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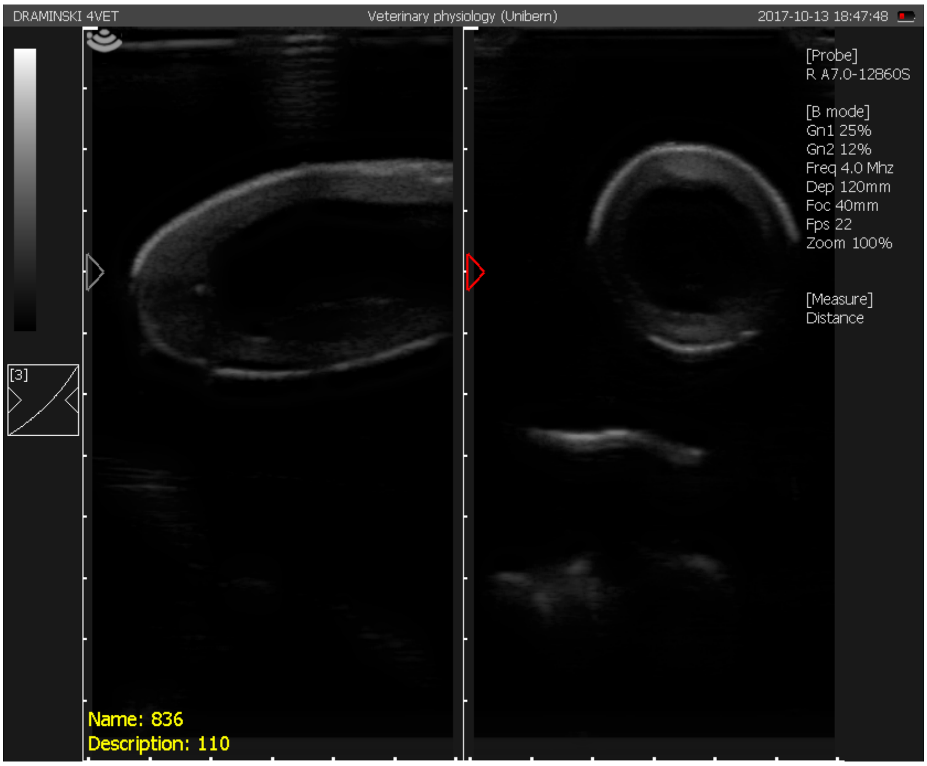

Department of Animal Nutrition and Management
Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, 647
Degree project 30 credits
Uppsala 2018

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Credits: 30 credits
Level: A2E
Course title: Degree project in Animal Science
Course code: EX0551
Course coordination department: Department of Animal Nutrition and Management
Programme: Animal Science

Place of publication: Uppsala
Year of publication: 2018
Cover picture: Jenny Löfstrand
Title of series / Number of part of series: Examensarbete/Sveriges lantbruksuniversitet, Institutionen för husdjurens utfording och vård, 647
Online publication: https://stud.epsilon.slu.se

Keywords: Teat changes, teat wall thickness, teat density, ultrasound, cutimeter, IMP

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Abstract

Udder health has a strong effect on profitability in milk productions. Udder diseases, especially mastitis, are among the most costly diseases and has a major economic impact on dairy production as well as it is an animal welfare issue. Mastitis can occur if bacteria enters the teat canal causing an infection. The teat has different mechanisms to protect itself from bacteria but changes in the teat condition can impair the defense. Milking machines has been seen to cause temporary changes in the teats after milking. Different changes have been observed by many scientists and in this study, focus was pointed on teat wall and teat density thickness. Teat wall thickness and teat tissue density increased after milking which is assumed to be due to accumulation of fluids in the teat defined as congestion. Temporary changes in the teat caused by machine milking has been seen to impair udder health and significantly increase microbial colonization of the teat canal. The results from this study showed that with routine milking the teat recovers from congestion within 30 minutes. After the 30 minutes the teat wall kept decreasing until next milking due to the increase of intramammary pressure. Teat changes can be measured with different methods and in this study, the measurements from a cutimeter were compared to the measurements from an ultrasound machine. The conclusion is that ultrasonography appears to be more sensitive to small variations and was able to reveal teat wall changes caused by both congestion and intramammary pressure. The cutimeter method was more easily implemented but did in this study only reveal teat density changes caused by congestion after milking.

Sammanfattning

För att bibehålla en lönsam mjölkproduktion är god juverhälsa av stor betydelse. Juversjukdomar är de hälsoproblem hos mjölkkor genom orsakar störst förluster i mjölkmängd och därmed lönsamhet på gården. Mastit är den vanligaste juversjukdomen och den kan orsakas av bakterier som tar sig in i juvret via spenkanalen. Spenkanalen har normalt sett ett naturligt skydd mot bakterier men förändringar i spenvävnaden kan försämra spenens eget försvar. Mjölkning med mjölkmaskin har i tidigare studies observerats ge tillfälliga förändringar av spenarna efter mjölkning. Olika spenförändringar har studerats av ett flertal forskare och i den här studien låg fokus på att undersöka spenväggarna och spenvävnadens densitet. Spenväggarnas tjocklek och spenvävnadens densitet ökar efter mjölkning vilket antas bero på ansamling av vätska i spenen. Tillfälliga spenförändringar orsakade av maskinmjölkning har setts försämra juverhälsan och signifikant öka mikrobiell tillväxt i spenkanalen. Vid normal mjölkning återhämtar sig spenen från svullnaden som uppstod i och med vätskeansamlingen, inom 30 minuter efter mjölkning. Spenväggen fortsätter sedan att minska i och med det ökade intrammamära trycket. Spenförändringar kan mätas med flera olika metoder och i den här studien genomfördes mätningar med en cutimeter och med en ultraljudsmaskin. Slutsatsen är att ultraljudsmetoden visade sig ge mer märkbara utslag även på små förändringar i spenväggen och uppvisade förändring orsakade både av vätskeansamling och intramammärt tryck. Cutimetermetoden var mer lättanvänd men uppvisade i den här studien endast densitetsförändringar orsakade av vätskeansamling och påverkades inte av intramammärt tryck.

