rate, the decline phase is reached. Finally, when milk ejection from the alveolar compartment to the cisternal compartment is lower than milk removal from the gland via the teat (Rasmussen, 2004), the over-milking phase is reached. The milk flow at this point is usually lower than 0.75 kg/min per udder quarter, which is equivalent

to 0.3 kg/min for the whole udder (Tančin *et al.*, 2006). When milking on whole udder level is performed, one or several udder quarters can be over-milked while one or several udder quarters are still insufficiently emptied. If cluster detachment on whole udder level is set to 0.3 kg/min, it has been found that individual quarters are over-milked on average for 62 s per milking (Tančin *et al.*, 2006). Front quarters have been found to be over-milked for longer times than hind quarters, which is most likely due to lower milk yield in front quarters (Tančin *et al.*, 2006; Weiss *et al.*, 2003).

Machine milking of dairy cows has long been performed on whole udder basis, and this is still the most common technique, but equipment for milking on quarter level is available and increasing in new installations of automatic milking. A milking machine is driven by vacuum. Each teat cup consists of a shell, a pulsation chamber and a liner. In whole udder-based milking, the four teat cups are connected in a cluster. The pulsation chamber of each teat cup is connected to the pulsator through a vacuum tube, and the pulsator regulates the vacuum in the pulsation chamber. The changes in pressure brought about by the pulsation causes the liner to expand and collapse. The liner is connected to the milk tube, and its alternating collapse and expansion massages the teat and extracts the milk.

The pulsation cycle has, like milk flow, four distinct phases; a, b, c and d. These are divided into the milking phase (a and b) and the massage phase (c and d), and the ratio between the milking and the massage phase is referred to as the pulsation ratio. During the b phase, the liner is completely open, while during the d phase, the liner is completely closed. During the a and c phases, the liner is in the process of opening and closing, respectively (Mein *et al.*, 1992).

Before the milking machine is attached, pre-stimulation is usually performed, either manually or mechanically. The common practice is that teat cleaning functions as pre-stimulation, as the teats are either wiped or cleaned with pressurised water before attachment of the teat cups. Some lag time may be left between the start of pre-stimulation and teat cups attachment, as this procedure reduces the risk of bimodal milk flow curves (Kaskous & Bruckmaier, 2011)

After milking, it is common to dip or spray the teats with a teat disinfectant, to reduce the risk of pathogens entering the teats and infecting the udder. The teat sphincters remain open for some time after milking and it is therefore beneficial if the cow remains standing for some time after milking.

Previous studies investigating the effect of feeding during milking show that at times it increases milk flow, decreases milking time and increases milk yield (Samuelsson *et al.*, 1993; Brandsma & Maatje, 1987). Svennersten *et al.*

(1990) found that feeding gave rise to a small increase in plasma oxytocin 5 min after feeding compared with 15 min before feeding. When feeding and milking are combined, it has been suggested that feeding enhances milk production through elevated and prolonged release of oxytocin (Svennersten *et al.*, 1995). A few studies have also found no effect of feeding, especially when fresh pasture is available or depending on time of day (Mačuhová *et al.*, 2003; Samuelsson *et al.*, 1993; Brandsma, 1978).

2.5 Factors Affecting Milking Efficiency in Automatic Milking

In automatic milking systems (AMS), cows are kept in loose housing and are able to influence when they eat, sleep and get milked. Cow traffic can be free or controlled, where cows need to pass through the milking unit (MU) on their way to the lying or feeding areas. Milking in AMS is dependent on high efficiency and the MU being visited around-the-clock. This places high demands on the functionality of the system, since any delays or disruption will decrease the number of milkings per cow, increase milking interval and decrease milk yield. Castro *et al.* (2012) concluded that the most important factors determining the milk yield per AMS and year were the number of cows and the milk flow, which together contributed to 87% of the variation in milk yield. If milk flow were to be increased by 0.1 kg/min, another 32,000 kg of milk could be harvested from the same number of cows during one year, while one more cow in the herd could increase yearly milk yield per AMS by 8 200 kg.

Milking efficiency can be measured in numerous ways, such as number of cows milked per hour or MU, milking time and average flow or harvest flow (yield of milk per minute spent in the MU). De Koning and Ouweltjes (2000) suggested that milk yield and milk flow are among the most important factors for maximising the capacity of an AMS. There are several factors that can influence milking efficiency measured as milk flow in a milking system (Blake & McDaniel, 1978), including pulsation rate, pulsation ratio (Gleeson *et al.*, 2007; Spencer *et al.*, 2007), vacuum level (Besier & Bruckmaier, 2016), milk ejection and (indirectly) the take-off level (Jago *et al.*, 2010; Rasmussen, 1999; Rasmussen, 1993).

The most common pulsation ratios used are 60:40 and 65:35. Both higher and lower pulsation ratios have been tested, but have often been found to cause strain and damage to the teat tissue. Milking time has been found to decrease when pulsation ratio is increased from 60:40 to 70:30 or 67:33 (Gleeson *et al.*, 2007; Spencer *et al.*, 2007; Thomas *et al.*, 1991). Thomas *et al.* (1991) also found that increasing the pulsation ratio from 50:50 to 70:30 increased milk

and fat yield, indicating better udder emptying. Bade *et al.* (2009) found that an increase in b phase duration from 200 to 800 ms increased peak milk flow, despite d phase duration being unchanged. A negative effect on teat condition and udder health has been found from higher pulsation ratios (Hamann & Mein, 1996; Østerås *et al.*, 1995), however, these studies have often been performed on whole udder level.

Besier and Bruckmaier (2016) tested the effect of high system vacuum and low claw vacuum, and calculated theoretical detachment levels in combination with the treatments applied. Their findings indicated that irrespective of the system vacuum, milking time could be decreased by 1.5 min from increasing the take off level to 1.0 kg/min at whole udder level, and that the milk yield loss was minimal. When take-off was simulated as 1.0 kg/min on whole udder level, 93% of available milk was harvested, while when take-off was simulated to 0.2 kg/min, 96% of available milk was harvested (Besier & Bruckmaier, 2016).

The take-off level in a cluster milking system is a compromise between sufficiently emptying as many glands as possible and not overmilking remaining glands. Different levels have been tested, and a shorter milking time has been found when increasing the take-off level from 0.2 to 0.4 kg/min (Jago *et al.*, 2010; Rasmussen, 1993) or from 0.48 or 0.6 to 0.8 kg/min (Magliaro & Kensinger, 2005), with minimal or no effect on milk yield. Edwards et al. (2013a; 2013b) found a decrease in milking time of over one minute by increasing the take-off level from 0.2 to 0.8 kg/min, with no effect on milk yield. However, strip yield was slightly increased. Also, all of these studies were performed on whole udder level, which still allows for individual quarters to be over- or under-milked. Weiss and Worstorff (2001) found that quarter level detachment of teat cups decreased the average cup-on time by around 20%, reducing or completely eliminating the occurrence of over-milking.

Many of the previous studies performed on milking efficiency have been performed on conventional whole udder milking. Since quarter level milking decreases the occurrence of over- and under-milking the results from whole udder milking systems may not be fully applicable to quarter level milking systems. For this reason, much of the older research needs to be repeated for milking systems where quarter milking is applied.

3 Aims

The overall aim of this thesis was to investigate the role of milk removal and the importance of residual milk for milk yield, milk composition and milking efficiency in dairy cows. Specific aims were to:

- Investigate the effect of milking frequency and residual milk removal on milk yield, milk composition and milk fatty acid composition. (**Paper I**)

- Investigate the effect of different pulsation ratios at quarter level on milking efficiency. (**Paper II**)

- Investigate the effect of take-off level on whole udder level, in combination with feeding during milking or no feeding during milking, on milking efficiency, milk fatty acid composition and susceptibility to lipolysis. (**Paper III**)

- Investigate the effect of take-off level on quarter level in combination with feeding during milking or no feeding during milking on milking efficiency and susceptibility to lipolysis. (**Paper IV**)

The overall hypothesis was that the milk retained in the udder, specifically the fat component of residual milk, is involved in the regulation of milk synthesis and secretion and that the removal of this milk would influence milk yield, composition and quality, measured as milk fatty acid composition and free fatty acid content.

4 Methods

This thesis is based on four studies conducted during the years 2010-2015, which are described in **Papers I-IV**. Three of these experiments (**Papers I, III** and **IV**) were conducted at the experimental facilities of the Swedish University of Agricultural Sciences, which have been certified for research use by the Swedish Board of Agriculture. One experiment (**Paper II**) was conducted on commercial farms in Sweden and Denmark. The experimental barn used in this thesis work was approved for use of animals for scientific purposes by the National Board of Agriculture in Sweden, which is the competent authority referred to in EU Directive 2010/63/EU. The specific animal experiments reported here were evaluated and approved by the Uppsala regional animal ethics committee and performed in accordance with EU Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

4.1 Paper I

The hypothesis of the study described in **Paper I** was that residual milk removal and increased milking frequency would have an additive or synergistic effect on milk yield, milk composition and milk fat composition. The study was conducted at the Kungsängen Research Centre, Swedish University of Agricultural Sciences, in 2010-2011, and included four cows of the breed Swedish Red.

The study had a Latin square design with a 2×2 factorial arrangement of treatments. Two milking frequencies (MF), 2 and 4 times daily milking (2x and 4x), were tested together with residual milk removal (RMR) or no RMR. Each treatment period was four days long and was preceded by a two-day long baseline period and a seven day long washout period, during which cows were milked twice daily with uneven milking intervals. Residual milk removal was performed at every milking throughout the RMR treatments, and milking was

performed using a custom milking machine allowing separation of the milk from different udder quarters and separate detachment of teat cups (DeLaval International AB, Tumba, Sweden). Milk yield was measured at each milking throughout the study, and milk was sampled for analysis of gross composition, somatic cell count (SCC) and FA composition throughout the study.

4.2 Paper II

The hypothesis of the study described in **Paper II** was that increased pulsation ratio increases milking efficiency without negative effects on teat and udder health, when applied in a quarter level milking system. The study was conducted on three commercial farms with automatic milking (DeLaval VMS™) in Sweden and two commercial farms in Denmark and included in total 356 cows of three breeds, Swedish Red, Swedish Holstein and Danish Jersey.

Three different pulsation ratios (60:40, 70:30 and 75:25) were tested against control pulsation (65:35) in a split-udder design during a six week treatment period. Milking efficiency, measured as milk flow, milk yield and machine-on time, was continually recorded by the AMS, while teat score, SCC and udder emptying were evaluated at the beginning of the experiment, three weeks into the experiment and at the end of the experiment.

