#### UNIVERSITY OF COPENHAGEN FACULTY OF HEALTH AND MEDICAL SCIENCES



# Master's Thesis in Animal Science

Simone Sohrbeck Thorsager Billing (kht342)

# Effects of changes in milk machine settings on milk yield, milking time, milk flow and teat end condition in Danish dairy cattle

Supervisor: Associate Professor, Hanne H. Hansen, Department of Veterinary and Animal Sciences, University of Copenhagen

Submitted on: 6<sup>th</sup> of August 2018

## Acknowledgements

This Master's thesis was written as the final part of the MSc-programme in Animal Science at the Faculty of Health and Medical Sciences, University of Copenhagen. It was conducted from November 2017 to August 2018 at the Department of Veterinary and Animal Sciences, University of Copenhagen.

I would like to thank my advisor Associate Professor Hanne H. Hansen at the Department of Veterinary and Animal Sciences, University of Copenhagen for guidance throughout the process, creating the contact to the milk company and help during the experimental period. Also, thanks to Christian Stilling Christensen at A/S S. A. Christensen & co., Kolding for creating contact to the farm, set up of experiments, help and professional inputs throughout the process.

The experiments were conducted in a commercial Danish dairy farm, and I am thankful to the owner Robert Mouritsen for the opportunity and collaboration. Without Robert's interest in new methods and risk taking, the project would not have been established. Furthermore, I owe my deepest gratitude to Lasse Emil Sembach and Simone Husballe Rasmussen, for helping me out during the experimental work and writing process. I could not have retrieved a sufficient amount of data, within the timeframe, without help from these two fellow students. I am thankful to Louise Kjær Hilligsøe, you are the reason I got started on the writing process to start with, and I am grateful for your reading of the thesis and comments. I am thankful to Nikolaj Peder Hansen for help with statistical analyses, careful reading of the thesis and constructive comments, I owe you my deepest thanks. Thanks to Anne Lønborg Hill for help with grammar. Lastly, thanks to my family and friends for believing in me and supporting me, through 9 months of irregular and intense working hours.

Simone Sohrbeck Thorsager Billing, kht342

## Abstract

In order to adapt to fluctuations of milk prices on the global market, it is important to reduce costs related to labour in dairy herds. Milking of cows can require a great amount of labour and therefore, methods of milking that can improve milk efficiency are needed. However, improved milk efficiency should not be on behalf of deterioration of teat end. The objective of the thesis was to investigate if three specific milk machine settings had a positive effect on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking in order to obtain a more efficient milking. In addition, it was investigated if milk machine settings had a positive effect on udder score. Three longitudinal cohort experiments were carried out in a commercial Danish dairy farm with 649 cows/year and with an external rotary. The three milk machine settings investigated were prestimulation pulsation, milk flow-controlled b-phase pulsation and fixed take-off time. Milk machine data concerning milk yield, milking time and milk flow was retrieved for statistical analyses. The statistical analyses compared milk production data for animals present both before and after experiment. Additionally, teat end condition was assessed before and after each experiment and combined to an udder score in order to determine if milk machine settings deteriorated teat ends. Experiment 1 found no effect of prestimulation pulsation on daily milk yield, but odds of deteriorated udder score increased after prestimulation pulsation. Experiment 2 found no effect of milk flow-controlled b-phase pulsation on total milking time/milking, but odds of deteriorated udder score increased after milk flow-controlled b-phase pulsation. Experiment 3 found increased milk flow/milking for multiparous cows after fixed take-off time was introduced, and odds of deteriorated udder score decreased after treatment. Milk flow-controlled b-phase pulsation improved milk efficiency through increased milk yield for primiparous cows. Fixed take-off time improved milk efficiency through increased milk yield for primiparous cows, and increased milk yield and milk flow for multiparous cows.

# List of abbreviations

ACR	Automatic cluster removal
Sec	Seconds
Min	Minutes
DIM	Days in milk
Milk flow	Average milk flow
Ms	Milliseconds
kPa	Kilo Pascal
TECT	Teat end callosity thickness
TECR	Teat end callosity roughness
ECM	Energy corrected milk
PMR	Partial mixed ration
LSM	Least square means
SEM	Standard error of means
SD	Standard deviation
OR	Odds ratio

# **Table of contents**

1.	Intr	oductio	n	5
2.	Lite	rature 1	ceview	7
	2.1.	Udder	physiology	7
	2.2.	Milk p	roduction	8
	2.3.	Milking	g methods	9
	2.3.1	1. P	restimulation	9
	2.3.2	2. P	ulsation rate and ratio1	0
	2.3.3	3. A	utomatic cluster removal1	2
	2.4.	Teat er	nd condition1	3
3.	Mat	erials a	nd methods1	.5
	3.1.	Experi	mental design1	5
	3.2.	Experi	ment 1 - prestimulation1	6
	3.3.	Experi	ment 2 – milk flow-controlled b-phase pulsation1	6
	3.4.	Experi	ment 3 – fixed take-off time1	7
	3.5.	Teat er	nd condition score1	7
	3.6.	Data c	ollection and calculations1	8
	3.7.	Statisti	cal analysis1	9
4.	Res	ults		1
	4.1.	Experi	ment 1 - prestimulation	1
	4.2.	Experi	ment 2 – milk flow-controlled b-phase pulsation2	2
	4.3.	Experi	ment 32	5
5.	Disc	ussion .		7
	5.1.	Experi	ment 1 – prestimulation pulsation2	7
	5.2.	Experi	ment 2 – milk flow-controlled b-phase pulsation2	8
	5.3.	Experi	ment 3 – fixed take-off time3	0
	5.4.	Teat er	nd condition score and methods	1
	5.5.	Other of	considerations	2
6.	Con	clusion		4
7.	Pers	spective	s 3	5
8.	Refe	erences.		6

## **1. Introduction**

An interview with the Danish dairy group Arla in 2017 revealed future variation in milk prices owing to fluctuations in milk prices on the global milk market, which Danish dairy farmers are an even greater part of now because of milk quota abolition (Brusgaard, 2017). In order to adapt to these conditions, Danish milk producers need methods to produce milk at a lower expense to become less vulnerable to milk price fluctuations. This can be done by improving milk efficiency in the herd, by increasing milk yield or by reducing milking time. In other words, milk speed - also known as milk flow - must increase.

In terms of effecting milk flow, several milk machine settings and milking methods have been reviewed in the literature. Among them are prestimulation, pulsation rate, pulsation ratio and automatic cluster removal (ACR). Manual and mechanic prestimulation can affect milk flow (Kaskous and Bruckmaier, 2011; Vetter et al., 2014; Watters et al., 2015). Pulsation rate and pulsation can affect milk yield and milking time (Ambord and Bruckmaier, 2009; Bade et al., 2009; Ferneborg and Svennersten-Sjaunja, 2015). ACR and truncation of milking time can be a potential and inexpensive method to improve milk efficiency in a dairy herd (Clarke et al., 2004).

Reduced milking time is associated with changes in teat end condition (Nørstebø et al., 2018). Teat ends are exposed and vulnerable in relation to milking and they are in risk of deterioration, hence in risk of mastitis (Mein et al., 2001; Neijenhuis et al., 2001). While improving milk efficiency, milking methods also need to consider the maintenance of healthy teat ends.

Based on the above and results of controlled experiments in the reviewed literature, a study was designed in order to investigate the effect of milk machine settings on milk characteristics and teat end condition in a commercial Danish dairy herd. Three treatments were tested: prestimulation pulsation, milk flow-controlled b-phase pulsation and fixed take-off time. Milk characteristics that were included in the experiments were daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking. Furthermore, teat end condition was included as an indicator of teat health.

The objective of this thesis was to investigate if three specific milk machine settings had a positive effect on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking in order to obtain a more efficient milking. In addition, it was investigated if milk machine settings had a positive effect on udder score.