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Introduction

A healthy udder is one of the main preconditions in how to maintain a profitable dairy production. The most common reason for udder diseases in dairy cows is mastitis, which also is the disease that has the strongest impact on the production economy (Halasa *et al*., 2007; Ruegg, 2012). Most cases of mastitis are caused by bacterial infection after milking when the teat canal is open and bacteria can enter and spread to the mammary gland. To protect the teat canal from bacteria entering and prevent infections it is important that the milking does not influence the teat in a negative way (Main, 2012). Many variables affect the udder health and especially during milking the udder and teats are exposed to strain from the milking machines leading to temporary changes in the teat wall thickness (Hamann & Stanitzke, 1990; Hamann & Mein 1990; Neijenhuis *et al*., 2001). This thickening reaction might also be connected to other negative teat conditions and may impair the udder health (Hamann & Mein, 1990; Zecconi *et al*., 1992; Zwertvaegher *et al*., 2013 and Neijenhuis *et al*., 2001). Multiple studies have been done to compare teat thickness before and after milking but fewer have been investigating the fluctuations of the teat thickness during the interval between two milkings. This is important in order to increase the understanding of what the impact from the milking machine is and what the impact from the IMP (intramammary pressure) is. A measurement right before milking could be affected by the previous milking or the IMP and this should be considered when comparing measurements. With more knowledge about the teat tissue and its reactions it could be possible to improve udder health which in turn will generate a positive effect on animal health and welfare as well as the production economy.

The goal of this study were to find out how the strain of the milking machines affects the teat and how the teat recovers. In order to facilitate for future research two different methods for making measurements were used, ultrasound measurements and cutimeter measurements, with the purpose of evaluating how compatible they were and if they differed in fields of application, Hamann & Stanitzke (1990) performed a study to evaluate the teats recovery after milking using a different method than this study and concluding the recovery time was longer than 30 minutes. This study investigates the recovery time further with different methods to get a more detailed image of the teat reactions.

The hypotheses were that the teat wall thickness and teat density would temporarily increase during milking and that the teat would be completely recovered from one milking to the following and that the IMP could have an effect on the teat leading to decreased teat wall thickness.

The objective was to, with help of this experiment, increase the understanding of the reaction on the teat wall after milking as well as the natural fluctuation during the day. With this knowledge, it might then be possible to further improve and adapt the milking machines and settings to reduce any negative effect of the milking machine on the teat.

Literature Review

Cows have successfully been bred to produce and store large amounts of milk.

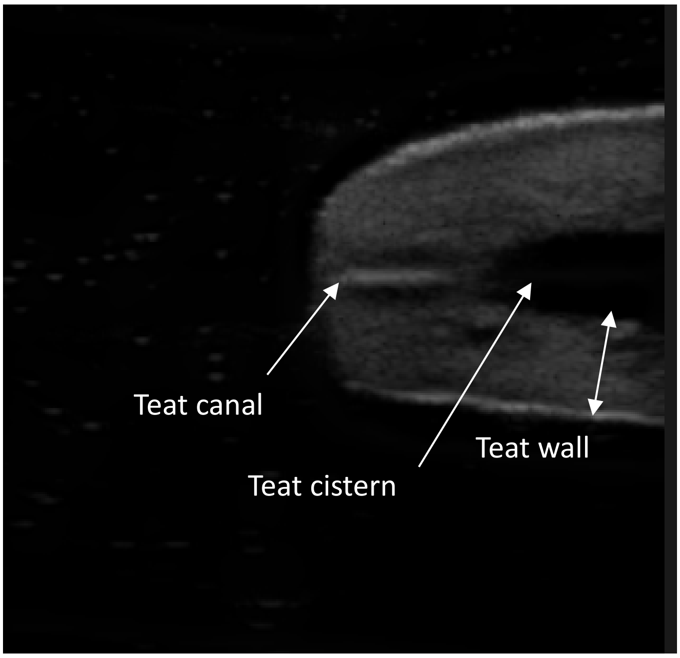

Figure 1) The image shows the basic anatomy of the teat which is mentioned repeatedly in the article

The main fraction of the milk (>80 %) is stored in the alveolar and the smaller fraction (< 20 %) is stored in the udder and teat cistern (Bruckmaier *et al.*, 1994). After milking when the udder has been emptied, the IMP is at its lowest point. As the milk starts to fill the udder again the IMP grows until the next milking where it is further increases by the release of oxytocin (Bruckmaier *et al.*, 1991) which occurs naturally by the prestimulation.

The teat has a dynamic tissue and has continuous visible rhythmic contractions during the day (Hamann & Mein, 1990). Teats also reacts to stimuli such as a light touch and changes in temperature (Hamann & Mein, 1990). Machine milking is known to cause contraction of the teat and thickening of the teat wall which can be interpreted as a non-natural reaction and may impair udder health (Hamann & Mein, 1990; Zecconi *et al.*, 1992; Zwertvaegher *et al.*, 2013; Neijenhuis *et al.*, 2001).

Teat reaction with machine milking compared to calf suckling

To clarify the mechanical load on the teats caused by the milking machine, compared to the response to a suckling calf Hamann & Stanitzke (1990) performed a study comparing these two events. Calf suckling measurements were described as the "biological reference values" and showed in opposite of machine milking a decreased teat diameter (measured with cutimeter) during and right after milking. It thereafter took between 15-30 minutes before the teat diameter returned to premilking value. During machine milking the teat diameter increased and the recovery time exceeded the 30 minutes of observation (Hamann & Stanitzke, 1990).

Machine milking was concluded to cause greater increases in teat diameter than calf suckling (Hamann & Stanitzke, 1990).