4.3 Paper III

The hypothesis of the study described in **Paper III** was that teat cup removal at a higher milk flow on whole udder level increases milking efficiency without negative effects on milk yield or milk fat quality if combined with feeding during milking. The study was conducted at the National Livestock Research Centre Uppsala-Lövsta and included 32 cows of the breed Swedish Red in an automatic milking system (DeLaval VMS™) with FeedFirst™ cow traffic.

Two different take-off levels (0.78 and 0.18 kg/min) were used in combination with feeding during milking (F) or no feeding during milking (NF). Treatments were in the published paper referred to as H+, H-, L+ and L-. The study had a Latin square design, with a 2×2 factorial arrangement of treatments, each treatment period was seven days long and data from the last two days of each treatment period were used in statistical analysis. The feed that was provided during milking was deducted from the daily ration of each cow. Milking efficiency was measured as milk flow, milk yield, milking time and harvest flow during the last three days during each treatment period, and residual milk removal was performed on 16 of the cows at the end of each

treatment period. Milk was sampled during the last two days of each treatment period for analysis of FFA, milk fat globule size, milk fat globule membrane (measured as activity of the membrane protein γ-glutamyl transpeptidase), fatty acid composition, gross composition, SCC and sodium and potassium concentration.

4.4 Paper IV

The hypothesis of the study described in **Paper IV** was that increased take-off level on quarter level increases milking efficiency without negative effects on milk yield or milk fat quality if combined with feeding during milking. The study was conducted at the National Livestock Research Centre Uppsala-Lövsta and included 30 cows of the breeds Swedish Red and Swedish Holstein in an automatic milking system (DeLaval VMS™) with FeedFirst™ cow traffic.

Three different take off levels (0.48, 0.3 and 0.06 kg/min and udder quarter) were used in combination with feeding during milking (F) or no feeding during milking (NF). The study had a Latin square design, with a 3×2 factorial arrangement of treatments, each treatment period was seven days long and data from the last two days of each treatment period were used in analysis. The feed that was provided during milking was deducted from the daily ration of each cow. Milking efficiency was measured as milk flow, milk yield and milking time during the last three days of each treatment period, and residual milk removal was performed on all cows at the end of every second treatment period. Milk was sampled during the last two days of each treatment period for analysis of FFA, milk fat globule size, γ-glutamyl transpeptidase, FFA composition, *β*-hydroxybutyrate content, cholesterol content, gross composition and SCC.

Table 1. *Summary of the experimental structure of the experiments used in Paper I-IV. SR=Swedish Red, SH=Swedish Holstein, DJ=Danish Jersey, RMR=residual milk removal, F=feeding during milking, NF=no feeding during milking*

	I	II	III	IV
No. cows	4	356	32	30
Location	Kungsängen, SLU	Commercial farms	Lövsta, SLU	Lövsta, SLU
Breed	SR	SR, SH, DJ	SR	SR, SH
Aim	Investigate effect of milking frequency and residual milk removal on milk yield and FA composition	Investigate effect of pulsation ratios on milk yield and milking effectivity	Investigate effect of take off level on udder level and feeding during milking on milking effectivity and milk quality	Investigate effect of take off level on quarter level and feeding during milking on milking effectivity and milk quality
Design	4*4 Latin square	Split-udder	4*4 Latin square	6*6 Latin square
Treatments	2x, 4x, 2xRMR, 4xRMR	60:40, 65:35, 70:30, 75:25	0.78F, 0.78NF, 0.18F, 0.18NF	0.48F, 0.48NF, 0.3F, 0.3NF, 0.06F, 0.06NF
Measurements:				
Milk yield	x	x	x	x
Gross composition	x	x	x	x
FA composition	x		x	
FFA			x	x
MFG			x	x
γ-glutamyl transpeptidase			x	x
Residual milk	x		x	x
Milk flow		x	x	x
Milking time		x	x	x
Udder emptying	x	x	x	x

4.5 Residual Milk Removal

Residual milk removal was performed in **Papers I**, **III** and **IV** by injections of oxytocin (Partoxin® vet. 17µg (10 IU)/ ml).

In **Paper I**, intravenous injections of 10 I.U. oxytocin were made in the jugular vein after each milking in the RMR treatments. In addition, residual milk removal was conducted on all cows on the last day of treatment in each treatment period by the same method, in order to harvest residual milk. Milking was re-initiated three minutes after the injection, without further pre-stimulation.

In **Paper III**, residual milk removal was performed on four cows in each group (total n=16) in the end of each treatment period. For this, 25 I.U. oxytocin was administered intramuscularly immediately after milking. Milking was re-initiated three minutes after injection using a bucket milking machine.

In **Paper IV**, residual milk removal was performed on all cows in the end of every second treatment period. In this case, 70 I.U. of oxytocin was administered intramuscularly immediately after milking. Milking was re-initiated three minutes after injection using a bucket milking machine.

4.6 Milk Analyses

Milk composition was, in **Papers I-III**, determined by mid-infrared spectroscopy (Fourier Transform Instrument, FT 120, Foss, Hillerød, Denmark) at the laboratory of the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences. SCC was determined using fluorescence-based cell counting (Fossomatic 5000, Foss) at the same laboratory. In **Paper IV**, milk composition and somatic cell count were analysed with the same equipment as above for the first two periods of the experiment. During the last four periods of the experiment milk composition and SCC was analysed using a CombiScope FTIR 300HP (Delta Instruments, The Netherlands).

Milk fatty acid composition was analysed at the National Resources Institute, Finland (**Paper I),** and at the Department of Food Sciences, Swedish University of Agricultural Sciences (**Paper III**). The analysis was performed according to Shingfield *et al.* (2003) using gas chromatography (6890N, Agilent Technologies and CP 3800, Varian, Walnut Creek, CA respectively) equipped with a CP-Sil 88 column (100 m × 0.25 mm i.d., 0.2 µm film thickness, Agilent Technologies) and a flame ionisation detector. The carrier gas used was hydrogen (**Paper I**) or helium (**Paper III**) respectively. Prior to

gas chromatography, the milk was extracted with diethyl ether and hexane. Organic extracts were combined, evaporated to dryness at 40°C under oxygen-free nitrogen, dissolved in hexane, and stored at -80°C prior to preparation of fatty acid methyl esters (FAME) and GC analysis. Lipid was transesterified to fatty acid methyl esters by incubation with methyl acetate and methanolic sodium methoxide (Shingfield *et al.*, 2003).

Free fatty acid content was analysed at the Department of Food Sciences, Swedish University of Agricultural Sciences in **Paper III**, and at Aarhus University, Denmark in **Paper IV**. In **Paper III**, the method used was a variation of the method devised by Deeth *et al.* (1975). The method involves extraction of milk with a mixture of two solvents (isopropanol:hexane:4NH2SO4, 40:10:1, and hexane) to separate the fat. The fat released was titrated with 0.002 N methanolic KOH and phenol red as an indicator. In **Paper IV**, a recently validated method based on an in-solution derivatization of milk was used (Amer *et al.*, 2013).

MFG size and amount of membrane material (**Papers III** and **IV**) were analysed at Aarhus University, Denmark. The size distribution of MFG was analysed by integrated light scattering using a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK) according to Wiking *et al.* (2004). The activity of the MFG membrane protein γ-glutamyl transpeptidase was examined as described by Wiking *et al.* (2004).

The β-hydroxybutyrate (**Paper IV**) content was analysed at Aarhus University, Denmark, using fluorometric determination (Larsen & Nielsen, 2005) and cholesterol was determined using the method described by Larsen (2012) at the same laboratory.

Sodium and potassium concentrations (**Paper III**) were analysed at AgriLab, Sweden. The method used was inductively coupled plasma - optical emission spectroscopy (ICP-OES) using a Spectro Blue FMS26 (Spectro analytical instruments, Germany). Prior to analysis, the samples were diluted 100-fold.

4.7 Data Handling and Statistical Analysis

4.7.1 Paper I

Measurements of milk yield and gross composition were analysed by ANOVA for repeated measures for a 4 × 4 Latin Square with a 2 × 2 factorial arrangement of treatments using the Mixed procedure in SAS (version 9.3, SAS Institute, Cary, NC) with a model that included the fixed effects of period, sampling day, increased milking frequency, residual milk removal, and their interaction, a covariate (measured over a 48 h period before treatments were

applied), and interactions of the covariate with milking frequency and removal of residual milk, and the random effect of cow assuming an Auto Regressive Order One Covariance Structure using the Kenward-Rogers correction method.

Milk FA composition data were analysed by ANOVA for a 4 × 4 Latin Square with a 2 × 2 factorial arrangement of treatments with a model that included the fixed effects of period, increased milking frequency, removal of residual milk, and their interaction, and the random effect of cow. Differences between FA composition of available and residual milk were tested using a Student's T-Test.

4.7.2 Paper II

Data were analysed using the Mixed and Glimmix procedures of SAS (version 9.3, SAS Institute, Cary, NC). The Mixed model was used for analysis of teat tissue thickness, milk yield, fat content, SCC, machine-on-time, peak milk flow and average milk flow. The model included the fixed effects of treatment and week, and the repeated effect of cow within herd. For analysis of milk flow measurements, milk yield was included as a covariate. Data on SCC were logarithmically transformed (log10) in order to achieve a normal distribution of the data.

The Glimmix procedure with logistic regression and binomial distribution was used for analysis of teat score. The model included the fixed effects of treatment and farm.

4.7.3 Paper III

Data were analysed by ANOVA for a 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments in a linear mixed-effects model in the statistical software R (r-project.org). The model included the fixed effects of period, lactation number, DIM, sample type (available or residual milk), take-off level, feeding and their interaction, and a random effect of cow. Milk fatty acid composition was analysed by MANOVA for a 4 × 4 Latin Square with a 2 × 2 factorial arrangement of treatments with a model that included the fixed effects of period, lactation number, DIM, take-off level, feeding and their interaction.

Measurements of sodium concentration, milking time, FFA content and SCC were natural log (ln)-transformed prior to analysis due to non-normal distribution.

4.7.4 Paper IV

Data were analysed by ANOVA for a 6 × 6 Latin square with a 2 × 3 factorial arrangement of treatments in a linear mixed-effects model using repeated measures in the statistical software SAS. The model included the fixed effects

of period, lactation number, days in milk, take-off level, feeding and their interaction and the random effect of cow within group. Cow within group was included as a repeated measure. Data on FFA content and SCC were log_{10}-transformed prior to analysis due to non-normal distribution. Differences in milk composition between harvested milk samples and residual milk samples were evaluated with a Student's T-Test. Posthoc means separation for significant main effects was done using the Tukey-Kramer adjustment.