The hypotheses of the thesis were:

- 1. Prestimulation pulsation increases daily milk yield
- 2. Milk flow-controlled b-phase pulsation reduces total milking time/milking
- 3. Fixed take off time increases milk flow/milking
- 4. Prestimulation pulsation, milk flow-controlled b-phase pulsation and fixed take off time decreases deterioration of teat ends

## 2. Literature review

The aim of this literature review was to describe how milk yield, milking time and milk flow in general can be influenced by some milk machine settings and milking management can influence milk production. In addition, the relation between milk machine settings and teat end condition are described.

#### 2.1. Udder physiology

Mammary glands are developed through foetal life, puberty and pregnancy. The udder consists of four teats with one mammary gland per teat. The alveolus are the functional parts of the gland and produce milk. Retention of milk in filled udders are caused by the smooth muscle cells surrounding the teat canal. During pregnancy, secretory tissue is stimulated to differentiate and grow. After calving, colostrum will be the first milk available in the udder and is most often used for calves in order to protect them against infectious agents. Hereafter, regular milk is produced and retrieved for production. (Sjaastad et al., 2010)

In order to completely remove milk from the udder, there is a need of milk ejection from alveolar and small ducts into the cisterns. The majority of milk in the udder is stored in the alveolar cavity (78-81%) while the cisternal cavity can store 12-17% of the milk depending on stage of lactation and parity (Pfeilsticker et al., 1996). Milk ejection is a neuroendocrine response caused by the release of the neuropeptide oxytocin into the blood from the posterior pituitary as a response to tactile stimulation of udder and teats, see Figure 2.1. Tactile stimulation can be caused by the calf, hand or milking machine (Bruckmaier, 2005). Milk ejection or milk let down is a shift of milk from the alveolar cavity to the cisternal cavity from where milk can be retrieved (Weiss et al., 2003). In order to have a continuous and fast removal of milk from the udder, prestimulation is essential, since it contributes to milk ejection (Sandrucci et al., 2007).

Milk ejection is influenced by intervals between milkings, stage of lactation and surrounding environment. According to Bruckmaier & Hilger (2001), milk ejection will occur fastest in early lactation at a milking interval of 12 hours and will be delayed by short milking intervals and late lactation. A delay of milk ejection can range from 40 seconds (sec) to 3 minutes (min) (Bruckmaier, 2005). Furthermore, milk ejection can be disturbed by inhibition of

oxytocin in the posterior pituitary or by oxytocin action in the mammary gland. Inhibition of oxytocin in the posterior pituitary can be caused by emotional stress in the cow (Bruckmaier, 2005).



Figure 2.1 The anatomy and physiology of milk let down. Oxytocin release and milk let down starts (Husvéth, 2011)

#### 2.2. Milk production

Milk yield, milking time and milk flow can be affected by stage of lactation and parturition. A field study by Sandrucci et al. (2007) investigated the effect of lactation stage on milk yield, milking time and milk flow. The study found that multiparous cows had greater milk yield, shorter milking time and greater milk flow than primiparous cows. In addition, cows less than 150 days in milk (DIM) had greater milk yield, longer milking time and greater milk flow than cows more than 150 DIM. This is supported by a study by Berry et al. (2013), that found that milk yield, milking time and milk flow will decrease through lactation, while bimodality increases.

Milk yield over time is the definition of milk flow and can be drawn as a milk flow curve, see Figure 2.2. Milk flow curves can be used as predictive indicators of udder health status.

Milk flow can drop shortly after milking has started owing to absent milk ejection. The phenomenon is called bimodal milk flow and is seen as a drop of milk flow between to peaks in milk flow, see Figure 2.2. A bimodal milk curve is an expression of delayed or missing shift of milk from alveolar compartments to cisternal cavity (Dzidic et al., 2004). According to Berry et al. (2013) bimodality increases through lactation owing to decreased milk yield and milk flow. Tamburini et al. (2010) found that animals with high frequency of bimodal milk curves within the first 100 days of lactation had the most deteriorated udder status during the lactation.



Figure 2.2 Milk flow curves. The solid line (black) shows a nonbimodal milk curve and the doubled line (grey) shows a bimodal milk curve (Edwards et al., 2014)

#### 2.3. Milking methods

#### 2.3.1. Prestimulation

Prestimulation is applied in order to stimulate the milk ejection reflex and ensure milk let down. Manual methods of prestimulation can be cleaning of udder and teats, premilking and lag time between cleaning and milk cluster attachment. Cleaning of the udder and teats is done to decrease risk of mastitis and to stimulate milk ejection reflex (Magnusson et al., 2006; Sandrucci et al., 2007). Premilking can be applied both before and after cleaning and is used to remove 1-2 strips of milk from the teat before milk cluster attachment, thereby apply tactile stimulation to the udder and decrease risk of bulk tank contamination (FAO, 2018). Hereafter, lag time between preparation and attachment of milk clusters can be applied in order to get proper effect of elevated oxytocin and milk let down (Kaskous and Bruckmaier, 2011; Watters et al., 2012). In automatic milking systems (AMS),

prestimulation methods can be performed by the milking robot. Alternatively, prestimulation can be applied mechanically as pulsations in milk cluster (Watters et al., 2015). Furthermore, prestimulation can be used in order to avoid bimodal milk flow and thereby increase milk flow (Bruckmaier and Blum, 1996).

Several studies have been investigating the effect of prestimulation on milk yield, milking time and milk flow. According to Sandrucci et al. (2007), preparation of udder prior to milking that included cleaning, forestripping and predipping resulted in greater milk yield, greater milk flow and shorter milking time compared to no preparation of udder prior to milking. Another study by Watters et al. (2015) found that forestripping in combination with 60 sec of lag time resulted in the shortest milking time compared to other combinations both with and without forestipping and lag times from 0 to 120 sec. In addition, the study found that cows in late lactation had reduced milking time when lag time increased compared to cows in early lactation. However, these results were not confirmed in the study by Vetter et al. (2014), who found that the combination of 15 secof prestimulation and 30 sec lag time did not affect milk yield, milking time and milk flow compared to no prestimulation and 45 sec of lag time. This is further supported by Kaskous & Bruckmaier (2011), who did not report any differences in milk yield, milking time and milk flow when investigating the effect of prestimulation for 15, 30 and 45 sec in combination with lag time of 30 and 45 sec. Based on the literature reviewed on prestimulation, it seems that prestimulation does not necessarily improve milk efficiency in terms of milk yield, milk flow and milking time.

#### 2.3.2. Pulsation rate and ratio

The milk cluster operates with four phases during milking. Phase a, b, c and d. The a-phase is a transition phase from the liner being closed to open. The b-phase is the open phase, where milk flows from the teats. The c-phase is the next transition phase, where the liner goes from open to closed. Lastly, the d-phase is the closed phase also called the massage-phase. Here, there is no flow of milk from the teat (Progressive Dairyman, 2017). All together the four phases are called a pulsation cycle, see Figure 2.3.

Pulsation rate is the setting that determines by which rate the pulsation cycles are repeated during a minute. Normally a pulsation rate is 50 to 65 cycles/minute (ISO, 2007). The length of a pulsation cycle can therefore vary from 923 to 1,200 milliseconds (ms). The length of

b-phase should not be less than 30% of the pulsation cycle and d-phase should not be less than 150 ms. Vacuum during d-phase should not be more than 4 kilopascall (kPa). (ISO, 2007)



Figure 2.3 Illustration of milk phases in milliseconds and variation of pulsation chamber vacuum in kPa. (Progressive Dairyman, 2017)

Pulsation ratio describes the relation between duration of a+b-phase and c+d-phase. Examples of pulsation ratios are shown in Table 2.1. A pulsation ratio of 65:35 indicates that duration of a+b-phase represent 65% of the pulsation cycles whereas the duration of c+dphase represent 35% of the pulsation cycle. The table shows how length of b-phase increases along an increased pulsation ratio, which is compensated for by a reduced length of d-phase. Another way to increase the length of b-phase could be to maintain the pulsation ratio and instead increase the pulsation rate. Thus, the total time spend on b-phases within a cycle will increase compared to a lower pulsation rate, which makes the liner collapse around the teat more often.