Different methods for measuring the teats´ reaction to machine milking

Over time different methods have been used to measure teat changes. One common method has been to use cutimeter (Hamann & Mein, 1988; Hamann & Stanitzke, 1990; Isaksson & Lind, 1992; Ambord & Bruckmaier, 2010) which can be constructed with or without a spring load. A cutimeter can be used for measurements of the teat diameter and equipped with a spring load it can also be used for density measurements of the teat tissue. With the spring, the cutimeter measurements reflects the density of the teat wall while the cutimeter without a spring load only gives information about the diameter. The measurements from a spring loaded cutimeter can vary depending on what pressure is applied by the cutimeter (Hamann & Mein, 1990).

An alternative method for teat tissue measurements is ultrasonography that produces images of the teat which then can be used for obtaining measurements (Bruckmaier & Blum, 1991; Neijenhuis *et al*., 2001; Ambord & Bruckmaier, 2010; Penry *et al*., 2017). With this method teat wall thickness can be examined and also measurements of the teat cistern, teat canal and vessels in the teat can be made (se figure 1).

Ambord and Bruckmaier (2010) compared measurements from a spring loaded cutimeter and from ultrasound images in a study on different vacuum levels in highline milking systems. They found that there was a correlation between the two methods.

Milking machine related changes of the teat and the consequenses

After machine milking, the teat diameter is in many cases changed compared to before milking (Hamann & Stanitzke, 1990; Isaksson & Lind, 1992; Hamann *et al*., 1993) and the same goes for teat wall density (Hamann & Mein, 1988, 1990) and teat wall thickness (Neijenhuis *et al*., 2001; Pařilová, *et al*., 2011). The time it takes for the thickening of the teat to return to premilking measurements has been investigated in different studies. Mein and Hamann (1988) found that it took between 1-4 hours for the teat wall density to return to premilking values (measured with a cutimeter) depending on different milking techniques. In the study by Neijenhuis *et al*. (2001) the recovery time of the teat wall thickness directly after milking until the teat reached premilking value was 6 hours (measured with ultrasound machine). The recovery time is important to know since the action of the milking machine on a non-recovered teat could lead to irreversible chronical changes in the teat tissue (Hamann & Østerås, 1994).

Machine milking induced teat wall thickening is caused by congestion and many believes it to also be caused by oedema. The reason for the development of congestion and oedema is the action of the milking machine which cause accumulation of blood and lymph fluids in the teat end and thereby thickening of the teat (Hamann & Mein, 1990; Hamann & Stanitzke, 1990). Congestion is defined as an intravascular accumulation of fluids which also is a precondition for the development of oedema which is defined as an extravascular accumulation of fluids

(Hamann & Mein, 1990). Congestion and oedema could weaken the body's immune defense by impairing the blood circulation and thus slow down the closure of the teat canal after milking (Hamann & Østerås, 1994). This would leave the teat canal open for bacterial entrance. Zecconi *et al*. (1992) found that machine milking induced teat thickness of more than 5 % (measured with a cutimeter) leads to a significantly increased microbial colonization of the teat duct. This conclusion was supported by Zwertvaegher *et al*. (2013) who discovered a significant correlation between changes in the teat barrel diameter after milking with increased somatic cell count. Machine milking causes thickening of the teat wall but the teat diameter can also change. In different studies teat diameter changes postmilking has been seen for mainly increased diameter (Hamann & Stanitzke, 1990; Isaksson & Lind, 1992), mainly decreased diameter (Hamann *et al*., 1993; Pařilová, *et al*., 2011; Guarin & Ruegg, 2016) or for both increases and decreases in different animals (Zwertvaegher *et al*., 2013). The reason for an increased diameter after milking could be because of the congestion and possibly oedema, while a decreased teat diameter could be an effect of reduced IMP in the teat (Hamann *et al*., 1993). Zwertvaegher *et al*. (2013) found that cows whose teat diameter increased had an elevated risk of high somatic cell count (SCC) compared to cows whose teat diameter had decreased after milking. High SCC levels is known occur for example when the udder is infected, and the udder health is impaired. SCC is therefor used regularly as an indication of udder health. Milking machine related teat diameter changes for the animals who had an increased diameter postmilking was related to higher SCC (Zwertvaegher *et al*., 2013). Even though the relation was established it is still not believed a too unlimited decrease in diameter is optimal either. It is expected to be a threshold for when also a decrease in diameter could impair the udder health (Zwertvaegher *et al*., 2013). Changes in the teat tissue after milking are assumed to affect the teat canal in a way that increases the risk of bacteria entering which can lead to intramammary infection of the udder (Neijenhuis *et al*., 2001). Thicker teats were likely to have a greater degree of congestion and oedema which according to Hamann and Østerås (1994) may impair the capacity of the teat to act as a barrier against pathogen invasion.

However, not every study has been able to prove any negative effects of milking machine induced teat changes. One recent study investigated anatomical teat conditions related to clinical mastitis and found no association between changes in teat diameter after milking with increased risk of clinical mastitis (Guarin & Ruegg, 2016).

Machine settings, milking routines and cow traits that affects the degree of thickening

The action of the milking machine affects the condition of the teat duct and teat skin which can increase the risk of mastitis (Main, 2012). Different settings of the milking machine have been found to affect the teats in different ways. For example, milking vacuum (Hamann & Mein, 1990, 1988; Hamann *et al*., 1993; Bade *et al*., 2009; Pařilová, *et al*., 2011; Penry *et al*., 2017; Nørstebø *et al*., 2018), pulsation rate (Hamann & Mein, 1990), cluster removal time (Rasmussen, 1993; Pařilová, *et al*., 2011) and different teat cups (Hamann & Mein, 1990) generate changes in the teat condition after milking. An increased vacuum level generates a greater degree of teat tissue changes in the teat and it also increases the recovery time needed for the tissue changes to recover after milking (Hamann & Mein, 1988). During milking, vacuum is controlling whether the liner should be contracted, which is the massaging phase when milk is removed, or if the liner should be open (b-phase) which is when congestion can occure (Besier et al., 2015). Bade *et al*. (2009) concluded in their paper that an increase of vacuum, b-phase and liner contraction would likely lead to increased hyperkeratosis, increased postmilking teat-end congestion and possibly more open teat-ends postmilking. However, the level of increase in vacuum, b-phase and liner contraction used in this study was greater than what is normally seen in conventional practice. In a more recent study by Penry *et al*. (2017) they could also see negative effects of a high mouthpiece vacuum. With the use of an ultrasound machine they were able to show the relationship between increased mouthpiece vacuum and increased teat congestion after milking.