Values presented are LsMeans±SEM unless otherwise stated.

5 Main Findings

The take-off levels used in **Papers III** and **IV** were aimed to be set at 0.2 and 0.8 kg/min for **Paper III** and 0.1, 0.3 and 0.5 kg/min in **Paper IV**. However, there were practical limitations with flow measurements that caused the actual take-off levels to be 0.18 and 0.78 kg/min in **Paper III** and 0.06, 0.3 and 0.48 kg/min in **Paper IV**.

5.1 Paper I

Increased milking frequency (MF) tended to increase milk yield, while there was no effect of residual milk removal (RMR) and no interaction between MF and RMR. Increased MF also tended to decrease protein content while RMR had no significant effect on gross composition or milk component yields. A significant interaction between MF and RMR was found for fat yield, causing an increased fat yield when increased MF and RMR were combined in the treatment 4xRMR.

No effects were found on SCC, fat content, lactose content, lactose yield or protein yield. SCC was very low in all treatments, which was also a prerequisite for cows participating in the study.

The residual milk yield and proportion increased in both treatments where residual milk removal was applied. In the treatment 2xRMR, the residual milk as a proportion of total milk increased from 20% on the first day of treatment to 33% on the last day. In treatment 4xRMR, residual milk proportion increased from 32% to 71% from the first to the last day of treatment.

The effects on milk FA composition were very diverse. Total proportions of saturated, mono- and poly-unsaturated, *de novo* and >C16 fatty acids were unaffected by treatments, in both available and residual milk. MF had small effects on a few fatty acids, and these effects were mostly found in residual milk. RMR had larger effect on milk FA composition in both available and

residual milk, and decreased the proportion of several short-chained fatty acids in both available and residual milk.

5.2 Paper II

The 70:30 and 75:25 pulsation ratios increased peak and average milk flow, while the 60:40 ratio decreased peak and average flow compared with the control. Machine-on time did not differ between control and the 70:30 treatment, but was shorter with 75:25 and longer with 60:40. In the 60:40 treatment, treated udder quarters had on average 14.1 ± 2.2 s longer machine-on time, while in the 75:25 treatment the machine-on time was 9.2 ± 2.1 s shorter. Milk yield was unaffected and fat content in strip milk did not differ between treatments, indicating no differences in udder emptying. No negative effects on teat condition or milk SCC were observed with any of the pulsation ratios applied during the study.

5.3 Paper III

Increasing the take-off level from 0.18 to 0.78 kg/min on whole udder level decreased milking time and increased harvest flow and average flow. Residual milk yield increased, but there were no effects on milk yield, peak flow or the activity of γ-glutamyl transpeptidase. The shortest milking time was observed with 0.78NF, while the longest milking time was observed with 0.18NF. FFA content was lower in the 0.78F treatment and lower in residual milk compared with available milk. The relative proportions of fatty acids C4:0, C6:0, C16:0, C18:3 (n-3) and C20:0 were affected by the interaction between feeding and take-off. Feeding during milking increased milk yield per day and decreased milking interval. High take-off decreased lactose content and tended to decrease fat and protein content. Sodium and potassium concentrations in milk were unaffected by treatments, indicating no loss of tight junction integrity, but did differ between available and residual milk. Average MFG size was larger and the activity of γ-glutamyl transpeptidase higher in residual milk. The proportion of C16:1 cis-9 was higher and the proportion of C18:3 (n-3) and C20:3(n-6) were lower in residual milk.

5.4 Paper IV

Increasing the take-off level from 0.06 to 0.48 kg/min on quarter level decreased milking time, while increasing take-off level from 0.30 to 0.48 kg/min tended to decrease milking time. However, there was no effect of

treatments on milk yield or peak flow. A slight decrease in content of lactose was found between take-off levels 0.48 and 0.3 kg/min. A similar decrease in lactose content was also found when feeding during milking was omitted.

Total FFA content, MFG size, cholesterol, β-hydroxybuturate and γ-glutamyl transpeptidase were unaffected by treatments. Take-off level 0.48 decreased the proportion of caproic (C6:0), palmitic (C16:0) and oleic (*cis*-9 18:1) acid, and tended to decrease the proportion of capric (C10:0), lauric (C12:0), myristic (C14:0) and heptadecanoic (C17:0) acid.

Table 2. *Summary of the results of Papers I-IV. MF=milking frequency, RMR=residual milk removal, TO=Takeoff, F=Feeding. NS=non-significant, - = not measured*

	I			II			III			IV		
	↑ MF	RMR	MF*RMR	60:40	70:30	75:25	↑ Takeoff	Feeding	TO*F	↑ Takeoff	Feeding	TO*F
Milk yield	(↑)	NS	NS	NS	NS	NS	NS	↑	NS	NS	NS	NS
Milk composition												
Fat %	NS	NS	NS	-	-	-	NS	NS	NS	NS	NS	NS
Protein %	(↓)	NS	NS	-	-	-	NS	NS	NS	NS	NS	NS
Lactose %	NS	NS	NS	-	-	-	↓	NS	NS	↓	(↓)	NS
Fat yield	NS	NS	4xRMR ↑	-	-	-	-	-	-	-	-	-
Protein yield	NS	NS	NS	-	-	-	-	-	-	-	-	-
Lactose yield	NS	NS	NS	-	-	-	-	-	-	-	-	-
SCC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Residual milk yield	NS	↑	-	-	-	-	↑	NS	NS	NS	NS	NS
FFA	-	-	-	-	-	-	NS	NS	0.78F↓	NS	NS	NS
MFG	-	-	-	-	-	-	NS	NS	NS	NS	NS	NS
FA composition	Yes	Yes	Yes	-	-	-	NS	NS	Yes	-	-	-
Machine-on time	-	-	-	↑	NS	↓	↓	NS	0.78NF↓	↓	NS	NS
Peak flow	-	-	-	↓	↑	↑	NS	NS	NS	NS	NS	NS
Average flow	-	-	-	↓	↑	↑	NS	NS	NS	NS	NS	NS

Arrows indicate significant effects of treatment, (↑=increase, ↓=decrease). Arrows within brackets indicate trends.

5.5 General Findings

5.5.1 Milk removal

In **Paper I**, increased milk removal did not increase milk yield, but tended to increase protein content and affected FA composition. When RMR was applied in combination with frequent milking, fat yield was increased despite fat content being unchanged, most likely due to the increased milk yield. As a response to the repeated residual milk removal, the proportion of residual milk compared with available milk increased drastically.

In **Paper III**, there were indications of a lower degree of udder emptying in high take-off level treatments. In **Paper III** the residual milk yield was higher, but in **Paper IV** there were no differences in milk yield or residual milk yield.

In **Paper II**, there were no effects on strip milk fat content from the treatments applied, indicating no effect on udder emptying.

5.5.2 Milking efficiency

In **Papers II**, **III** and **IV**, milking efficiency was affected by the treatments applied. By increasing pulsation ratio or increasing take-off level, it proved possible to increase milk flow and shorten milking time. In **Paper III**, milking time was on average one minute shorter with the higher take-off level (0.78 kg/min) compared with the lower (0.18 kg/min), in **Paper IV** a similar time reduction was seen when increasing take-off from the lowest to the highest level (0.06 kg/min to 0.48 kg/min and udder quarter). In **Paper II**, milking time was 21 s shorter in the high pulsation treatment (75:25 compared with 60:40).

5.5.3 Milk lactose

In **Papers III** and **IV**, lactose content was affected by the treatments applied. Increasing take-off level from 0.18 to 0.78 kg/min on whole udder level or 0.3 to 0.48 kg/min on quarter level significantly decreased lactose content, by 0.02 and 0.06% in **Paper III** and **IV**, respectively. While these changes in lactose were very small and of little relevance in terms of dairy production, changes in lactose content is an indicator of altered integrity of the blood:milk barrier in the mammary gland.

5.5.4 Milk fat

Milk fatty acid composition

Milk fat composition was affected by treatments applied in **Paper I** and **III**.

In **Paper I**, RMR had a significant impact on the FA composition, while MF had almost no effect. RMR increased total C16, cis 16:1, total 16:1 and C16:0 in available milk, while increased MF decreased 24:0 in avaibable milk. RMR also decreased the relative proportions of several short-chained fatty acids in both available and residual milk. The relative proportions of 6:0 and 8:0 decreased in available and residual milk, and that of 4:0 also decreased in residual milk. The relative proportion of 4:0 was also affected by MF, decreasing with 4x milking. The most pronounced differences between available and residual milk were found in the treatment 2xRMR, where the proportion of several short-chained FAs (C4:0, C6:0, C8:0, C9:0, 10:0, *cis*-9 10:1 and C11:0) were greater in residual milk. However, the shift of milk from available to residual must be considered in interpretation of these results.

In **Paper III**, the proportions of fatty acids C4:0, C6:0, C16:0, C18:3 (n-3) and C20:0 were affected by the interaction between feeding and take-off level. The proportions of C4:0 and C6:0 were lower with 0.78NF, while the content of C16:0 was lower with 0.18F. The proportion of C16:1 cis-9 was higher and the proportion of C18:3 (n-3) and C20:3(n-6) were lower in residual milk. The β-hydroxybutyrate content of milk was unaffected, indicating no changes in milk fat synthesis due to treatments.

Milk fat globules and free fatty acids

In both **Paper III** and **IV**, MFG size distribution and the activity of γ-glutamyl transpeptidase were unaffected by treatments. In **Paper IV**, cholesterol was also tested, but no significant effects of treatments were found. This clearly indicates no effect of take-off level or feeding on milk fat globules in milk. As expected, MFG size was found to be larger and the activity of γ-glutamyl transpeptidase higher in residual milk (**Paper III**).

In **Paper III**, there was a significant interaction between feeding and take-off level, with a high take off level resulting in higher FFA content if not combined with feeding, but lower FFA content if combined with feeding. Low take-off level resulted in a high FFA content if combined with feeding, but a lower FFA content if not combined with feeding during milking.

In **Paper IV** there were no differences in total free fatty acids, but slight differences in individual free fatty acid content between the three take-off levels. A take-off level of 0.48 kg/min decreased the content of caproic (C6:0), palmitic (C16:0) and oleic (*cis*-9 18:1) acid, and tended to decrease the proportion of capric (C10:0), lauric (C12:0), myristic (C14:0) and heptadecanoic (C17:0) acid.