Studies have been investigating the effect of increased pulsation ratio as increased length of b-phase and milk flow-controlled b-phase on milk yield, milking time and milk flow. Gleeson et al. (2004) found an increase of milk yield as pulsation ratio increased. Increased pulsation ratio increased length of b-phase at the expense of d-phase length. A study by Bade et al. (2009) found that milk flow increased when length of b-phase increased. In contrast, a study by Ferneborg & Svennersten-Sjaunja (2015) found that an increase in pulsation ratio

did not have an effect on milk yield, but did increase milking time. In addition, increased pulsation ratio did not affect teat end condition. Alternatively, Ambord & Bruckmaier (2009) investigated the effect of milk flow-controlled b-phase, where b-phase was adjusted during milking in accordance to milk flow. Hence, increase in milk flow would increase pulsation ratio and decrease pulsation rate. Increase of pulsation ratio increased length of b-phase at the expense of d-phase length. The study found an increased milk flow after milking with milk flow-controlled b-phase compared to milking without milk flow-controlled b-phase. Milk yield and milking time was not affected by milk flow-controlled b-phase compared to milking without milk flow-controlled b-phase. Based on this, an increase of b-phase length may increase milk flow, but milk yield is not necessarily affected. Milking time may increase owing to milk flow-controlled b-phase. However, teat end conditions were not affected by increased length of b-phase.

Table 2.1 Duration of the milk phases (a-d) for four pulsation ratios (Pulsation rate 60 cycles/minute, systemvacuum 46 kPa). (Ferneborg and Svennersten-Sjaunja, 2015)

	Pulsation phase length (ms)			
Ratio	А	В	С	D
60:40	146	443	108	304
65:35	144	494	108	253
70:30	142	546	108	204
75:25	140	589	107	165

#### 2.3.3. Automatic cluster removal

ACR can be used to reduce milking time and improve milk efficiency or to reduce the effect of slow milking cows and thereby as an alternative strategy to culling them. A study by Clarke et al. (2004) investigated the effect of fixed take-off time on milk yield, milk flow and milking time. The study found no effect on milk yield when ACR was set at a threshold of 8.7 and 10.5 min compared to control with ACR threshold of 0.200 kg/min. However, milk flow increased for fixed take-off times compared to a control group with fixed take-off level, which suggests that cows compensate for reduced milking time by increasing milk flow. Another study by Jago et al. (2010) investigated ACR with fixed take-off level was set to a milk flow of 0.35 kg/min and fixed take-off time was set by determining the milking time of

the 70<sup>th</sup> percentile cow, when ranked from fastest to slowest, irrespective of yield. The maximum milking time was decreased throughout the experiment in order to keep approximately 30% of individual milkings reaching the threshold of maximum milking time. The fixed take-off time was 560 sec in the beginning of experiment and ended on 402 sec for milking performed in the morning. For evening milking, the take-off time started at 371 sec and ended on 300 sec. The study found that there was no difference in milk yield between fixed take-off time and level, and there was no difference in teat end condition between the two groups. Based on this, ACR at fixed take-off time will not affect milk yield and teat end condition. Cows may compensate for reduced milking time by increasing milk flow. ACR may potentially improve milk efficiency.

#### 2.4. Teat end condition

Teat end callosity and hyperkeratosis are conditions where keratin from the teat canal is removed and tissue around the teat canal is damaged. It creates a raised ring around the teat canal, which can increase in thickness and become rough (Neijenhuis et al., 2000), see Figure 2.4. Teat end callosity and hyperkeratosis are associated with increased risk of mastitis (Mein et al., 2001).



Figure 2.4 Four levels of teat end; 1-4. 1: no ring or roughness, 2: Raised ring and smooth or slightly rough surface. 3: Raised and rough ring with isolated fronds of keratin. 4: Very raised and roughed ring with fronds of keratin. Cracked with a "flowered" appearance. With modifications by Mein et al. (2001).

A study by Neijenhuis et al. (2000) investigated the longitudinal development of teat end callosity thickness (TECT) and teat end callosity roughness (TECR) in early lactation. The study found that increases of TECT and TECR were associated with parity and DIM. Primiparous cows had a less thick callosity ring compared to multiparous cows. The callosity ring of multiparous cows became rough later compared to primiparous cows. The callosity ring turned from smooth to rough, on average, 56 days into lactation. In addition, callosity ring became thicker and rougher when milking time became longer.

Hyperkeratosis can be caused by overmilking. According to Rasmussen (2004) overmilking starts when flow of milk to the cisterns is lower than the milk flow out of the teat canal. A reverse pressure gradient across the teat canal can occur during overmilking owing to mouthpiece chamber vacuum. If the vacuum in the teat canal for a shorter period is greater than vacuum beneath the teat end, this can cause a reverse pressure gradient across the teat canal. This can increase the risk of bacterial invasion into the teat canal (Rasmussen et al., 1994). A study by Hillerton et al. (2002) investigated teat condition associated with overmilking and found that all teat end parameters were worsened when overmilking was allowed in 2 and 5 min. In addition, Tancin et al. (2006) concluded that the duration of overmilking phase increased from first to third month of lactation after which it decreased through lactation. This is in agreement with findings of Neijenhuis et al. (2004) concluded that the effect of overmilked teats on mastitis seemed to be small.

### 3. Materials and methods

#### 3.1. Experimental design

Three longitudinal cohort experiments were carried out in a commercial Danish dairy herd, Sædding Storgaard, located in Nørre Nebel, Jutland. The farm consists of 649 cows/year of which 76% are crossbreeds between Danish Holstein, Red Danish, and Danish Red Holstein and 24% are pure breed of either Danish Holstein, Red Danish, or Danish Red Holstein. Average milk yield/cow/year during the past 12 months was 12,353 kg energy corrected milk (ECM) (SEGES, 2018). All cows are milked three times a day (5 AM, 12 PM, and 8 PM) in a SAC external rotary parlor with a capacity for 32 cows. The animals are housed in a loose-house system with solid floors and automatic manure scrapers. During the experiments, the cows were fed a partial mixed ration (PMR) ad libitum and offered a concentrate mix in the rotary during milking. Feed plans are assumed to be unchanged or with only minor modifications throughout the experimental period. The employees at the farm carried out the milking of the cows, which included a briefly wash of all teats for less than 30 sec with a cotton cloth before attachment of milk clusters without any premilking. Milking procedures prior to cluster attachment remained unchanged throughout the experimental period. An illustration of the experimental period is shown in Figure 3.1. If not otherwise stated, milk machines settings are set to have a pulsation rate of 60 cycles/min, a-+b-phase constitute 66% of pulsation duration and ACR occurs at 1250 g/min.



Figure 3.1: Timeline of the experimental period with three experiments from 7<sup>th</sup> of December 2017 to 11<sup>st</sup> of May 2018. Experiments are separated with periods of time without treatment.

In order to identify effects of treatment, milk data before and in the end of the experiments were compared. Averages of milk data (daily yield, DIM, total milking time/milking and milk flow/milking) from three times daily milking across three days (9 milking sessions) were calculated for each cow before start of experiment and in the end of the experiment, see Table 3.1. For experiment 1, averages of milk data were made based on milk data 10 days prior to experiment, due to delayed start of experiment 1.

Table 3.1 Three experiments were conducted from 27<sup>th</sup> of November 2017 to 11<sup>th</sup> of May 2018. Periods before and after experiments of which milk data were collected for calculation of averages in daily milk yield, milk yield/ milking, DIM, total milking time/milking and milk flow/milking in order to compare and identify effects of treatments.

Experiment	Before experiment	After experiment
1. Prestimulation	25 <sup>th</sup> -27 <sup>th</sup> November	9th-11th of January 2018
	2017	
2. Milk flow-controlled	21 <sup>st</sup> -23 <sup>rd</sup> of January	21 <sup>st</sup> -23 <sup>rd</sup> of March 2018
b-phase	2018	
3. Fixed take-off Time	$31^{st}$ of March 2018 - $2^{nd}$	9 <sup>th</sup> -11 <sup>th</sup> of May 2018
	of April 2018	

#### 3.2. Experiment 1 - prestimulation

The first experiment investigated a treatment of pre-stimulation built into the milk cluster prior to milking and included 581 cows. The experiment lasted 37 days. Milk clusters pulsated at 120 cycles/min when milk flow was below 600 g/min, which meant that the teats were prestimulated by milk cluster until milk flow reached above 600 g/min. However, if milk flow dropped below 600 g/min at other times during milking, the prestimulation pulsations would start again. When milk flow was above 600 g/min, pulsation rate was 60 cycles/min and a-+b-phase constituted 66 % of pulsation duration.