Cluster removal time can be a machine setting if automatic cluster removal is an option or otherwise a milking routine. Regardless of which, it can influence the teat health according to the study by Rasmussen (1993). He found that cluster removal at a higher milk flow decreased the teat thickness changes postmilking compared to premilking in first lactation cows. There was also a tendency for reduced clinical mastitis in older cows when cluster removal at a higher milk flow was practiced (Rasmussen, 1993). A more recent study by Pařilová, *et al*. (2011) also showed significant changes in the teat postmilking due to different vacuum and different milk flows at cluster removal. Lower vacuum and cluster removal at a higher milk flow combined generated less changes in teat diameter, teat length, teat wall thickness and teat canal length.

Nørstebø *et al*. (2018) observed that cows with a low milk flow had an impaired teat condition even with standard vacuum compared to cows with mid or high milk flow. An increased vacuum level would likely result in even more severe teat end condition (Nørstebø *et al*., 2018). Anatomical traits in cows teat can influence the milk flow rate and thereby the postmilking thickening. Weiss *et al*. (2004) could in their study see a reduced milk flow in cows with short teat canals.

Materials and Methods

The study was conducted at the Swiss Federal Research Station Agroscope in Posieux. The treatments were approved by the veterinary office of the Canton of Fribourg (2017-17-FR). The experiment took place from the 31st of August to the 13th of October 2017.

Animals and Housing

Twelve Holstein dairy cows (five Holstein Friesian and 7 Red Holstein) were used. The same cows were used for all treatments. Three cows were in their first lactation, four were in their second and six were in their third or higher lactation (the oldest cow was in her eighth lactation). They were between 123 and 384 DIM and produced between 17 and 28 kg milk per day. All cows were free from mastitis. During the experimental period the cows were kept in a tie-up stall. Between one and three cows were sampled at the same milking.

Milking

The cows were milked twice a day, once in the morning at 5.45 (13 h after previous milking) and once in the evening at 16.45 (11 h after previous milking). The milking was performed by the same person who also did the premilking and the measurements. The premilking (cleaning with a disinfectant tissue, forestripping and manual teat stimulation) lasted for 1 minute and after that the cluster was attached. The cows were milked with bucket milking and manual cluster removal was done with visual assessment at an estimated milkflow of 0.2 kg/min. The milking equipment which was used in the experiment was a standard bucket milker. Experimental milkings were performed with standard milking units (Cluster Uniflow 3 SS light; SAC, Kolding, Denmark) and standard liners (Uniflex, product no. 252.15.022; SAC) attached to a bucket milker. The system vacuum was 42 kPa.

Teat measurements

Two methods were used for evaluating changes on the teats; ultrasound cross-sectioning images allowed measurements of the teat wall thickness while cutimeter measurements provided information of teat tissue density measurements. One teat per cow were used for measuring and it was always the front left teat. The ultrasound images took about 30 seconds to make while the cutimeter measurement were performed in about 10 seconds.

Treatments

Five different treatments were included and every treatment was carried out during one morning and one evening milking for every cow meaning each cow had 10 milkings recorded within the study.

During these treatments, ultrasonic images and cutimeter measurement were made. At every measuring point for the ultrasonic measurements two images were taken, one longitudinal and one transverse. For the thickness measurements, a cutimeter was used. Also recorded for every milking was milking duration and yield.

The following treatments were carried out in randomized order for each animal.

Treatment 1) Detection of normal condition before milking

Ultrasound and cutimeter measurements were taken before and after milk let down. At the time of milking, prior to any premilking, one measurement was carried out. Then premilking was performed for 1 minute and the next measurements were carried out. Milking was then continued as normal routine.

Treatment 2) Detection of normal condition and change in teat thickness according to the normal routine milking

The premilking and milking routine was performed as normal. After milking measurements were taken continuously every five minutes starting directly after cluster removal and continuing for one hour. After one hour measurements were taken every ten minutes for an additional hour.

Treatment 3) Detection of teat thickness after prolonged milking time

After the milk flow decreases below 0.2 kg/min the milking unit is kept on for an additional five minutes. Measurements are then carried out for two hours as in treatment 2. During the morning milking measurements are, after the two hours, taken once every hour until next milking.

Treatment 4) Detection of teat thickness during milking

After two minutes of milking the cluster is removed and one measurement is taken. Milking is then carried out as normal.

Treatment 5) Detection of teat thickness during milking

After the milk flow rate has peaked and starts to noticeably decrease, the cluster is removed and one measurement is taken. The milking plateau was estimated to be at least more than 2 minutes into the milking to provide a different result from treatment 4. Milking is then carried out as normal.

For the last five cows in the study, two extra treatments were added. The first additional measurements were carried out for 2 hours like in treatment 2 but without the cutimeter. For the second additional treatment measurements were carried out three times, 0, 60 and 120 minutes after milking, both with the ultrasound and the cutimeter. The reasons for the additional treatments were to find out if frequent measuring points or use of the cutimeter affected the result.