5.5.5 Residual milk

In **Paper I**, the proportion of residual milk increased due to residual milk removal, while the proportion of available milk decreased. The proportion of residual milk increased more when RMR was applied in combination with increased MF. Marked differences in FA composition were seen between available and residual milk, changes that were most prominent in the 2xRMR treatment, where there was a shift in residual milk proportion due to treatments.

In **Paper III**, several significant differences were found between residual and available milk, with residual milk having a higher sodium content, lower potassium content, higher SCC, larger MFG, higher activity of γ-glutamyl transpeptidase, lower FFA content, higher proportions of C16:1 cis-9, and lower proportions of 18:3 n-3 and 20:3 n-6.

In **Paper IV**, the yield of residual milk did not differ between treatments, and residual milk fat content did not differ between treatments.

6 General Discussion

6.1 Milk Removal

The most pronounced effect of more complete milk removal in **Paper I** was a rapid increase in residual milk proportion. This has been reported earlier and been suggested to be due to central or peripheral inhibition of milk ejection (Bruckmaier, 2003; Donker *et al.*, 1954). The combination of increased MF and RMR increased fat yield, which was most likely an effect of the numerical, but on RMR statistically nonsignificant, increase in milk yield. A trend for increased milk yield was seen with both RMR and the interaction of MF and RMR. However, this trend could not be proven statistically. Since the cows used in the study were in mid-lactation, it is likely that the storage capacity of the udder was not a limiting factor for milk synthesis, and results on milk yield could have been more pronounced if the cows used had been in peak lactation. Also, a higher number of cows in this study could have demonstrated the effect, or lack thereof, more clearly.

In **Papers I**, **III** and **IV**, injections of oxytocin were used to harvest residual milk, but different injection sites and doses were used. The reason for using intravenous injections in **Paper I** was that we wanted to obtain a rapid response to the injection and to keep the doses in the lower range due to the frequency of injections. In **Papers III** and **IV**, there were practical limitations to using intravenous injections, since the cows in these studies were milked in an AMS instead of a stanchion barn, which was used in **Paper I**. We therefore chose to use intramuscular injections instead, and the dose was increased, which is recommended when changing injection site from intravenous to intramuscular. However, the dose was only slightly increased in **Paper III**, and to ensure maximal emptying of the udder, we chose to increase the dose further in **Paper IV**. From the results on residual milk proportion in **Paper I**, it is evident that the dose and frequency of oxytocin injections affected milk ejection and udder emptying, an effect that was apparent already on day two of

treatment. However, in **Papers III** and **IV**, injections were performed once a week or every other week, respectively, and it is not likely that these spot injections had any influence on the effects of treatments, especially since the first days after each injection were not included in the statistical analysis of treatment effects. The dose could have affected the residual milk yield, but since there were no effects on residual milk yield in **Paper IV**, where the highest dose was used, this is not likely. However, several very low residual milk yields and proportions were registered in **Paper IV**. These low yields could be due to failed injections or very high udder emptying in quarter level milking systems. The data were analysed using only residual yields over 500 or 1000 ml, but no treatment differences were found.

In **Paper III**, the residual milk yield was higher when take-off level 0.78 kg/min was applied, a result that was not repeated in **Paper IV**, where there were no differences in residual milk yield. It is likely that these differing results were caused by the whole udder level milking that was applied in **Paper III**. With whole udder level milking and a high take-off level, some quarters may still be in the plateau phase or in the beginning of the decline phase, with high milk flow, when the cluster is taken off. These quarters will then have a high yield of residual milk, while other quarters may be more or less completely emptied. When a higher take-off level is applied in quarter milking systems, as in **Paper IV**, all quarters will have reached the decline phase and lower milk flows before the teat cups are detached. Thus, any difference in residual milk yield in **Paper IV** would reflect the milk that is harvested from flow 0.48 to 0.3 or 0.06 kg/min. As indicated by Besier and Bruckmaier (2016), the actual yield difference would be very small, less than 1% of total yield. This difference was not found in **Paper IV**, indicating no difference in udder emptying

The average flow rate was not affected by take-off level in **Papers III** and **IV**, which is surprising since milking time was decreased in both studies, while milk yield was unaffected. An actual effect on flow rate was not expected in these studies, but an indirect effect of milking time on average flow. It is likely that an effect could have been shown if a larger number of cows had been used in the studies.

With residual milk removal in **Paper I**, the proportion of residual milk was increased to levels comparable to when a higher milking frequency was applied (33% in 2xRMR and 39% in 4x, respectively). This could be an effect either of a partial inhibition of milk ejection, and/or of lower udder fill.

6.2 Milking Efficiency

In **Papers II**, **III** and **IV**, there was an effect on milking efficiency from the treatments applied. In **Papers III** and **IV**, milking time could be reduced by approximately one minute, while in **Paper II** milking time was shortened by approximately 20 s when pulsation ratio was increased from 75:25 to 60:40. The milking times in **Paper III** and **IV** were very similar, 6.5-7 min for high take-off level and 7-8 min for lower take-off levels, irrespective of whether take off was done on whole udder or quarter level.

Milking time is a crucial aspect in all milking systems, both to maximise the use of the milking system and to minimise the influence of the whole process surrounding milking (waiting, entering, exiting and handling time) on the time budget of dairy cows. The time spent with milking and milking related activities has been found to significantly affect the time budget of dairy cows in freestall systems, consuming on average of 2.7 h/day, ranging from 0.5 to 6 h (Gomez & Cook, 2010). In stanchion barns, parlour and rotary milking systems, the total milking time also affects the work of the herdsmen. Shorter milking times in AMS, parlour and rotary systems have the potential to increase the number of milkings per 24 hours and to reduce the time spent by each cow in the waiting area. In the waiting area, cows are not able to eat or rest, and it is therefore important that waiting times are as short as possible. Assuming a typical automatic milking herd of 60 cows milked 2.5 times per day, a one minute reduction in milking time per cow saves in total 150 minutes per day. Assuming an average milking time of 7 minutes, this would enable another 21 milkings per day. The number of cows milked per hour would also increase by one, from 7.5 to 8.5, reducing time in the waiting area for all cows in the system.

Out of the time spent in the milking unit, several minutes are used for activities other than milking (Bava *et al.*, 2005). This time is used for pre-stimulation, attachment of teat cups, teat spraying and time spent entering and exiting the milking unit. Since pre-stimulation is combined with cleaning, very little time can be saved there, although cows with high udder fill are less dependent on pre-stimulation (Kaskous & Bruckmaier, 2011). Prescott *et al.* (1998) showed that high yielding dairy cows increase milking frequency when feed is provided in the MU. A teaser feed ration in the milking unit can therefore make cows enter the MU more quickly, but also exit more slow, if feed is left in the feed trough. The milking consumes most of the time in the MU, and it is therefore the activity in the MU with the highest potential to save time. In **Paper III** and **IV**, an attempt was made to shorten the milking time by using a higher take-off level, and this proved to be successful. Prior to the experiment there were some concerns regarding udder emptying and therefore

tests were performed on combining the take-off level treatments with feeding, to increase milk removal and thereby get maximal emptying of the udder despite higher take-off level. The results supported findings by Besier and Bruckmaier (2016), who observed a very small yield loss but a larger time gain when simulating a higher take-off level.

Increased pulsation ratio was able to increase both peak and average flow and decrease milking time (**Paper II**), although the effect on milking time was smaller than observed with increased take-off levels. Combining a higher pulsation ratio with a higher take off level and no feeding during milking could perhaps increase milking efficiency further, without negative effects on teat tissue or udder emptying.

6.3 Milk Lactose

Lactose is the milk component showing the smallest variation in healthy udders (Forsbäck *et al.*, 2010). A decreased content of lactose is usually related to disturbances in the udder, causing lactose to leak across the blood:milk barrier. These disturbances include intramammary infections (Forsbäck *et al.*, 2010; Berglund *et al.*, 2007; Linzell & Peaker, 1972), prolonged milking intervals (Delamaire & Guinard-Flament, 2006; Stelwagen *et al.*, 1997) and drying off. Dutreuil *et al.* (2016) showed that lactose content in milk is lower for extremely short (4 h) or extremely long (24 or 36 h) milking intervals. The effect of a short interval in their study could have been fully or partly attributed to a dilution effect of fat, since fat content was found to be higher at the short milking interval and protein content, alike lactose, was lower. The effect of long milking intervals (18 h or more) on lactose content has been well established and has been confirmed to be due to leakage over the blood:milk barrier (Stelwagen *et al.*, 2008; Davis *et al.*, 1999; Stelwagen *et al.*, 1997). Lactose content has also been found to decrease with increased milking frequency (Hale *et al.*, 2003) and with decreased milking frequency (Bernier-Dodier *et al.*, 2010). To the best of my knowledge, the effects of incomplete milkings or large amounts of residual milk have not been investigated previously. Rather surprisingly, the lactose content of milk was affected by treatments in several experiments reported in this thesis (**III** and **IV**). In **Papers III** and **IV**, lactose content decreased with higher take-off level. Higher take-off level should theoretically lower udder emptying, since milk yield is slightly reduced (Besier & Bruckmaier, 2016). Lactose content should theoretically increase with higher take-off level, since lactose content is lower in residual milk compared to available milk (Ontsouka *et al.*, 2003).

While lactose content was affected by several of the treatments applied in this thesis, the magnitude of the changes was small and may not be of any practical or biological importance. Due to the low day-to-day variation in lactose content in milk from healthy glands, very small variations are required to cause significant differences. More data and probably also detailed studies on blood:milk barrier integrity are necessary to understand the observed responses in lactose content. In future studies, it would be interesting to add measurements of lactose in urine or plasma in order to rule out or confirm leakage over the blood:milk barrier.

6.4 Milk Fat

6.4.1 Fatty acid composition

In **Paper I**, several effects on fatty acid composition were found, mainly among short chained fatty acids (<C16) in both available and residual milk and total C16 in available milk. Proportions of 4:0, 6:0 and 8:0 were decreased by removal of residual milk, indicating a decrease in *de novo* synthesis. On the other hand, no effect on total fatty *de novo* acids (C4-14) was found. The 16:0, cis 16:1, total 16:1 and total C16 fatty acid proportions in available milk were all increased by RMR. C16 isomers can originate from either *de novo* synthesis or from feed, and total *de novo* proportion was unaffected in this study. It is therefore not possible to draw any conclusions regarding *de novo* fatty acid synthesis from these results.