#### 3.3. Experiment 2 – milk flow-controlled b-phase pulsation

The second experiment investigated treatment with milk flow-controlled b-phase pulsation and included 510 cows. The experiment lasted 59 days. For milk flow above 2000 g/min, the pulsation rate was 63 cycles/min and a-+b-phase constituted 65% of pulsation duration. For milk flow below 2000 g/min, the pulsation rate was 57 cycles/min and a-+b-phase constituted 67% of pulsation duration. ACR occurred at a milk flow of 1250 g/min. After the experiment, the changes made for the treatment were kept as a permanent part of the milk machine setting.

#### 3.4. Experiment 3 – fixed take-off time

The third experiment investigated the effect of a treatment with fixed take-off time. Milk clusters were removed automatically after a specific milking time and included 577 cows. The experiment lasted 36 days. The treatment was introduced gradually through three steps. In step 1, milk clusters were removed after cluster attachment time of 10 min. Step 2 reduced cluster attachment time to 9 min. Lastly, in step 3, milk clusters were automatically removed after 8 min. The treatment was kept as a permanent part of milk machine settings after end experiment.

#### 3.5. Teat end condition score

Before and after each experiment, all milking cows had teat end hyperkeratosis score based on a the 5-point score system modified from Mein et al. (2001). The teat end score consisted of five scores: 1-5. 1 was a smooth teat end with no ring, 2 was a smooth raised ring or slightly rough ring with no keratin fronds, 3 was a raised roughened ring with isolated fronds of old keratin and 4 was a raised ring with rough fronds of old keratin, the ring is rough and cracked giving the teat end a "flowered" appearance. The score 5 was added for dry teats, missing teats or teats not milking for various reasons. In order to compare teat ends before and after experiment, only cows present both before and after an experiment were included in the data analysis. Additionally, the highest observed teat end score on an individual udder was chosen to represent the total udder score and will be reported as udder score prospectively. Udder scores from after experiment were used as udder scores before next experiment. In Table 3.2 an overview of dates with udder scores before and after experiment is presented.

Experiment	Before experiment	After Experiment
	_	_
1. prestimulation	27 <sup>th</sup> of November	11 <sup>th</sup> of January 2018
	2017	
2. milk flow-controlled b-	11 <sup>th</sup> of January 2018	23 <sup>rd</sup> of March 2018
phase pulsation		
3. fixed take-off time	23 <sup>rd</sup> of March 2018	11 <sup>th</sup> of May 2018

Table 3.2 Periods before and after treatment for three experiments in which teat ends were scored on all milking cows.

#### 3.6. Data collection and calculations

Milk data generated during milking was logged and saved in the SAC program Saturnus. Data from Saturnus was exported as excel sheets for further processing. Data contained: cow identification, milk yield/milking, time of milking start, date of milking, date of parturition, time of milking end and milk flow/milking. However, time of start and end for each milking was measured as time of which the cow entered the rotary and time of which the cow leaved the rotary. Therefore, total milking time/milking was calculated based on yield/milking and milk flow/milking:

5. Total milking time/milking =  $\frac{\text{Yield/milking}}{\text{average milk flow/milking}}$ 

Milk data for 3 times milking/day for 3 days (9 sessions) before and after experiment were calculated into the mean values for each cow: milk yield/milking, daily milk yield, DIM, total milking time/milking and milk flow/milking. The following four formulas were used:

6. 
$$Mean_{Milk yield/milking} = \left(\frac{Milk yield_{milking 1} + Milk yield_{milking...} + Milk yield_{milking 9}}{9}\right)$$
7. 
$$Mean_{Daily yield} = \left(\frac{Yield_{milking 1} + Yield_{milking...} + Yield_{milking 9}}{9}\right) * 3$$

7. 
$$Mean_{Daily yield} = \left( - \frac{Maining 1}{2} \right)$$

- 8.  $Mean_{DIM} = \frac{DIM_{day 1} + DIM_{day 2} + DIM_{day 3}}{3}$
- 9.  $Mean_{total milking time/milking} =$ Total milking time/milking<sub>milking1</sub>+Total milking time/milking<sub>milking</sub>...+Total milking time/milking<sub>milking 9</sub>

## 10. Mean<sub>milk flow/milking</sub> = <u>Total milk flow/milking<sub>milking1</sub>+milk flow/milking<sub>milking...</sub>+milk flow/milking<sub>milking9</sub></u>

Due to lactation cycle dynamic, not all cows were present at each experiment. Therefore, observations were paired in each experiment, so that cows included in the data analysis were present both before and after treatment. This means that less cows are used in the data analysis than there are present in the experiments. Furthermore, if cows had one or more records of 0 kg milk yield during the 9 milking sessions, they were excluded from data. Cows in first parity were defined as primiparous and all other cows were defined as multiparous.

Udder score 1-2 was categorized as not deteriorated and score 3-5 was categorized as deteriorated in calculations of odds and odds ratio (OR). Odds and OR were calculated for udder scores. The equation described by Szumilas (2010) was used in order to determine the probability of an udder score due to treatment or no treatment:

11.  $OR = \frac{(n)treated \ cases/(n)untreated \ cases}{(n)treated \ non-cases/(n) untreated \ non-cases}$ 

Where treated cases include cows that have been exposed to treatment (after treatment) and untreated cases includes cows that have not been exposed to treatment (before treatment). Thus, treated cases are cows with deteriorated udder scores after treatment, unexposed cases are cows with deteriorated udder scores before treatment, exposed non-cases are cows with not deteriorated udder scores after treatment and unexposed non-cases are cows with no deteriorated udder score before treatment.

#### 3.7. Statistical analysis

The statistical analyses for milking data were conducted in SAS (version 7.1, SAS Institute Inc., Cary, NC) using the MIXED procedure for response variables within each experiment. Results are presented as least square means (LSM) and standard error of means (SEM). LSM and SEM were estimated using the PDIFF option in the LSMEANS statement in SAS and are reported in tables unless other is stated. Statistical significance was accepted at P $\leq$ 0.05 and 0.05<P $\leq$ 0.10 was considered a tendency. There was found a significant interaction between parity and DIM and therefore the final model used, is for either primi- or

multiparous cows, to estimate daily milk yield, milk yield/milking, total milking time and milk flow:

$$Y_{ik} = \mu + TREAT_i + DIM + C_k + e_{ik}$$

In which Y is the response variable (Daily yield, Total milking time/milking and milk flow/milking),  $\mu$  is the overall mean, TREAT is the fixed effect of treatment (i=no, yes), DIM is the regression coefficient for DIM, C is the random effect of cow (*k*=cow number) and *e* is the random residual error assumed to be independent and normal distributed.

## 4. Results

#### 4.1. Experiment 1 - prestimulation

Out of 602 cows, 416 cows were used in the analysis of milk data. 162 cows were primiparous and 254 cows were multiparous. Before experiment 1, primiparous cows averaged ( $\pm$ SD) 152  $\pm$ 110 in DIM (range: 5-571) and multiparous cows averaged ( $\pm$ SD) 136  $\pm$ 93.9 in DIM (range: 4-516).

In Table 4.1 and Table 4.2, the results of the statistical analysis for experiment 1 can be seen for primi- and multiparous cows, respectively. There was no effect of treatment on parameters for either primi- or multiparous cows. Increase of DIM significantly (P<0.03) reduced daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking for both multi- and primiparous cows.