Procedure of the ultrasonic examination

The ultrasonographic images were taken with a DRAMINSKI 4 Vet diagnostic ultrasound scanner (2015, Input: 100-240 VAC, 50-60 Hz, max 1,2 A, DRAMINSKI, ul.Owocowa 17, PL 10-860 Olsztyn, Poland) connected to a linear array 5 MHz probe. During scanning the teat was held in a plastic cup of hand-warm water and the probe was held on the outside of the cup moistened with contact gel. The described procedure allows teat scans to be made without deforming the teat. For the longitudinal images the probe was held lateral to the teat and for the transvers images the probe was held approximately two centimeters above the teat tip. This method is established and has been used for a long time (Bruckmaier & Blum, 1992). From the images teat diameter and teat wall thickness measurements (calculated by subtracting the diameter of the teat cistern from the teat diameter and dividing by two) were made. The diameter measurements were all made two centimeters above the teat tip on the longitudinal image.

Procedure of the cutimeter examination

The cutimeter measurements were made with a spring-loaded caliper as described by Hamann and Main (1988). The cutimeter measurements were performed after the ultrasound scans to not affect the images. In this experiment, the jaws of the cutimeter was placed approximately two centimeters above the teat tip.

Statistical Analyses

All data analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC) with cow treated as the experimental unit. A repeated measures mixed model analysis using the MIXED procedure was performed to examine the effects of cutimeter and ultrasound measurements. For the first set of data, the model included fixed effects as treatment (Before, AfterLD, 2Min, Plateau, After and Over) and morning or evening measurements. For the second set of data, fixed effects were treatment (normal or over milking), time, morning or evening measurements and their interactions. On both sets, cow was the specific term for the repeated statement and subject. A compound symmetry structure was used. The PDIFF option was used to separate least square means. A simple linear regression analysis using the REG procedure was performed to evaluate the relationship of cutimeter and ultrasound measurements. The model included time as fixed effect. All significance was declared at $P \leq 0.05$. Data are reported as least squares means ± SEM.

Results

There were no differences in teat wall thickness between morning and evening milking for any of the treatments and therefore morning and evening are not separated in the graphs.

With the ultrasound method, there were significant differences between measurements before milk let-down and after milk let-down ($p < 0.05$) as well as after milk let-down and 2 minutes into milking ($p < 0.001$). There was also a tendency for increased thickening between 2 minutes into milking and after the plateau ($p < 0.1$) and between the plateau and directly after milking ($p < 0.1$). From the plateau until directly after over milking the increase of teat wall thickness was significant (< 0.0001) (Figure 1). For the cutimeter method one significantly different measurement was found. The measurement directly after over milking differed from all the previous ones (*Figure 2*).

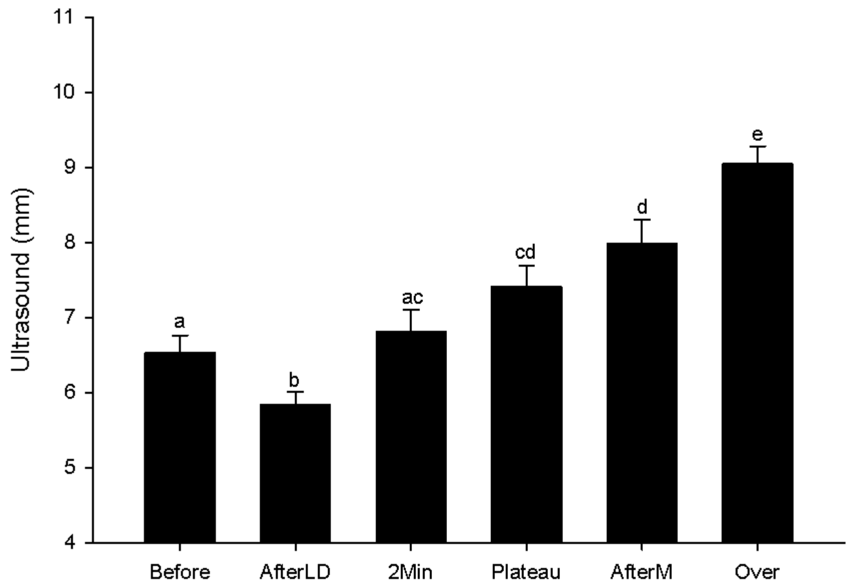

Figure 1)
The graph shows ultrasound measurements taken before milk let-down (Before), after milk let-down (AfterLD), 2 minutes into milking (2Min), after the plateau (Plateau), directly after milking with normal milking time (AfterM) and directly after milking with 5 minutes over milking (Over)

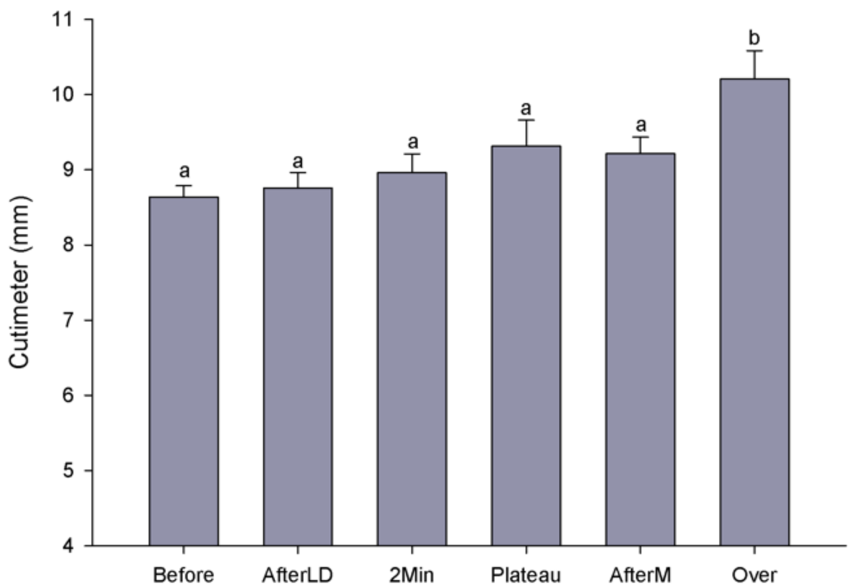

Figure 2)
The graph shows cutimeter measurements taken before milk let-down (Before), after milk let-down (AfterLD), 2 minutes into milking (2Min), after the plateau (Plateau), directly after milking with normal milking time (AfterM) and directly after milking with 5 minutes over milking (Over). Only the last measurement (over) was significantly different from the other ones.