Differences in the FA composition between available and residual milk in **Paper I** were mainly detected for the treatment 2xRMR, where residual milk was removed twice daily. Also, the differences were mainly found among short-chained fatty acids, i.e. those synthesized *de novo*. Dutreuil *et al.* (2016) found that the proportion of short-chained fatty acids was positively correlated with MFG size, which is also supported by the results of Briard *et al.* (2003). Unfortunately, MFG size was not evaluated in **Paper I**. In 2xRMR, there was a gradual shift in the proportions of the two milk fractions, resulting in residual milk comprising 33% of total milk on the last day of treatment, whereas residual milk comprised 18, 39 and 71% of total milk on the last day of treatment for the treatments 2x, 4x and 4xRMR respectively. Across all treatments, a significant difference between available and residual milk was seen for 4:0 and 6:0, indicating that these fatty acids are more predominant in residual milk. These results are in contrast to the results of Gómez-Cortés *et al.* (2011) and Dill *et al.* (1974), who found no major differences in fatty acid composition between available and residual milk in ewes and cows respectively. In **Paper III**, these short chained fatty acids were found in higher

proportion in the 0.78NF treatment, but no difference between available and residual milk was found. Comparing the proportions of these fatty acids between **Paper I** and **III**, the proportions in residual milk were similar between the studies, but not the proportions in available milk, which were lower in **Paper I**. This could partly be an effect of differences in the precision of the analysis at the different laboratories.

In treatment 2xRMR, the proportion of short-chained fatty acids was higher in residual milk, but the proportion of some of these fatty acids in both available and residual milk was decreased by RMR. Overall, the proportions of these FA (C4:0, C6:0, C8:0, C9:0, 10:0, *cis*-9 10:1 and C11:0) were low in 2xRMR compared with the other treatments.

The increased proportion of SCFA in residual milk was in contrast to findings by Kernohan *et al.* (1971), who found no differences in SCFA, but a higher proportion of long-chained unsaturated fatty acids in residual milk. However, it must be considered that the methods used today with long polar capillary columns allows far better resolution of specific FA in milk fat.

The studies included in this thesis only begin to describe the effect of milk removal on milk fatty acid composition. Various differences were found, but further studies on larger numbers of cows are needed to determine why certain fatty acids are affected, while others are not.

6.4.2 Milk fat globules and free fatty acids

Milk fat globule size and the activity of γ-glutamyl transpeptidase did not differ between treatments in any of the studies where these parameters were tested (**Papers III** and **IV**). This indicates no effect on MFG stability of the treatments applied, which in **Paper IV** was confirmed by the lack of effect on total FFA content. In **Paper III**, 0.78F gave rise to a lower FFA content, while in **Paper IV**, the concentrations of several FFA were lower when higher take-off level was applied, even if total FFA was unaffected. This could indicate that a different composition of MFG were subjected to lipolysis, or that less milk with a high FFA content was harvested. However, FFA content was found to be lower in residual milk, and udder emptying was found to be lower with high take-off level treatments in **Paper III**. This could indicate a redistribution of milk in the udder rather than a smaller proportion of residual milk being harvested.

In **Paper IV**, cholesterol was also tested and did not differ between treatments. A change in γ-glutamyl transpeptidase or cholesterol could have indicated a change in MFG membrane composition. In **Paper III**, MFG size was found to be larger in residual milk, which is in line with previous findings (Kernohan & Lepherd, 1969). The activity of γ-glutamyl transpeptidase was

found to be higher in residual milk, which probably reflects the higher fat content, since large MFG have less membrane material per g fat compared with small MFG (Briard *et al.*, 2003).

6.5 Residual milk

Contrary to expectations, the yield and proportion of residual milk did not differ between treatments in **Paper IV**, but was higher with take-off at a lower milk flow level in **Paper III**. In **Paper I**, residual milk proportion increased in both RMR treatments, and it increased more when increased MF and RMR were combined. On the 4xRMR treatment, the proportion of residual milk increased to levels comparable to those when there is a lack of endogenous milk ejection (Bruckmaier, 2005), while in 2xRMR, residual milk proportion was close to that of 4x. Similar results were found by Donker *et al.* (1954) after hourly injections of oxytocin, and were attributed to possible de-sensitisation of oxytocin receptors. In **Paper I**, this resulted in a need for supraphysiological doses of oxytocin to elicit a milk ejection. However, when the treatment periods ended and cows went back to standard management, normal amounts of milk were harvested within a couple of days.

There were some inconsistencies in the results between papers in this thesis regarding residual milk fat composition. In **Paper I**, the proportions of some short chained FA were higher in residual milk across treatments, while in **Paper III**, higher cis-9 C16:1, and lower 18:3 n-3 and 20:3 n-6 was found in residual milk. This could be an effect of the treatments applied, or a reflection of the differences in precision in the methods used for FA determination in different laboratories.

Some expected differences were found between available and residual milk in **Papers III** and **IV**, these include larger MFG size (Kernohan & Lepherd, 1969), higher activity of γ-glutamyl transpeptidase and higher sodium concentrations (Ontsouka *et al.*, 2003). In contrast to Ontsouka *et al.* (2003), potassium concentration was found to be lower in residual milk.

Several previous studies have found a so-called residual effect from short milking intervals of up to 6 h (Dutreuil *et al.*, 2016; Stelwagen *et al.*, 2008; Stelwagen *et al.*, 1996). The residual effect is described as a dilution effect from the high fat content of the residual milk, which causes lactose and protein content to be lower after a short milking interval. Fat content is of course also higher. A similar effect on the first milkings in the 4x and 4xRMR treatments was observed in **Paper I** (data not presented), where fat content appeared to be higher in the first two milkings with 6 h intervals. It is rather surprising that this effect was also seen for the 4xRMR treatment, since residual milk was

removed and could not have caused the effect. This indicates that the residual effect reported in literature might not only be an effect of remaining residual milk in the gland, but that it also may be an effect of a higher milk fat secretion, as indicated in the results on the higher total milk fat yield in the 4xRMR treatment in **Paper I**.

6.6 Feeding During Milking

Feeding during milking has been proposed as a means to facilitate cow traffic in automatic milking systems and is suggested to be a part of a good milking routine. As previously mentioned Svennersten *et al.* (1990) found that feeding gave rise to a small increase in plasma oxytocin 5 min after feeding compared with 15 min before feeding. The increase in oxytocin levels from feeding has been suggested to improve milk ejection, but oxytocin has also been suggested to affect milk synthesis (Lollivier *et al.*, 2006; Svennersten-Sjaunja & Olsson, 2005; Lollivier *et al.*, 2002)

In many of the studies available in the literature, it is not specified whether the cows were accustomed to feeding during milking or not. If feeding during milking was their normal routine, results from an experimental treatment without feeding at milking would show effects caused not by the provision of feed, but by the absence of the feed ration, which could cause either confusion or disappointment and might be comparable to a novel surrounding response. Although no behavioural observations were conducted in **Paper IV**, experimental staff noted that cows started moving around when they had finished their feed ration, and that cows that did not receive feed pounded their heads against the feed through to make pellets fall down. It has previously been shown that cows subjected to aversive treatment or unfamiliar surroundings during milking decrease the yield of available milk and increase residual milk yield (Rushen *et al.*, 2001; Rushen *et al.*, 1999). In a study by Johansson *et al.* (1999a), where behaviour and cortisol levels of cows fed before, during or after milking were investigated, it was found that cows fed 1.5 h after milking had a lower frequency of social interactions and a higher oral activity during the time from milking until feeding. Furthermore, cows that were fed after milking had higher levels of cortisol during the 30-60 min after feeding and higher milking-related cortisol secretion compared with cows fed during milking. Cooper *et al.* (2008) showed that as little as 2 h of deprivation of feed and lying resulted in clear signs of discomfort among cows. In the studies by Johansson *et al. (1999a; 1999b)*, the group that was fed after milking, as opposed to cows fed before or during milking, was kept without feed for 1.5 h before milking (3 h in total), which in itself could have affected milk ejection. Cows that were fed

before milking had access to feed during milking, and were not deprived of feed at all, in contrast to cows fed during milking. It is therefore possible that the provision of feed again after 1.5 h of feed deprivation in the group that was fed during milking gave rise to a surge of oxytocin that was not seen in the other groups.

In the study by Johansson *et al.* (1999b), cows that were fed during milking were provided with the feed as the teat cups were attached, not during pre-stimulation. This could indicate that the effect of feeding during milking acts more to maintain, rather than to enforce, milk ejection. Svennersten *et al.* (1995) found that cows fed during milking had both prolonged and elevated release of oxytocin compared with cows that were fed 1.5 h after milking. On the other hand, Schams *et al.* (1984) found no relationship between oxytocin level in plasma and milk flow, indicating that even small amounts of oxytocin can elicit a full milk ejection and that milk ejection is a threshold phenomenon. They also suggested that for milk removal, the timing of oxytocin release is more important than the actual concentrations.

Samuelsson *et al.* (1996) found that cows that were feed deprived for 20 h or more had a lower response area for oxytocin and elevated cortisol during the first 10 minutes after initiation of milking. This confirmed the results from Svennersten *et al.* (1995), that feed deprived cows had a lower milking-related oxytocin increase.

In the study by Johansson *et al.* (1998), it was found that cows that were fed concentrates at the same time as milking was initiated showed a rapid increase in plasma oxytocin, while cows that only received hand stimulation had a lower surge of oxytocin. Despite this, milk, protein and lactose yield and average flow were higher in stimulated cows than in cows that received no hand stimulation, irrespective of whether they were fed or not. This indicates that the high surge of oxytocin that was seen in fed cows did not facilitate milk ejection.

The possibility of using a concentrate ration, given at the onset of milking, in combination with a higher take-off level to obtain a sufficient milk removal at a shorter milking time and at the same time avoid over-milking was tested in **Papers III** and **IV**. In **Paper IV**, there were no positive effects of feeding, while in **Paper III** feeding increased daily milk yield and reduced milking interval. The cows in these studies were held in a Feed First™ system, and were thereby coming directly from the feeding area to milking. (Brandsma, 1978) reported that the 'concentrates effect' was less pronounced or completely absent when there was a lot of fresh grass available, a claim later confirmed by Samuelsson *et al.* (1993), who found that the effect of feeding during milking disappeared in the evening milking when cows had been on

pasture during the day. Also, Mačuhová *et al.* (2003) found no effect of concentrate feeding on oxytocin release when pre-stimulation was removed.