Item	No	Drestingulation	SEM	Р-
	prestimulation	Presumulation	SEM	Value
Daily milk yield (kg)	35.0	34.6	0.55	0.36
Milk yield/milking (kg)	11.7	11.5	0.18	0.36
Total milking time/milking (min)	4.25	4.22	0.07	0.70
Milk flow/milking (kg/min)	2.87	2.82	0.06	0.42

Table 4.1 Effect of prestimulation prior to milking on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking for 162 primiparous cows.

Item	No Prestimulation		SFM	Р-
Ittm	prestimulation	Trestinuation	<b>BENI</b>	Value
Daily milk yield (kg)	46.8	46.0	0.57	0.12
Milk yield/milking (kg)	15.6	15.3	0.19	0.12
Total milking	5.66	5.61	0.33	0.90
time/milking (min)				
Milk flow/milking	3.50	3.39	0.08	0.27
(kg/min)				

Table 4.2 Effect of prestimulation prior to milking on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking for 254 multiparous cows.

In experiment 1, 587 cows were present for assessment of teat end condition, which resulted in udder scores, see Table 4.3. Odds of deteriorated and not deteriorated udder score was 1.12 and 0.95, respectively, when udder scores before and after treatment was compared. Thus, OR was 1.19 and odds of deteriorated udder score after treatment is therefore more likely compared to odds of deteriorated udder score before prestimulation.

 Table 4.3 Number of udders with deteriorated and not deteriorated udder score before and after treatment with prestimulation. Where udder score 1-2 is not deteriorated and udder score 3-5 is deteriorated.

	Outcome status			
	Deteriorated udder Not deteriorated udder			
	score	score		
No prestimulation	181	406		
Prestimulation	203	384		

#### 4.2. Experiment 2 - milk flow-controlled b-phase pulsation

Out of 510 cows, 423 cows were used in the analysis of milk data. 159 cows were primiparous and 264 cows were multiparous. Before experiment 2, primiparous cows averaged ( $\pm$ SD) 149  $\pm$ 115 in DIM (range: 6-628) and multiparous cows averaged ( $\pm$ SD) 136  $\pm$ 100 in DIM (range: 3-573).

In Table 4.4 and Table 4.5 the results of the statistical analysis for experiment 2 can be seen for primi- and multiparous cows, respectively. For primiparous cows, daily milk yield and milk yield/milking increased significantly (P<0.01) after treatment, whereas total milking time/milking and milk flow/milking were not affected by treatment. For multiparous cows, daily milk yield, milk yield/milking and total milking time/milking were not affected by treatment. However, there was a tendency (P<0.10) to a decrease in milk flow/milking after treatment for multiparous cows. Increase of DIM significantly (P<0.01) reduced daily milk yield and milk yield/milking for both primi- and multiparous cows. For multiparous cow, milk flow/milking was significantly (P<0.01) reduced by increase of DIM. Milk flow/milking was not affected by DIM for primiparous cows. DIM did not affect total milking time/milking for either multi- or primiparous cows.

	No milk flow-	Milk flow-	SEM	Р-
Item	controlled b-	controlled b-		Value
	phase pulsation	phase pulsation		
Daily milk yield	36.5	38.1	0.61	< 0.01
( <b>kg</b> )				
Milk	12.2	13.0	0.21	< 0.01
yield/milking (kg)				
Total milking	5.54	4.99	0.57	0.50
time/milking				
(min)				
Milk	2.85	2.88	0.08	0.76
flow/milking				
(kg/min)				

Table 4.4 Effect of milk flow-controlled b-phase pulsation on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking for 159 primiparous cows.

Item	No milk flow- controlled b- phase pulsation	Milk flow- controlled b- phase pulsation	SEM	P- Value
Daily milk yield (kg)	48.0	48.0	0.64	0.95
Milk yield/milking (kg)	16.0	16.0	0.21	0.95
Total milking time/milking (min)	6.39	5.48	0.91	0.34
Milk flow/milking (kg/min)	3.22	3.12	0.52	0.08

Table 4.5 Effect of milk flow-controlled b-phase pulsation on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking for 264 multiparous cows.

In experiment 2, 411 cows were present both before and after milk flow-controlled b-phase pulsation for assessment of teat end condition for udder scores, see Table 4.6. Odds of deteriorated and not deteriorated udder score were 1.49 and 0.75, respectively, when udder scores before and after treatment were compared. Hence, OR was 1.99 and odds of deteriorated udder score after milk flow-controlled b-phase stimulation is greater than odds of deteriorated udder score before milk flow-controlled b-phase stimulation.

Table 4.6 Distribution of individual udder based on udder score before and after milk flow-controlled b-phase stimulation, where udder score 1-2 is not deteriorated and udder score 3-5 is deteriorated.

	Outcome status			
	Deteriorated udder score	Not deteriorated udder		
	Deteriorated udder score	score		
No milk flow-controlled b-	138	273		
phase stimulation	150	275		
Milk flow-controlled b-	206	205		
phase stimulation	200	203		

#### 4.3. Experiment 3

Out of 577 cows, 500 cows were used in the analysis of milk data. 173 cows were primiparous and 327 cows were multiparous. Before experiment 2, primiparous cows averaged ( $\pm$ SD) 157  $\pm$ 117 in DIM (range: 5-637) and multiparous cows averaged ( $\pm$ SD) 138  $\pm$ 105 in DIM (range: 6-556).

In Table 4.7 and Table 4.8 the results of the statistical analysis for experiment 3 can be seen for primi- and multiparous cows, respectively. For primiparous cows, there was a significant (P<0.01) increase of daily milk yield and milk yield/milking after treatment, but no effect of treatment on total milking time/milking and milk flow/milking. For multiparous cows, there was a significant (P<0.01) increase of daily milk yield and milk yield, milk yield/milking and milk flow/milking after treatment, but no effect of treatment on total milking time/milking. For multiparous cows, there was a significant (P<0.01) increase of daily milk yield, milk yield/milking and milk flow/milking after treatment, but no effect of treatment on total milking time/milking. Increase of DIM significantly (P<0.01) reduced daily milk yield, milk yield/milking and milk flow/milking for both primi- and multiparous cows. Furthermore, increase in DIM gave significant (P<0.01) longer total milking time/milking for primiparous cows, whereas total milking time/milking for multiparous cows were not affected by DIM.

Itom	No fixed take-	Fixed take-	SEM	Р-
Item	off time	off time		Value
Daily milk yield (kg)	35.7	37.4	0.55	< 0.01
Milk yield/milking (kg)	11.9	12.5	0.18	< 0.01
Total milking	4.53	4.57	0.07	0.32
time/milking (min)				
Milk flow/milking	2.84	2.88	0.06	0.59
(kg/min)				

Table 4.7 Effect of fixed take-off time on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking for 173 primiparous cows.

Item	No fixed take- Fixed take-	Fixed take-	SEM	P-
	off time	off time		Value
Daily milk yield (kg)	48.1	50.1	0.51	< 0.01
Milk yield/milking (kg)	16.0	16.7	0.17	< 0.01
Total milking	3.95	6 52	1 73	0.30
time/milking (min)		0.52	1.75	0.50
Milk flow/milking	3 31	3 51	0.06	< 0.01
(kg/min)	5.51	5.51	0.00	NU.U1

Table 4.8 Effect of fixed take-off time on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking for 327 multiparous cows.

In experiment 3 with fixed take-off time, 485 cows were present both before and after when teat ends were assessed, see Table 4.9. Odds of deteriorated udder score were 0.84, whereas odds of not deteriorated udder score were 1.16. Which gives an OR of 0.72, thus odds of deteriorated udder score after fixed take-off time is therefore less than odds of deteriorated score before treatment. This indicates that fixed take-off time may be gentler to teat ends compared to no fixed take-off time.

 Table 4.9 Distribution of individual udder based on udder score before and after fixed take-off time, where udder score 1-2 is not deteriorated and udder score 3-5 is deteriorated.

	Outcome status		
	Deteriorated udder score	Not deteriorated udder	
	Deteriorated udder score	score	
No fixed take-off time	246	239	
Fixed take-off time	207	278	

## **5.** Discussion

The current experimental study was designed to examine the effect of milk machine settings on daily milk yield, total milking time/milking and milk flow/milking in a Danish commercial dairy herd. In addition, it was designed to investigate if milk machine settings had effect on udder score.