Measurements of teat diameter on each cow were obtained during calculations of the teat wall thickness. Those measurements showed that the teat diameter was shorter directly after milking compared to before milking ($p < 0.0001$).

The hourly measurement from two to ten hours after milking showed a consistent and significant ($p < 0.05$) decrease in teat thickness over the day with the ultrasound method. With the cutimeter no significant ($p > 0.05$) changes were found (Figure 3).

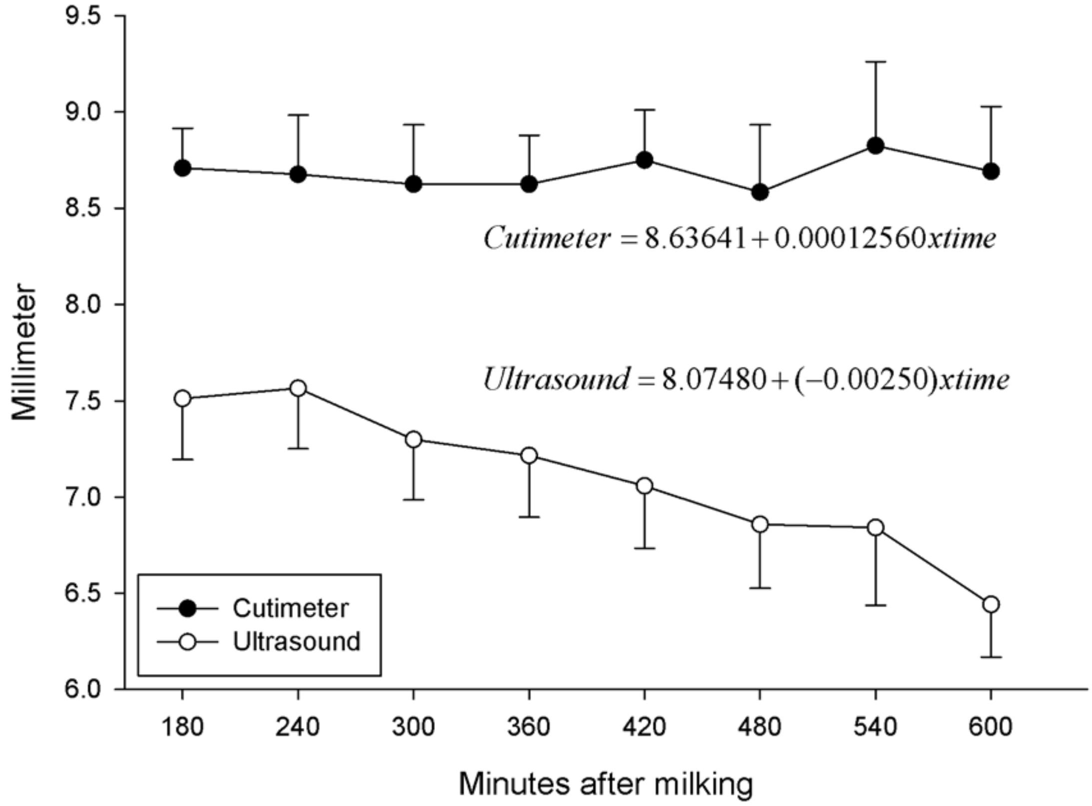

Figure 3) The graphs shows teat tissue density changes measured with a cutimeter and teat wall changes measured with a ultrasound machine for the time two hours after milking until ten hours after milking. For the ultrasound method time partially explains the change but for the cutimeter method time does not affect the teat tissue density.

Five minutes over milking gave significant differences compared to the normal milking measurements where over milking caused a greater thickness of the teat wall (obtained with both cutimeter and ultrasound method) (Figure 4 and 5).

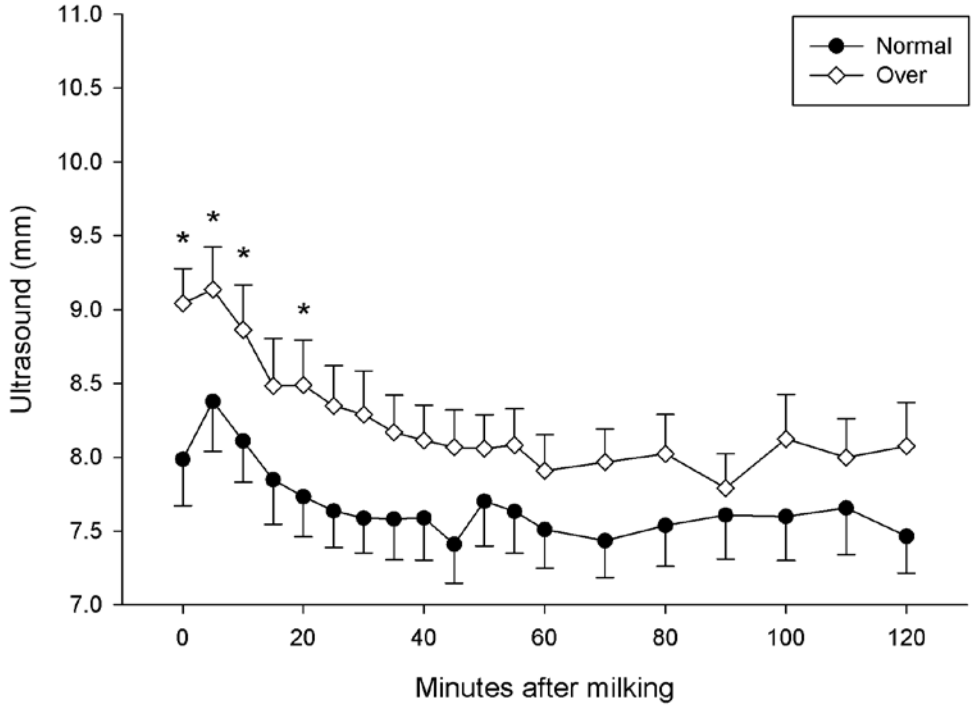

Figure 4) Teat wall thickness measured with ultrasound directly after milking until two hours after milking for normal routine milking (normal) and five minutes over milking (over). With the ultrasound method, over milking has a significantly higher teat wall density than the normal routine milking values at 0, 5, 10 and 20 minutes after milking.