Previous studies on feeding during milking often lacks information on the amount fed and whether the cows were able to consume feed during the whole milking. In **Papers III** and **IV** of this thesis, feed was provided in small amounts during milking, and frustration in cows from not having any feed left could have affected the results. In addition, the cows used in these studies were accustomed to receiving feed during milking, which could have affected the results. In **Paper I**, the cows were fed at the same time as pre-stimulation was initiated, but residual milk yield and proportion still increased on both RMR treatments. It was impossible to distinguish whether feeding had any positive effect on milk ejection in **Paper I**, but it was not able to fully compensate for the effects of frequent oxytocin injections.

The rather contradictory effects of feeding in **Papers III** and **IV** could be due either to the previously mentioned frustration or to feeding actually not enforcing milk ejection in these cows. Most previous findings have comprised an effect on average flow, milk yield and milking time, which are all strongly related to each other, and peak flow has often been unaffected. It could be the case that feeding during milking prolongs rather than enforces milk ejection, as supported by the prolonged release of oxytocin found in the study by (Svennersten *et al.*, 1995). Feeding would then be causing not a higher peak flow, but a longer duration of the plateau phase. This would enable higher average flow, shorter machine-on time and, if udder emptying is improved, higher milk yield. There could also be an effect on the occurrence of bimodal milk flow curves, whereby the feeding-induced surge in oxytocin could compensate for a delayed stimulation-induced surge of oxytocin high enough to elicit a milk ejection. However, based on findings by Johansson *et al.* (1998) it is not likely that feed could compensate in full for milk ejection. Despite the many papers published on the effects of feeding during milking, there is still some confusion regarding what the treatments actually tested. More research is needed to clarify when feeding during milking can facilitate milk removal, for which cows it can be beneficial, and whether feeding during milking should or should not be used in automatic milking systems. Future studies should also account for effect of time since previous feeding, the customary routine of the cows and the total eating time in the MU in relation to milking time.

6.7 Quarter Milking

Quarter milking was used, and was an important part of the experimental design, in three of the papers in this thesis (**Papers I, II** and **IV**).

It is well known that milk yield, milking time and flow can differ quite a lot between quarters. Weiss *et al.* (2004) showed that milk yield was lower, milking time shorter and average flow lower in front quarters than in rear quarters. They also showed that the plateau phase was shorter and the decline phase longer in front quarters. This has important practical implications, especially when milking is performed on whole udder level instead of quarter level. In the study by Weiss *et al.* (2004), milking time was found to be one minute shorter in front quarters, which clearly shows that when milking is performed on whole udder level, settings need to be carefully considered in order to minimise the risk of over-milking. This is also supported by Weiss and Worstorff (2001), who found a significant decrease in cup-on time from using quarter level detachment of teat cups.

Total milking times were comparable in **Papers III** and **IV**, where udder milking and quarter milking was applied respectively. The take-off levels in these papers differed, with 0.18 and 0.78 kg/min being used in **Paper III** and 0.06, 0.3 and 0.48 kg/min being used in **Paper IV**. It could have been beneficial in **Paper IV** to use take off levels more similar to those in **Paper III**, for instance 0.045 and 0.195 kg/min to make the results more comparable, but unfortunately there were practical limitations for this.

Many of the previous studies on both pulsation ratio and take off levels have been performed on udder level rather than quarter level, which influences the interpretation of the results. For instance, previous studies on pulsation ratio have often shown a negative effect on teat condition and udder health (Hamann & Mein, 1996; Østerås *et al.*, 1995), which could be an effect of over-milking due to udder level milking. Tančin *et al.* (2006) stated that the over-milking phase starts at a flow rate of 0.075 kg/min. If take-off is set to this level or higher, over-milking should be completely avoided in quarter level milking, and the risk of teat end damage should be significantly reduced.

Although quarter milking is common in automatic milking, udder level milking is still more commonly used over the world. Despite it being beneficial for udder health, quarter milking is often not viable for economical, practical or technological reasons. However, in order to make recommendations on management and milking technique in quarter milking systems, it is important that research performed on milking in whole udder milking systems is repeated for quarter milking systems.

7 Conclusions

The overall conclusion of this thesis is that residual milk removal in mid-lactation dairy cows does not affect milk yield, but that the relative proportions of specific short chained fatty acids increase when residual milk is removed. It can also be concluded that milking efficiency in an automatic milking system can be increased with higher take off levels and that the effect of feeding during milking and impact of oxytocin on milk synthesis needs to be investigated further.

- Residual milk removal affects milk fatty acid composition, mainly by a decrease in the proportion of short-chained fatty acids in available and residual milk. Increased milking frequency affects milk composition, and there are no additive effects of increased milking frequency and residual milk removal.

- Increased pulsation ratio can be applied in quarter level milking systems to increase milk flow and shorten milking time without effects on udder emptying, teat ends or somatic cell count

- Increased take-off level on whole udder level can shorten milking time without negative effects on milk yield or composition, specifically the susceptibility to lipolysis. Feeding during milking may counteract effects of low take-off level when milking is performed on whole udder level.

- Increased take-off level on quarter level can shorten milking time without negative effects on milk yield, milk composition or the susceptibility to lipolysis. Feeding during milking may not affect udder emptying when quarter milking is applied.

8 Populärvetenskaplig sammanfattning

Mjölk – en vit vätska som produceras för att bli föda till en unge till ett däggdjur, och ett livsmedel som finns i var mans kylskåp. Mjölk är ett viktigt livsmedel för oss människor, och oavsett om den konsumeras i sin renaste, oförändrade form eller om den är förädlad till attraktiva produkter såsom ost och smör, så måste råmaterialet, den mjölk vi får från korna, vara en högkvalitativ produkt som produceras till ett så lågt pris som möjligt. Korna behöver vara hållbara och må bra, och belastningen på miljön och klimatet minimeras. I ett automatiskt mjölkningssystem finns möjlighet för korna själva att påverka när de vill äta, vila och mjölkas. Mjölkningen sker i en mjölkningsrobot, som mjölkar varje spene individuellt. Ofta ges en liten mängd kraftfoder under mjölkningen. Kraftfodret ges både för att locka kon till mjölkningsstationen och för att påverka mjölknedsläppet. Mjölknedsläppet innebär att mjölken transporteras från de övre delarna av juvret (alveolerna), där mjölken huvudsakligen produceras, till den nedre delen av juvret (cisternen), där mjölken huvudsakligen lagras, och vidare ut till spenen, varifrån den kan mjölkas ut. Utan mjölknedsläpp kan ungefär 20% av mjölken i juvret mjölkas ut, så det är en viktig process för att vi ska få ut så mycket mjölk som möjligt. Efter ett bra mjölknedsläpp kan man mjölka ut 80-90% av mjölken, och den mjölk som blir kvar kallas residualmjölk. Residualmjölken är en gräddig mjölk, som innehåller mycket fett, mindre vatten, och fettet är i form av stora fettkulor.

Under mjölkningen flödar mjölken ur spenarna i fyra distinkta faser: ökningsfasen, platåfasen, minskningsfasen och övermjölkningsfasen. Under ökningsfasen ökar mjölkflödet snabbt tills platåfasen nås, för att sedan ligga på en stabil nivå under större delen av mjölkningen. När majoriteten av mjölken har mjölkats ut så når flödet minskningsfasen, där det minskar snabbt och mjölkmängden ökar i långsammare takt. Till slut, när flödet av mjölk från alveolerna till cisternen har blivit lägre än flödet från cisternerna ut genom spenen har övermjölkningsfasen nåtts. Denna fas kan slita på spenarna, och

därmed öka risken för juversjukdomar. Det är därför viktigt att denna fas är så kort som möjligt eller undviks helt. När alla spenkoppar sitter ihop i ett kluster och sätts på och tas av samtidigt, innebär det ofta att en eller flera spenar hinner nå övermjölkningsfasen, eftersom både flöde och mjölkmängd skiljer sig mellan juverdelarna, så kan en juverdel vara urmjölkad långt före eller efter de övriga. Det gör att mjölkningen blir ojämn, och att en eller flera juverdelar kan vara övermjölkade samtidigt som en eller flera andra juverdelar kan vara ofullständigt tömda. I vissa system mjölkas varje juverdel individuellt, där spenkopparna sätts på och tas av en och en, vilket minskar risken för ojämn mjölkning, och i synnerhet över- och undermjölkning. Tidpunkten för när spenkopparna tas av bestäms av mjölkflödet. Om avtagningen av spenkopparna sker när mjölkflödet är högt, vid en högre avtagningsnivå, finns risk för att juvret inte töms ordentligt medan en lägre avtagningsnivå ger risk för övermjölkning.

En mjölkningsmaskin fungerar genom vakuum. I varje spenkopp finns ett spengummi som leder till en slang där mjölken ska flöda. Mellan spengummit och spenkoppens innervägg finns en vakuumkammare, och tryckskillnaden i vakuumkammaren gör att spengummit pulserar och därigenom masseras spenen. Tryckskillnaden, eller pulseringen, kommer från en pulsator, som genom en slang är förbunden med vakuumkammaren i varje spenkopp. Förenklat så slår pulsatorn av och på vakuumet enligt en förutbestämd takt. Liksom mjölkflödet har pulseringen fyra faser, a, b, c och d. Dessa fyra faser delas in i mjölkningsfasen (a+b) och massagefasen (c+d). Under b-fasen är spengummit helt öppet, och under d-fasen är det helt stängt, medan det under a- och c-fasen öppnar respektive stänger sig. När spengummit är öppet så flödar mjölken ut genom mjölkslangen, och när det är stängt så masseras spenen, och får vila från vakuumets sug. Hur långa de olika faserna är kan justeras, en vanlig pulseringskvot är 65:35, vilket innebär att mjölkningsfasen utgör 65% av tiden, medan massagefasen utgör 35%. Om man ökar längden på mjölkningsfasen ökar också belastningen på spenen i synnerhet vid övermjölkning, men det finns möjlighet för att snabbare mjölkningen, eftersom man utvinner mjölk under större del av varje minut. Vid övermjölkning är det mjölkningsfasen som sliter mest på spenarna, och om man använder en lång mjölkningsfas är det därför extra viktigt att undvika övermjölkning.

Studierna i den här avhandlingen har testat effekten av tömning av juvret på mjölkmängd, mjölksammansättning och mjölkningseffektivitet. Det har gjorts genom fyra olika studier.

I studie ett testades om mer frekvent mjölkning och mer fullständig tömning av juvret påverkade mjölkmängd, mjölksammansättning och mjölkfettsammansättning. Resultaten visade inga effekter på mjölkmängd, men

mjölkfettsammansättningen ändrades vid mer fullständig tömning av juvret. När residualmjölken mjölkades ut så ökade andelen av vissa kortkedjiga fettsyror. Vi såg också att andelen residualmjölk ökade drastiskt vid den mer fullständiga tömningen av juvret, så att det första mjölknedsläppet knappt hade någon effekt över huvud taget.