#### 5.1. Experiment 1 – prestimulation pulsation

Experiment 1 investigated the effect of prestimulation pulsation implemented in milk clusters on daily milk yield. There was no effect of treatment on parameters for either primior multiparous cows. Therefore, the hypothesis that prestimulation pulsation will result in increased daily milk yield is rejected. Furthermore, there was increased odds of deteriorated udder score after prestimulation pulsation compared to before treatment with prestimulation pulsation. The hypothesis that prestimulation pulsation decreases deterioration of teat end conditions is therefore rejected.

A study by Watters et al. (2015) found that milk yield/milking was significantly (P<0.05) higher when milk clusters were attached immediately without any wash or premilking compared to both manual and mechanical prestimulation. This agrees with findings of the current experiment, where no prestimulation tended to give greater daily milk yield and milk yield/milking. However, in contrast to experiment 1, Watters et al. (2015) saw that milk flow rate was higher when cows had been prestimulated either manually and mechanically compared to no prestimulation. This is in contrast to experiment 1, where milk flow/milking was reduced after prestimulation. In addition, another study by Watters et al. (2012) states that the lag time between prestimulation and milk cluster attachment is crucial in order to decrease total milking time/milking and increase the amount of milk harvested in the first 2 min of milking.

During experiment 1, employees pointed out that milk clusters fell off during prestimulation pulsation and had to be reattached. They experienced it as if the milk clusters did not probably grip around the teat and had difficulties in maintaining the grip, perhaps owing to the fast prestimulation pulsation. This could happen both in beginning of milking but also later, which in some cases resulted in an interrupted milking session both for the concerned cow and other cows waiting for first attachment of milk clusters. However, these problems did not seem to have affected total milking time/milking, since this was not affected by prestimulation pulsation.

The lack of effect of prestimulation pulsation on milk flow/milking may be due to bimodality. Since bimodal milk flow is a drop of milk flow in the beginning of milking, bimodality may increase total milking time/time, thereby decreasing milk flow. If bimodal milk flow is the case in this dairy herd, prestimulation should be able to stimulate milk ejection reflex and ensure continuous milk let down through the milking. However, owing to problems with milk clusters falling off in experiment 1, milk ejection reflex may not have been stimulated sufficiently and bimodality would still be the case in the herd. This could be the reason that milk flow/milking and total milking time were not affected by the treatment.

Odds of deteriorated udder score increased after prestimulation pulsation compared to before. This could be due to the fact, that milk clusters did not get a proper grip around the teat, but also that the liner would collapse faster around the teats during the prestimulation pulsation. It might be possible, that these machine factors deteriorated the teats to a greater extent. Since there was no control group of cows, it is impossible to determine if the increased odds of deteriorated udder score were caused by machine factors or natural factors as change in stage of lactation or weather conditions. However, according to Mein et al. (2001), premilking udder preparations may increase roughness of teat end, which could also be the reason for increase of odds of 'bad' udder score in this experiment.

#### 5.2. Experiment 2 – milk flow-controlled b-phase pulsation

Experiment 2 investigated the effect of milk flow-controlled b-phase pulsation on total milking time/milking. There was no effect of treatment on total milking time/milking for either primi- or multiparous cows. Therefore, the hypothesis that milk flow-controlled b-phase pulsation can reduce total milking/milking is rejected. However, daily milk yield and milk yield/milking increased significantly (p<0.01) after treatment for primiparous cows. Odds of deteriorated udder score increased after milk flow-controlled b-phase pulsation and the hypothesis on decreased odds of deteriorated udder score is rejected.

A similar study by Ambord & Bruckmaier (2009) reported no difference in total milk yield and main milking time when investigating milk flow-controlled b-phase. In terms of total milking time/milking, the study agrees with the findings of experiment 2. However, experiment 2 is in contrast to the results by Ambord & Bruckmaier (2009), since daily yield and milk yield/milking increases significantly (P<0.01) for primiparous cows after treatment in experiment 2. In addition, Ambord & Bruckmaier (2009) found that milk flow only increased for cows that already showed high milk flow under standard conditions and as long as milk was available in cisternal cavities when investigating milk flow-controlled b-phase. Since cows in experiment 2 were not grouped by high or low milk flow prior to the experiment, it was not possible to determine if changes in milk flow/milking for multiparous cows was caused by milk flow-controlled b-phase pulsation.

The effect of increased DIM on daily milk yield and milk yield/milking was in accordance with the literature. The reviewed literature states, that milk yield is reduced through lactation and that multiparous cows have a greater milk yield and milk flow compared to primiparous cows. However, increase of DIM only reduced milk flow/milking for multiparous cows, and increase of DIM did not affect total milking time/milking for either multi- or primiparous cows. This is in contrast to finding of the reviewed literature.

Milk flow-controlled b-phase pulsation was expected to reduce total milking time/milking owing to adaption to the milk flow. If pulsation allows milk to be retrieved faster from the udder during increased milk flow, it could be possible that milking time was shortened. In experiment 2, there is only a numerically reduction of total milking time/milking for both primi- and multiparous cows.

Treatment with milk flow-controlled b-phase pulsation increased odds of deteriorated udder score compared to no milk flow-controlled b-phase pulsation, which suggests that this milk machine setting deteriorates the teat ends to a greater extent than no treatment. This could be caused by the increase of pulsation cycles/min when milk flow is above 2000 g/min, because cluster liners will then collapse faster around the teat and thereby create a beating effect on the teat. Another reason could be because of extended b-phase when flow is below 2000 g/min, because teats have a decreased amount of time without pressure and rest. Or perhaps a combination of these two reasons could be the explanation for increased odds of deteriorated udder score after treatment. However, a fourth reason for increased odds of deteriorated udder scores after treatment with milk flow-controlled b-phase pulsation could

be, that teats undergo a natural deterioration owing to milking in general and this is what the results describes. This will be discussed to a greater extent later in the thesis.

Based on the discussion for experiment 2, milk flow-controlled b-phase pulsation do not reduce total milking time/milking, and odds of deteriorated udder score is not reduced. However, milk efficiency increases for primiparous cows because milk yield increases without changes in milking time and milk flow.

#### 5.3. Experiment 3 – fixed take-off time

Experiment 3 investigated the effect of fixed take-off time on milk flow/milking. There was a significantly (P<0.01) increase of milk flow/milking after treatment for multiparous cows. Therefore, the hypothesis that fixed take-off time increases milk flow/milking is accepted for multiparous cow, but not for primiparous cows. Odds of deteriorated udder score decreased after treatment and the hypothesis that fixed take-off time decreases odds of deteriorated udder score is accepted.

A study by Clarke et al. (2004) investigated the effect of fixed ACR time on milk yield and milking time in dairy cattle. The study found that milking time was reduced with 26-30 % when ACR time was set as mean milking duration for the 5<sup>th</sup> slowest milking cow in a group of 16 cows, which was between 8.7 and 10.5 min. The milk yield was not significantly affected by the reduction of milking time (Clarke et al., 2004). However, if milking time is reduced in the study by Clarke et al. (2004) and milk yield is unaffected, then milk flow must have increased, which agrees with findings of experiment 3 for multiparous cows.

Results show that fixed take-off time did not affect total milking time/milking significantly for either primi- or multiparous cows. But for multiparous cows, there was a numerically increase of 2.57 min in total milking time despite fixed take-off time. This is contrary to the idea of fixed take-off time. However, the increase in total milking time/milking could explain the reason that daily milk yield and milk yield/milking increases significantly (P<0.01) for multiparous cows. Milk yield possibly increases because multiparous cows are milked 2.57 min more after treatment. Additionally, milk flow/milking increases significantly (P<0.01) for multiparous cows after treatment, which suggest that milk efficiency improves through increased milk yield and milk flow despite longer total milking time/milking. However, the significantly (P<0.01) increase in daily milk yield and milk yield/milking for primiparous cows despite no changes in total milking time/milking and milk flow/milking could indicate

that primiparous cows empty their udders to a greater extent or that milk production simply is increased because of treatment. This also suggests, that milk efficiency for primiparous cows are improved through increased milk yield.