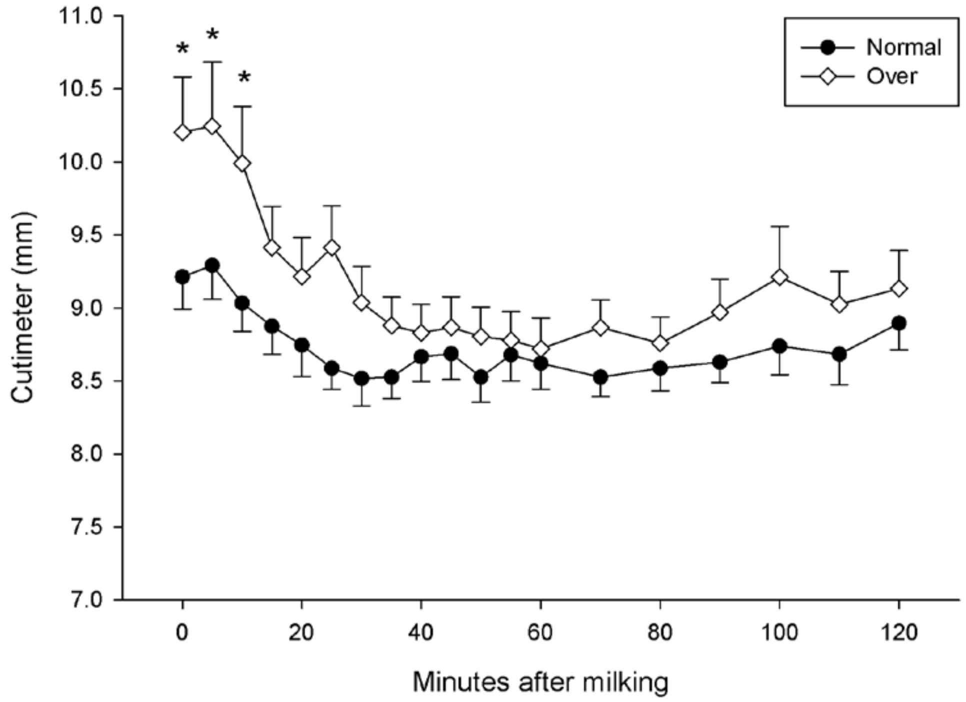

Figure 5) Teat tissue density measured with a cutimeter directly after milking until two hours after milking for normal routine milking (normal) and five minutes over milking (over). With the cutimeter method over milking has a significantly higher teat wall thickness than the normal routine milking values at 0, 5 and 10 minutes after milking.

Table 1) Regression analyses shows that between 0 – 60 minutes after milking, time partially explains the changes in cutimeter and ultrasound for both normal and over milking.

Measuring method	Milking length	Regression	R^2	P
Cuti	normal	9,07637 + (-0,01032) * time	0,0439	0,0002
US	over	8,07038 + (-0,01079) * time	0,022	0,0098
Cuti	normal	10,03456 + (-0,02590) * time	0,1186	< 0,0001
US	over	8,96267 + (-0,01914) * time	0,0717	< 0,0001

The two additional methods (two hours measurements without cutimeter and two hours with only three measurements points) for treatment 2 that were performed on the five last cows hade a too low power to show any significance.

Discussion

Factors that may have been affecting the results could be touch of the teat such as when the cows were moving or kicking towards the udder. During those happenings the teat sometimes temporarily thickend. Different kinds of stress were also observed to increase the teat thickness such as when the cows were fed and when some other cows were moved from the barn. The ambient temperature also differed between morning and evening. During treatment 2 and 3 measurements were taken in a frequent interval which included dipping the teat into water. The teat then dried through evaporation which in turn leads to cooling of the teat that can affect the teat density and teat wall thickness. Effort was put in to keep the water that was used for dipping the teat in had to have a hand-warm temperature. Cold water was observed in this study as well as in others (Bruckmaier & Blum, 1991) to contract the teat considerably.

In this study, it was chosen not to exclude any of the results although it seemed clear during the time of measuring that confounding factors caused great impact on some single measuring times. Most often they were caused by the cow moving her legs towards the udder in an attempt to remove flies. If those measurements would have been removed the result could have shown an even greater significance and a more stable decrease in teat thickness over the day.