I studie två testades om förlängd mjölkningsfas kunde användas på fjärdedelsnivå utan att skada spenspetsarna och påverka juverhälsan, samt om mjölkningstiden kunde minska. Resultaten visade att den längre mjölkningsfasen inte verkade ha negativ effekt på spenarna, och att mjölkningarna dessutom kunde bli ungefär 20 sekunder snabbare.

I studie tre testades effekterna av en hög och en låg avtagningsnivå på heljuvernivå i kombination med utfodring under mjölkning. Resultaten visade att juvertömningen minskade något med hög avtagningsnivå, men att mjölkmängden var opåverkad. Däremot blev mjölkningstiden kortare, och de kor som fick foder under mjölkning hade något högre mjölkavkastning per dag och gick till mjölkning oftare. Laktoshalten i mjölken minskade något med högre avtagningsnivå, men inga skillnader fanns i övrig mjölksammansättning.

I studie fyra undersöktes effekterna av tre olika avtagningsnivåer på juverdelsnivå i kombination med utfodring under mjölkning. Även i denna studie fann vi att mjölkmängden var opåverkad men att mjölkningstiden blev kortare, samt att laktoshalten i mjölken minskade något med ökad avtagningsnivå, men mjölksammansättningen var i övrigt opåverkad. I denna studie sågs inga effekter på residualmjölkmängd.

Sammantaget har studierna i den här avhandlingen undersökt hur olika mjölkningsjusteringar påverkar tömningen av juvret och mjölkproduktionen samt hur en mer fullständig tömning av juvret påverkar mjölkbildningen. Slutsatsen är att flera av de testade mjölkningsjusteringarna kan hjälpa till att effektivisera mjölkningen utan att inverka negativt på mjölkmängd, mjölksammansättning eller juverhälsa. En mer fullständig tömning av juvret gav förändringar som vi inte riktigt än kan förstå, men det står klart att residualmjölken är spännande och att vi har mycket kvar att lära om betydelsen av denna fraktion av mjölken.

References

Amer, B., Nebel, C., Bertram, H.C., Mortensen, G., Hermansen, K. & Dalsgaard, T.K. (2013). Novel method for quantification of individual free fatty acids in milk using an in-solution derivatisation approach and gas chromatography-mass spectrometry. *International Dairy Journal,* 32(2), pp. 199-203.

Bade, R.D., Reinemann, D.J., Zucali, M., Ruegg, P.L. & Thompson, P.D. (2009). Interactions of vacuum, b-phase duration, and liner compression on milk flow rates in dairy cows. *Journal of Dairy Science,* 92(3), pp. 913-21.

Bava, L., Sandrucci, A., Tamburini, A. & Succi, G. (2005). Milk ejection during automatic milking in dairy cows. *Italian Journal of Animal Science,* 4(sup2), pp. 218-220.

Berglund, I., Pettersson, G., Östensson, K. & Svennersten-Sjaunja, K. (2007). Quarter milking for improved detection of increased SCC. *Reproduction in Domestic Animals,* 42(4), pp. 427-432.

Bernier-Dodier, P., Delbecchi, L., Wagner, G.F., Talbot, B.G. & Lacasse, P. (2010). Effect of milking frequency on lactation persistency and mammary gland remodeling in mid-lactation cows. *Journal of Dairy Science,* 93(2), pp. 555-64.

Besier, J. & Bruckmaier, R.M. (2016). Vacuum levels and milk-flow-dependent vacuum drops affect machine milking performance and teat condition in dairy cows. *Journal of Dairy Science,* 99(4), pp. 3096-102.

Blake, R.W. & McDaniel, B. (1978). Relationships among rates of milk flow, machine time, udder conformation, and managemental aspects of milking efficiency: a review. *Journal of Dairy Science,* 61(4), pp. 363-378.

Brandsma, S. The relation between milking, residual milk and milk yield. In: *Proceedings of Annual meeting National Mastitis Council, Inc. National Mastitis Council, inc*1978.

Brandsma, S. & Maatje, K. (1987). The importance of milking routines and machines for udder evacuation and production. *NJF Seminar.* Helsinki, Finland.

Briard, V., Leconte, N., Michel, F. & Michalski, M.-C. (2003). The fatty acid composition of small and large naturally occurring milk fat globules. *European Journal of Lipid Science and Technology,* 105(11), pp. 677-682.

Bruckmaier, R.M. (2003). Chronic oxytocin treatment causes reduced milk ejection in dairy cows. *Journal of Dairy Research,* 70(1), pp. 123-126.

Bruckmaier, R.M. (2005). Normal and disturbed milk ejection in dairy cows. *Domestic Animal Endocrinology,* 29(2), pp. 268-73.

Bruckmaier, R.M. & Blum, J.W. (1996). Simultaneous recording of oxytocin release, milk ejection and milk flow during milking of dairy cows with and without prestimulation. *Journal of Dairy Research,* 63(02), pp. 201-208.

Bruckmaier, R.M. & Hilger, M. (2001). Milk ejection in dairy cows at different degrees of udder filling. *Journal of Dairy Research,* 68(03), pp. 369-376.

Bruckmaier, R.M., Schams, D. & Blum, J.W. (1994). Continuously elevated concentrations of oxytocin during milking are necessary for complete milk removal in dairy cows. *Journal of Dairy Research,* 61(03), pp. 323-334.

Castro, A., Pereira, J.M., Amiama, C. & Bueno, J. (2012). Estimating efficiency in automatic milking systems. *Journal of Dairy Science,* 95(2), pp. 929-36.

Collier, R.J., Hernandez, L.L. & Horseman, N.D. (2012). Serotonin as a homeostatic regulator of lactation. *Domestic Animal Endocrinology,* 43(2), pp. 161-70.

Cooper, M.D., Arney, D.R. & Phillips, C.J.C. (2008). The effect of temporary deprivation of lying and feeding on the behaviour and production of lactating dairy cows. *Animal,* 2(02), pp. 275-283.

Davis, S., Farr, V. & Stelwagen, K. (1999). Regulation of yield loss and milk composition during once-daily milking: a review. *Livestock Production Science,* 59(1), pp. 77-94.

De Koning, K. & Ouweltjes, W. (2000) Maximising the milking capacity of an automatic milking system. In: *Proceedings of Robotic milking: the International Symposium*, Lelystad, The Netherlands, pp. 38-46.

Deeth, H.C., Fitz-Gerald, C.H. & Wood, A.F. (1975). A convenient method for determining the extent of lipolysis in milk. *Australian Journal of Dairy Technology,* 30(3), pp. 109-111.

Delamaire, E. & Guinard-Flament, J. (2006). Longer milking intervals alter mammary epithelial permeability and the udder's ability to extract nutrients. *Journal of Dairy Science,* 89(6), pp. 2007-2016.

Dill, C., Lane, G. & Hartsfield, S. (1974). Influence of repeated oxytocic treatments on composition of bovine milk fat. *Journal of Dairy Science,* 57(10), pp. 1164-1169.

Donker, J., Koshi, J. & Petersen, W. (1954). The effect of exogenous oxytocin in blocking the normal relationship between endogenous oxytocic substance and the milk ejection phenomenon. *Science,* 119, pp. 67-68.

Dutreuil, M., Guinard-Flament, J., Boutinaud, M. & Hurtaud, C. (2016). Effect of duration of milk accumulation in the udder on milk composition, especially on milk fat globule. *Journal of Dairy Science,* 99(5), pp. 3934-44.

Edwards, J.P., Jago, J.G. & Lopez-Villalobos, N. (2013a). Milking efficiency for grazing dairy cows can be improved by increasing automatic cluster remover thresholds without applying premilking stimulation. *Journal of Dairy Science,* 96(6), pp. 3766-73.

Edwards, J.P., Jago, J.G. & Lopez-Villalobos, N. (2013b). Short-term application of prestimulation and increased automatic cluster remover threshold affect milking characteristics of grazing dairy cows in late lactation. *Journal of Dairy Science,* 96(3), pp. 1886-93.

Ely, F. & Petersen, W.E. (1941). Factors involved in the ejection of milk. *Journal of Dairy Science,* 24(3), pp. 211-223.

Forsbäck, L., Lindmark-Månsson, H., Andren, A., Åkerstedt, M., Andree, L. & Svennersten-Sjaunja, K. (2010). Day-to-day variation in milk yield and milk composition at the udder-quarter level. *Journal of Dairy Science,* 93(8), pp. 3569-77.

Gleeson, D.E., O'Brien, B., Boyle, L. & Earley, B. (2007). Effect of milking frequency and nutritional level on aspects of the health and welfare of dairy cows. *Animal,* 1(1), pp. 125-32.

Gómez-Cortés, P., Bodas, R., Mantecón, A.R., de la Fuente, M.A. & Manso, T. (2011). Milk composition and fatty acid profile of residual and available milk from ewes fed with diets supplemented with different vegetable oils. *Small Ruminant Research,* 97(1-3), pp. 72-75.

Gomez, A. & Cook, N.B. (2010). Time budgets of lactating dairy cattle in commercial freestall herds. *Journal of Dairy Science,* 93(12), pp. 5772-5781.

Hale, S., Capuco, A. & Erdman, R. (2003). Milk yield and mammary growth effects due to increased milking frequency during early lactation. *Journal of Dairy Science,* 86(6), pp. 2061-2071.

Hamann, J. & Mein, G.A. (1996). Teat thickness changes may provide biological test for effective pulsation. *Journal of Dairy Research,* 63(02), pp. 179-189.

Hernandez, L.L., Collier, J.L., Vomachka, A.J., Collier, R.J. & Horseman, N.D. (2011). Suppression of lactation and acceleration of involution in the bovine mammary gland by a selective serotonin reuptake inhibitor. *Journal of Endocrinology,* 209(1), pp. 45-54.

Hernandez, L.L., Stiening, C.M., Wheelock, J.B., Baumgard, L.H., Parkhurst, A.M. & Collier, R.J. (2008). Evaluation of serotonin as a feedback inhibitor of lactation in the bovine. *Journal of Dairy Science,* 91(5), pp. 1834-44.

Jago, J.G., Burke, J.L. & Williamson, J.H. (2010). Effect of automatic cluster remover settings on production, udder health, and milking duration. *Journal of Dairy Science,* 93(6), pp. 2541-9.

Jensen, R.G. (2002). The composition of bovine milk lipids: January 1995 to December 2000. *Journal of Dairy Science,* 85(2), pp. 295-350.