Before the experiment, 95 % of the cows in the herd had a total milking time/milking below 6 min. However, since the experiments were carried out in agreement with farmers preferences and willingness to take risks, a fixed take-off time of 8 min was agreed on. This suggests, that an even further reduction of fixed take-off time could be possible in the herd without decrease in milk yield.

Milk machine settings from experiment 2 with milk flow-controlled b-phase pulsation was carried over to experiment 3 as a permanent setting. Because of that, it is not possible to say if results of experiment 3 are caused exclusively by fixed take-off time or if an interaction between milk flow-controlled b-phase pulsation and fixed take-off time is the reason for increase in milk production.

Odds of deteriorated udder score decreased after treatment with fixed take-off time compared to before treatment. This indicates that fixed take-off time could be gentler to teat ends, or that the development seen in teat end condition is the natural development of teat end condition. According to Mein et al. (2001) increased machine-on time increases overmilking which can cause deteriorated teat ends. Thus, the study agrees with results of experiment 3.

Based on this, fixed take-off time do no reduce total milking time/milking but improves milk efficiency through increased milk yield for primiparous cows, and increased milk yield and milk flow for multiparous cows.

#### 5.4. Teat end condition score and methods

Normally, OR calculations are used in case-control studies where two similar groups participate. One group is exposed to e.g. a treatment, whereas the other group works as a control and therefore are not exposed. In this way, it is possible to calculate the odds of a specific outcome for both the exposed and non-exposed group and compare these to outcomes in an OR. Furthermore, it allows to investigate if a certain outcome is caused by the exposure. In these three experiments, there was no control group to compare with, thus

no knowledge about udder score if cows were not exposed to treatment. This means, that the relation between exposure and outcome may not uniquely be caused by treatment, which the OR calculations are not able to consider.

Calculations of OR were made under the assumption that only experimental treatment could be the reason for worsening of teat end condition. However, it is unknown if other factors could have affected teat end condition negatively. In terms of that, a study by Neijenhuis et al. (2000) states that teat end condition can be affected by teat end shape, parity, DIM and milk yield. Additionally, during scorings of teat end condition, it was stated by a present milk quality advisor, that some teats was infected with a wart-like condition on teat and teat end, which made it difficult to determine teat end score. It is unknown, if the development of this condition affected teat end condition toward a score 5. If that is the case, then odds of 'bad' udder score is not exclusively a result of milk machine setting. Further and more specific investigations may be needed in order to establish the causality between the three specific milk machine settings and teat end condition.

In conclusion, teat end condition scores and udder scores may be used as an indicator of problems in milk machine settings. However, it is not possible to conclusively determine, what changes in teat end condition is caused by.

#### 5.5. Other considerations

The longitudinal experimental design was chosen in order to measure the effect of treatments in a dairy herd, also called a longitudinal cohort study. This means, that the same variables for the same animals were observed over a period of time, and that all animals were exposed to the same treatment. This was done, since it was the only possibility to conduct an experiment in the current herd. Furthermore, a longitudinal experiment allows to follow change over time. However, this experimental method can cause problems with animals leaving the herd before experiment is ended. This could e.g. be due to calving or culling. Where culling could be caused by the treatment, which is therefore not expressed in results.

Milk yield is often expressed as kg of ECM, which describes the content of protein and fat in milk. Additionally, farmers are paid by dairies based on ECM delivered. An increase in milk yield is often more interesting if it is seen as increase in kg ECM, since this in the end can increase the farmer's income from milk. However, in these three experiments milk yield is only expressed as kg milk and the value of the increase is therefore unknown. However, no literature reviewed reports on changes in milk composition owing to changes in milk machine settings. Therefore, the effect of treatments on milk yield is assumed to be of value for the farmer.

In general, the employees on the farm seemed to have different perceptions of the executions of procedures. Hence, total duration of milking sessions varied from 3 to 5.5 hour for the 6 visits to the farm where milkings where observed. For some employees it was observed, that they paused the rotary in order to smoke or drink coffee. This prolonged the duration of the milking session to some extent and the effect of this on results is unknown.

The experimental period took place during autumn, winter and spring, which in Denmark can cause great variation to the weather. During winter, there were problems with leakage of water pipes in the barn, which caused 30-40 cm of water for the cows to walk in. That meant, that cows in the waiting area prior to rotary, stayed in water without chance to move for up to 1 hour. The effect of this on general health and udder health is unknown.

Overall, despite different weaknesses and possible errors in the three experiments, it is a strength that the experiments have been tested in a commercial farm. Because it proves that the positive results of treatments are possible to obtain for other farmers, and that changes owing to milk machine settings potentially can improve milk efficiency despite variation in herd composition and milking management.

## 6. Conclusion

The objective of this thesis was to investigate if three specific milk machine settings had a positive effect on daily milk yield, milk yield/milking, total milking time/milking and milk flow/milking in order to obtain a more efficient milking. In addition, it was investigated if milk machine settings had a positive effect on udder score.

Experiment 1 investigated the effect of prestimulation pulsation on milk yield and found that daily milk yield and milk yield/milking was not affected by prestimulation pulsation for either primi- or multiparous cows. Furthermore, odds of deteriorated udder score increased after prestimulation pulsation. Based on this, prestimulation pulsation does not improve milk efficiency or udder score.

Experiment 2 investigated the effect of milk flow-controlled b-phase pulsation on milking time and found that milking time was not affected by milk flow-controlled b-phase pulsation. However, milk efficiency was improved through increased milk yield for primiparous cows. In addition, the odds of deteriorated udder score increased after milk flow-controlled b-phase pulsation.

Experiment 3 investigated the effect of fixed take-off time on milk flow and found that milk flow increased after treatment for multiparous cows. In addition, milk efficiency was improved through increased milk yield for primiparous cows, and increased milk yield and milk flow for multiparous cows. Additionally, odds of deteriorated udder decreased after fixed take-off time.

## 7. Perspectives

In order to validate the positive results of fixed take-off time, further research is recommended. A case-control study could reveal if effects are actually caused by treatment or perhaps other factors. In general, an economic analysis of results in terms of costs of labour may contribute to the decisions on implementation of fixed take-off time. Furthermore, fixed take-off time should be tested on several farms in order to see if the treatment depends on other herd factors such as ECM/cow/year, breed, cows/year, etc.

The effect of DIM on production results after milk machine setting experiments could be examined further. SEGES has made standard lactation curves for cows in different parities, which predict the milk yield for a cow in a specific lactation stage. These lactation curves could have been compared to lactation curves for cows in the experiment, that received a milk machine setting treatment. Comparison of the differences between 'normal' and 'treatment' lactation curves could reveal, if changes in milk yield is caused by treatment or normal development in lactation. Furthermore, a consideration of the milk yield of the individual cow in the statistical analysis could contribute to better description of data.

Further investigations of teat end condition through the life cycle of a cow could contribute into a better understanding of teat end condition. If standard development of teat end condition could be described, this could be compared to the development seen after experiments, which perhaps could determine if milk machine setting treatments caused damage to teat end condition. Alternative, a logistic regression for teat end condition could reveal the effect of other factors on teat end condition.