Previous comparison between ultrasound and cutimeter measurements have shown a correlation between the two methods (Ambord & Bruckmaier, 2010). However, in their study measurements were only taken during a short period of time where the influence of IMP was small. In this study measurements from a longer time interval were compared and no correlation between the two methods was found. Although, in accordance with Ambord and Bruckmaier (2010) a closer relationship between the two methods were seen in the equations of the short time interval between 0 – 60 minutes after milking where the IMP is believed to be insignificant. Still, the cutimeter and the ultrasound methods differed a lot in execution and results. The ultrasound method allowed smaller fluctuations to be visible and thereby also gave more significant results than the cutimeter method. For the execution of the measurements the cutimeter method were far more efficient than the ultrasound. The cutimeter method required minimum equipment (only the cutimeter) while for the ultrasound method a large investment had to be made to purchase the ultrasound machine and the probe. Extra equipment needed was contact gel, transparent cups and hand-warm degassed water. In total, the ultrasound method required more preparation. During the execution of the measurements the cutimeter method were somewhat faster than the ultrasound method where the image had to be of good quality to enable measurements to be made from them later on. A lot of time was spent on making the measurements of the teat wall on the ultrasound images while with the cutimeter the measurements were noted directly as they were taken in the barn. With the cutimeter method more cows can be measured on the same day which enables larger number of cows to participate in the study. Other studies have been able to acquire significant measurements with the cutimeter method (Hamann & Mein, 1988; Hamann & Stanitzke, 1990; Isaksson & Lind, 1992; Ambord & Bruckmaier, 2010) but in this study most differences were small. It would be of interest to see if the cutimeter method could provide significant result in a study with a larger number of cows. In conclusion, the cutimeter method was much easier, faster and cheaper while the ultrasound method gave more defined results and allowed for more different measurements to be made such as teat diameter, teat wall thickness, teat cistern dimeter, teat canal length. With the ultrasound machine, it is also possible to examine amount of fluid in the teat wall tissue and vessels.

The results showed no differences in treatments in morning or evening milking for teat wall thickness. However, there were tendencies for a greater teat wall thickness after the morning milkings. The reasons for this may be that the mean milking yield and machine-on time were higher in the morning than in the evening, although the differences were not big enough to be significance. A longer milking time is believed to cause greater teat wall thickness (Rasmussen, 1993; Pařilová, *et al*., 2011).

It can be discussed whether the thickening of the teat wall is due to congestion and oedema or congestion alone. One argument for the development of oedema was discussed by Hamann and Mein (1988). Their opinion was that since the cutimeter applied a pressure of 20-25 kPa to the teat apex it should have eliminated fluids in the blood vessels as the mean arterial pressure is only about 12 kPa. However, just as discussed by Hamann and Mein (1988) it is not yet known how rapidly the vessels will be emptied. It is also not certain that the pressure applied to the teat wall by the cutimeter is the same as what is applied on the vessels. The opinion of Isaksson and Lind (1992) is that the thickening of the teat is not caused by oedema but instead of active hyperaemia which they also think is part of the teats natural reaction which has no pathologically negative effect. One thing they thought supported their opinion was the complete recovery of the teat between two milkings. On the ultrasound images taken in this study there were no signs of oedema which would have been seen as fluids outside of the vessels.

Earlier studies have compared teat measurements from directly after milking with premilking values to see the effect of the milking machine. Premilking measurements was considered by Isaksson and Lind (1992) to represent the normal teat value. The opinion of Hamann and Mein (1990) also suggest that the premilking value is the normal teat value as they expressed that due to the fact that the time it took for the teat thickness to recover to premilking values was so slow, this is proof of oedema rather than congestion. The result found in the current study implicates that the premilking value is not the baseline value for the normal teat thickness. It was clear with the ultrasound images that the teat wall was decreasing rapidly directly after milking and then a slow decrease continued during the rest of the day. The reason for this may be due to the IMP which increases as the milk is continuously filling the udder. The IMP would then be high right before milking and insignificant right after milking. One theory is that it is not possible for the teat to reach the premilking values until the IMP is high enough. Milk ejection leads to an even greater IMP in the teat cistern due to the milk let down which fills the udder and teat cistern with milk (Bruckmaier *et al*., 1991). The increased IMP explains why in the current experiment the teat wall thickness decreased significantly after premilking.

To perform a study on the differences of teat thickness before and after milking without the interference caused by the milking machine the udder could be emptied with drainage by a cannula. This has been done by Hamann and Mein (1990) and it showed significant decrease in teat thickness (measured with cutimeter with 2.5 kPa pressure) after draining the udder with a cannula. Without the action from the milking machine the teat tissue density in this study decreased after emptying of the udder in comparison to the studies who had used a milking machine where the teat density increased (Hamann & Mein 1988, 1990). From these results, it can be concluded that the IMP has an impact on the teat tissue density and that the thickening of the teat after milking is due to the process of the milking machine.

Calf suckling has earlier been proven to decrease the teat diameter directly after suckling compared to pre-suckling measurements while machine milking in the same study increased the diameter (Hamann & Stanitzke, 1990). Those findings supported the conclusion that machine milking has a greater negative effect on the teat and thereby also udder health than the natural calf suckling. This might still be true however, in this study as well as others which were performed more recently, there was a decrease in teat diameter after machine milking (Pařilová, *et al*., 2011; Guarin & Ruegg, 2016). In this study, the teat wall thickness and density did increase but the diameter decreased due to the contraction of the teat. It is therefore apparent that the diameter of the teat is not positively correlated with the teat wall thickness. Different milking equipment and settings have been seen to affect the teat thickness after milking (Hamann *et al*., 1993; Hamann & Mein, 1990, 1988; Bade *et al*., 2009; Pařilová, *et al*., 2011; Penry *et al*., 2017) which possibly can be a reason for the different result in teat diameter in the different studies.

Unfortunately, the two additional treatments that were performed to see if a frequent use of the cutimeter had an impact on the teat and thereby also the ultrasound images were implemented on too few cows to show any valid results. It would be interesting to redo the treatments with more cows to see if any differences would be detected. For future research, it would be important to know if a frequent use of the cutimeter affects the teat and thus the ultrasound images. If so, the frequency of the cutimeter measurements should be decreased and the two methods should be used separately.

Conclusion

The conclusions from this study were that the teat tissue density and teat wall thickness recovers from congestion within 30 minutes after milking. The teat wall continues to decrease until the next milking likely due to the increase in IMP. The milk let down appears to increase the IMP further and the decrease in teat wall thickness peaks after the milk let down. The ultrasound method shows teat wall changes both due to congestion and IMP pressure while the cutimeter method, due to the high-pressure spring load, only shows the tissue density changes which occurs by congestion after milking. Overmilking for five minutes caused a greater thickening of the teat after milking and the recovery time from congestion was prolonged compared to normal milking.

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