Johansson, B., Olofsson, J., Wiktorsson, H., Uvnäs-Moberg, K. & Svennersten-Sjaunja, K. (1998). A comparison between manual prestimulation versus feeding stimulation during milking. *Swedish Journal of Agricultural Research,* 28, pp. 177-187.

Johansson, B., Redbo, I. & Svennersten-Sjaunja, K. (1999a). Effect of feeding before, during or after milking on dairy cow behaviour and the hormone cortisol. *Animal Science,* 68, pp. 597-604.

Johansson, B., Uvnäs-Moberg, K., Knight, C.H. & Svennersten-Sjaunja, K. (1999b). Effect of feeding before, during and after milking on milk production and the hormones oxytocin, prolactin, gastrin and somatostatin. *Journal of Dairy Research,* 66(02), pp. 151-163.

Kaskous, S. & Bruckmaier, R.M. (2011). Best combination of pre-stimulation and latency period duration before cluster attachment for efficient oxytocin release and milk ejection in cows with low to high udder-filling levels. *Journal of Dairy Research,* 78(01), pp. 97-104.

Kernohan, E.A. & Lepherd, E. (1969). Size distribution of fat globules in cow's milk during milking, measured with a Coulter counter. *Journal of Dairy Research,* 36(02), pp. 177-182.

Kernohan, E.A., Wadsworth, J. & Lascelles, A. (1971). Changes in the composition of bovine milk fat during milking. *Journal of Dairy Research,* 38(01), pp. 65-68.

Knight, C.H., Hirst, D. & Dewhurst, R.J. (1994). Milk accumulation and distribution in the bovine udder during the interval between milkings. *Journal of Dairy Research,* 61(02), pp. 167-177.

Larsen, T. (2012). Enzymatic–fluorometric quantification of cholesterol in bovine milk. *Food Chemistry,* 135(3), pp. 1261-1267.

Larsen, T. & Nielsen, N.I. (2005). Fluorometric Determination of β-Hydroxybutyrate in Milk and Blood Plasma. *Journal of Dairy Science,* 88(6), pp. 2004-2009.

Linzell, J. & Peaker, M. (1971). Mechanism of milk secretion. *Physiological. Reviews,* 51(3), pp. 564-597.

Linzell, J. & Peaker, M. (1972). Day-to-day variations in milk composition in the goat and cow as a guide to the detection of subclinical mastitis. *British Veterinary Journal,* 128(6), pp. 284-295.

Lollivier, V., Guinard-Flament, J., Ollivier-Bousquet, M.l. & Marnet, P.-G. (2002). Oxytocin and milk removal: two important sources of variation in milk production and milk quality during and between milkings. *Reproduction Nutrition Development,* 42(2), pp. 173-186.

Lollivier, V., Marnet, P.G., Delpal, S., Rainteau, D., Achard, C., Rabot, A. & Ollivier-Bousquet, M. (2006). Oxytocin stimulates secretory processes in lactating rabbit mammary epithelial cells. *Journal of Physiology,* 570(Pt 1), pp. 125-40.

Mačuhová, J., Tančin, V.r. & Bruckmaier, R.M. (2003). Oxytocin release, milk ejection and milk removal in a multi-box automatic milking system. *Livestock Production Science,* 81(2-3), pp. 139-147.

Magliaro, A. & Kensinger, R. (2005). Automatic cluster remover setting affects milk yield and machine-on time in dairy cows. *Journal of Dairy Science,* 88(1), pp. 148-153.

Mather, I.H. & Keenan, T.W. (1998). Origin and Secretion of Milk Lipids. *Journal of Mammary Gland Biology and Neoplasia,* 3(3), pp. 259-273.

Mein, G., Bramley, A., Dodd, F. & Bramley, J. (1992). Basic mechanics and testing of milking systems. In: Bramley, A., Dodd, F., Mein, G. & Bramley, J. (eds) *Machine milking and lactation*. Vermont, USA: Insight books, pp. 235-284.

Ontsouka, C., Bruckmaier, R. & Blum, J. (2003). Fractionized milk composition during removal of colostrum and mature milk. *Journal of Dairy Science,* 86(6), pp. 2005-2011.

Peaker, M. & Wilde, C.J. (1996). Feedback control of milk secretion from milk. *Journal of Mammary Gland Biology and Neoplasia,* 1(3), pp. 307-315.

Phillips, D. (1978) The role of environmental factors in stimulating the milk ejection reflex. In: *Proceedings of Annual meeting-National Mastitis Council, Inc. National Mastitis Council, inc (USA)*.

Prescott, N.B., Mottram, T.T. & Webster, A.J.F. (1998). Relative motivations of dairy cows to be milked or fed in a Y-maze and an automatic milking system. *Applied Animal Behaviour Science,* 57(1–2), pp. 23-33.

Rasmussen, M.D. (1993). Influence of switch level of automatic cluster removers on milking performance and udder health. *Journal of Dairy Research,* 60(03), pp. 287-297.

Rasmussen, M.D. (1999) Benefits from early removal of the milking unit. In: *Proceedings of Proc. Brit. Conf. on Mastitis, Stoneleigh, UK*, pp. 55-61.

Rasmussen, M.D. (2004) Overmilking and teat condition. In: *Proceedings of NMC Annual Meeting Proceedings*, pp. 169-175.

Rushen, J., De Passille, A. & Munksgaard, L. (1999). Fear of people by cows and effects on milk yield, behavior, and heart rate at milking. *Journal of Dairy Science,* 82(4), pp. 720-727.

Rushen, J., Munksgaard, L., Marnet, P.G. & DePassillé, A.M. (2001). Human contact and the effects onf acute stress on cows at milking. *Applied Animal Behaviour Science,* 73, pp. 1-14.

Samková, E., Spicka, J., Pesek, M., Pelikanova, T. & Hanus, O. (2012). Animal factors affecting fatty acid composition of cow milk fat: A review. *South African Journal of Animal Science,* 42(2), pp. 83-100.

Samuelsson, B., Uvnäs-Moberg, K., Gorewit, R. & Svennersten-Sjaunja, K. (1996). Profiles of the hormones somatostatin, gastrin, CCK, prolactin, growth hormone, oxytocin and cortisol. II. In dairy cows that are milked during food deprivation. *Livestock Production Science,* 46(1), pp. 57-64.

Samuelsson, B., Wahlberg, E. & Svennersten, K. (1993). The effect of feeding during milk production and milk flow. *Swedish Journal of Agricultural Research,* 23, pp. 101-106.

Schams, D., Mayer, H., Prokopp, A. & Worstorff, H. (1984). Oxytocin secretion during milking in dairy cows with regard to the variation and importance of a threshold level for milk removal. *Journal of endocrinology,* 102(3), pp. 337-343.

Shingfield, K., Ahvenjarvi, S., Toivonen, V., Arola, A., Nurmela, K., Huhtanen, P. & Griinari, J.M. (2003). Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Animal Science,* 77(1), pp. 165-180.

Spencer, S.B., Shin, J.W., Rogers, G.W. & Cooper, J.B. (2007). Short communication: Effect of vacuum and ratio on the performance of a monoblock silicone milking liner. *Journal of Dairy Science,* 90(4), pp. 1725-8.

Stelwagen, K., Farr, V., McFadden, H., Prosser, C.G. & Davis, S. (1997). Time course of milk accumulation-induced opening of mammary tight junctions and blood clearance of milk components. *American Journal of Physiology Regulatory Integrative Comparative Physiology,* 273, pp. R379-R386.

Stelwagen, K., Farr, V.C., Nicholas, G.D., Davis, S.R. & Prosser, C.G. (2008). Effect of milking interval on milk yield and quality and rate of recovery during subsequent frequent milking. *Livestock Science,* 114(2), pp. 176-180.

Stelwagen, K., Knight, C.H., Farr, V.C., Davis, S.R., Prosser, C.G. & McFadden, T.B. (1996). Continuous versus single drainage of milk from the bovine mammary gland during a 24 hour period. *Experimental physiology,* 81(1), pp. 141-149.

Svennersten-Sjaunja, K. & Olsson, K. (2005). Endocrinology of milk production. *Domestic Animal Endocrinology,* 29(2), pp. 241-58.

Svennersten, K., Gorewit, R.C., Sjaunja, L.O. & UvnÄS-Moberg, K. (1995). Feeding during milking enhances milking-related oxytocin secretion and milk production in dairy cows whereas food deprivation decreases it. *Acta Physiologica Scandinavica,* 153(3), pp. 309-310.

Svennersten, K., Nelson, L. & Uvnäs-Moberg, K. (1990). Feeding-induced oxytocin release in dairy cows. *Acta Physiologica Scandinavica,* 140(2), pp. 295-296.

Tančin, V., Ipema, B., Hogewerf, P. & Mačuhová, J. (2006). Sources of variation in milk flow characteristics at udder and quarter levels. *Journal of Dairy Science,* 89(3), pp. 978-988.

Thomas, C.V., Force, D.K., Bremel, D.H. & Strasser, S. (1991). Effects of pulsation ratio, pulsation rate, and teatcup liner design on milking rate and milk production. *Journal of Dairy Science,* 74(4), pp. 1243-1249.

Weiss, D., Dzidic, A. & Bruckmaier, R. (2003). Quarter specific milking routines and their effect on milk removal in cows. *Milchwissenschaft,* 58(5-6), pp. 238-242.

Weiss, D., Weinfurtner, M. & Bruckmaier, R. (2004). Teat anatomy and its relationship with quarter and udder milk flow characteristics in dairy cows. *Journal of Dairy Science,* 87(10), pp. 3280-3289.

Weiss, D. & Worstorff, H. (2001). Effects of quarter take-off with and without machine stripping under simulated automatic milking conditions. *Milchwissenschaft,* 56(8), pp. 427-430.

Wellnitz, O. & Bruckmaier, R.M. (2001). Central and peripheral inhibition of milk ejection. *Livestock Production Science,* 70(1), pp. 135-140.

Wiking, L., Stagsted, J., Björck, L. & Nielsen, J.H. (2004). Milk fat globule size is affected by fat production in dairy cows. *International Dairy Journal,* 14(10), pp. 909-913.

Wilde, C.J., Addey, C., Boddy, L.M. & Peaker, M. (1995). Autocrine regulation of milk secretion by a protein in milk. *Biochemical Journal,* 305, pp. 51-58.

Østerås, O., Rønningen, O., Sandvik, L. & Waage, S. (1995). Field studies show associations between pulsator characteristics and udder health. *Journal of Dairy Research,* 62(01), pp. 1-13.

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