## 8. References

- Ambord, S., and R.M. Bruckmaier. 2009. Milk flow-controlled changes of pulsation ratio and pulsation rate affect milking characteristics in dairy cows. J.Dairy Res. 76:272– 277. doi:10.1017/S0022029909003963.
- Bade, R.D., D.J. Reinemann, M. Zucali, P.L. Ruegg, and P.D. Thompson. 2009. Interactions of vacuum, b-phase duration, and liner compression on milk flow rates in dairy cows.
  J. Dairy Sci. 92:913–921. doi:10.3168/jds.2008-1180.
- Berry, D., B. Coughlan, B. Enright, S. Coughlan, and M. Burke. 2013. Factors associated with milking characteristics in dairy cows. J. Dairy Sci. 96:5943–5953. doi:10.3168/jds.2012-6162.
- Bruckmaier, R.M. 2005. Normal and disturbed milk ejection in dairy cows. Pages 268–273 in Domestic Animal Endocrinology.
- Bruckmaier, R.M., and J.W. Blum. 1996. Simultaneous recording of oxytocin release, milk ejection and milk flow during milking of dairy cows with and without prestimulation.J. Dairy Res. 63:201. doi:10.1017/S0022029900031708.
- Bruckmaier, R.M., and M. Hilger. 2001. Milk ejection in dairy cows at different degrees of udder filling. J. Dairy Res. 68:369–376. doi:10.1017/S0022029901005015.
- Brusgaard, L. 2017. Arla: Priserne vil svinge. BOVI.
- Clarke, T., E.M. Cuthbertson, R.K. Greenall, M.C. Hannah, E. Jongman, and D. Shoesmith. 2004. Milking regimes to shorten milking duration.. J. Dairy Res. 71:419–426. doi:10.1017/S0022029904000421.
- Dzidic, A., J. Macuhova, and R.M. Bruckmaier. 2004. Effects of Cleaning Duration and Water Temperature on Oxytocin Release and Milk Removal in an Automatic Milking System. J. Dairy Sci. 87:4163–4169. doi:10.3168/jds.S0022-0302(04)73559-6.
- Edwards, J.P., J.G. Jago, and N. Lopez-Villalobos. 2014. Analysis of milking characteristics in New Zealand dairy cows. J. Dairy Sci. 97:259–269. doi:10.3168/jds.2013-7051.
- FAO. 2018. Milk Hygiene. Accessed. http://www.fao.org/docrep/004/T0218E/T0218E03.htm.
- Ferneborg, S., and K. Svennersten-Sjaunja. 2015. The effect of pulsation ratio on teat condition, milk somatic cell count and productivity in dairy cows in automatic milking.J. Dairy Res. 82:453–459. doi:10.1017/S0022029915000515.
- Gleeson, D.E., E.J. O'Callaghan, and M. V Rath. 2004. Effect of liner design, pulsator setting, and vacuum level on bovine teat tissue changes and milking characteristics as

measured by ultrasonography. Ir. Vet. J. 57:289–296. doi:10.1186/2046-0481-57-5-289.

- Hillerton, J.E., J.W. Pankey, and P. Pankey. 2002. Effect of over-milking on teat condition.J. Dairy Res. 69:81–84. doi:10.1017/S0022029901005386.
- Husvéth, F. 2011. Physiological and Reproductional Aspects of Animal Production. Accessed July 26, 2018. https://www.tankonyvtar.hu/en/tartalom/tamop425/0010\_1A\_Book\_angol\_05\_termel eselettan/ch14s06.html.
- ISO. 2007. INTERNATIONAL STANDARD ISO Milking machine installations.
- Jago, J.G., J.E. McGowan, and J.H. Williamson. 2010. Effect of setting a maximum milking time, from peak lactation, on production, milking time and udder health. N. Z. Vet. J. 58:246–252. doi:10.1080/00480169.2010.69298.
- Kaskous, S., and R.M. Bruckmaier. 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.. J. Dairy Res. 78:97–104. doi:10.1017/S0022029910000816.
- Magnusson, M., A. Christiansson, B. Svensson, and C. Kolstrup. 2006. Effect of different premilking manual teat-cleaning methods on bacterial spores in milk. J. Dairy Sci. 89:3866–3875. doi:10.3168/jds.S0022-0302(06)72429-8.
- Mein, G.A., F. Neijenhuis, W.F. Morgan, D.J. Reinemann, J.E. Hillerton, J.R. Baines, I. Ohnstad, M.D. Rasmussen, L. Timms, J.S. Britt, R. Farnsworth, N. Cook, and T. Hemling. 2001. Evaluation of Bovine Teat COndition in Commerical Dairy Herds: 1. Non-Infectious Factors. Proc. 2nd Int. Symp. Mastit. Milk Qual. 2:347.
- Neijenhuis, F., H.W. Barkema, H. Hogeveen, and J.P.T.M. Noordhuizen. 2000. Classification and Longitudinal Examination of Callused Teat Ends in Dairy Cows. J. Dairy Sci. 83:2795–2804. doi:10.3168/jds.S0022-0302(00)75177-0.
- Neijenhuis, F., H.W. Barkema, H. Hogeveen, and J.P.T.M. Noordhuizen. 2001. Relationship Between Teat-End Callosity and Occurrence of Clinical Mastitis. J. Dairy Sci. 84:2664–2672. doi:10.3168/jds.S0022-0302(01)74720-0.
- Nørstebø, H., A. Rachah, G. Dalen, O. Rønningen, A.C. Whist, and O. Reksen. 2018. Milkflow data collected routinely in an automatic milking system: An alternative to milkingtime testing in the management of teat-end condition?. Acta Vet. Scand. 60. doi:10.1186/s13028-018-0356-x.

Pfeilsticker, H.-U., R.M. Bruckmaier, and J. rg W. Blum. 1996. Cisternal milk in the dairy

cow during lactation and after preceding teat stimulation. J. Dairy Res. 63:509–515. doi:doi:10.1017/S0022029900032040.

- Progressive Dairyman. 2017. The Four Phases of the Pulsation Cycle Make up Liner Open and Close Times for Optimal Milk Harvest. Accessed April 11, 2018. https://www.progressivedairy.com/images/downloads/2017/04/20/0817pd-walker-fg-1.pdf.
- Rasmussen, M.D. 2004. Overmilking and Teat Condition. NMC Annu. Meet. Proc. 169–175.
- Rasmussen, M.D., E.S. Frimer, and E.L. Decker. 1994. Reverse Pressure Gradients Across the Teat Canal Related to Machine Milking. J. Dairy Sci. 77:984–993.
- Sandrucci, A., A. Tamburini, L. Bava, and M. Zucali. 2007. Factors Affecting Milk Flow Traits in Dairy Cows: Results of a Field Study. J. Dairy Sci. 90:1159–1167. doi:10.3168/jds.S0022-0302(07)71602-8.
- SEGES. 2018. DMS.
- Sjaastad, Ø. V., O. Sand, and K. Hove. 2010. Physiology of Domestic Animals. 1st ed. Scandinavian Veterinary Press.
- Szumilas, M. 2010. Explaining odds ratios. J. Can. Acad. Child Adolesc. Psychiatry 19:227–229. doi:10.1136/bmj.c4414.
- Tamburini, A., L. Bava, R. Piccinini, A. Zecconi, M. Zucali, and A. Sandrucci. 2010. Milk emission and udder health status in primiparous dairy cows during lactation. J. Dairy Res. 77:13–19. doi:10.1017/S002202990990240.
- Tančin, V., B. Ipema, P. Hogewerf, and J. Mačuhová. 2006. Sources of Variation in Milk Flow Characteristics at Udder and Quarter Levels. J. Dairy Sci. 89:978–988. doi:10.3168/jds.S0022-0302(06)72163-4.
- Vetter, A., H.A. van Dorland, M. Youssef, and R.M. Bruckmaier. 2014. Effects of a latency period between pre-stimulation and teat cup attachment and periodic vacuum reduction on milking characteristics and teat condition in dairy cows. J. Dairy Res. 81:107–112. doi:10.1017/S0022029913000617.
- Watters, R.D., R.M. Bruckmaier, H.M. Crawford, N. Schuring, Y.H. Schukken, and D.M. Galton. 2015. The effect of manual and mechanical stimulation on oxytocin release and milking characteristics in Holstein cows milked 3 times daily.. J. Dairy Sci. 98:1721–9. doi:10.3168/jds.2014-8335.
- Watters, R.D., N. Schuring, H.N. Erb, Y.H. Schukken, and D.M. Galton. 2012. The effect of premilking udder preparation on Holstein cows milked 3 times daily.. J. Dairy Sci.

95:1170-6. doi:10.3168/jds.2011-4388.

Weiss, D., A. Dzidic, and R.M. Bruckmaier. 2003. Effect of stimulation intensity on oxytocin release before, during and after machine milking. J. Dairy Res. 70:349–354. doi:10.1017/S0022029903006216.