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EFFECT OF MILKING VACUUM LEVEL AND OVERMILKING ON COWS' TEAT CHARACTERISTICS

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Abstract

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The influence of milking vacuum and milk flow level (resp. detachment level) on cows' teat characteristics were studied in four experiments. The MIXED procedure was used to test treatment effects on the level of teat length, teat thickness at the base and half-way between the teat end and the base of udder, teat canal length, teat end width, teat wall thickness, teat cistern width after milking and on differences between these teat characteristics measured before and after milking. A total of 51 cows were included in all experiments. All the cows had clinically healthy udders. Some cows were involved in two or more experiments. Finally, 330 teat measurements of 165 cows were taken and statistically processed. Vacuum and milking with or without overmilking significantly ($P < 0.05$-0.001) influence monitored parameters. Milking vacuum has an influence on two of three measured external teat parameters: teat diameter measured at the base of the teat and half-way between the udder base and the teat tip. Change in teat length measured before and immediately after milking was higher when higher vacuum of 45 kPa was used. Detachment level also has an influence on teat proportions. Overmilked teats were longer and narrower compared to non-overmilked teats. Interaction between milking vacuum and detachment level influences external teat parameters as well.

cow's teat, milking vacuum level, milk flow level, overmilking, teat characteristics, ultrasound, parity

The implementation of machine milking has simplified the care of animals and increased milk quality, but on the other hand, it can induce many disorders of the udder (Twardon *et al.*, 2001). The economic results of milk production have been insufficient due to the low price of milk during recent years. Breeders are looking for any reserves which may still exist in dairy cow breeding (Pařilová *et al.*, 2010). One of the areas where the milking process could be more effective is shortening of the milking duration. This might be achieved, for example, by using a higher vacuum level for milking. However, changes in the physiological status of the teat tissue can be evoked by technology or frequency of milking and increases the risk of infection to the udder (Ipema and Benders, 1992). It could definitely be summarized that all cows have a biological limit for a positive reaction to vacuum. Exceeding these limits may lead to damage of the teat tissue or, on the other hand, slipping of milking clusters off the teat, which would extend milking time and result in improper milking. The changes in the udder may be irreversible if cows are exposed to improper milking for a long period, and these cows are at much higher risk of mastitis or/and culling.

The bovine teat canal is highly specialised in its unique function of preventing both leakage of milk and entry of bacteria (Paulrud, 2005). The internal environment of the mammary gland remains sterile most of the time, despite frequent soiling and contamination of the outer teat skin. Considering the relatively short length of the teat canal, this specialised epithelial structure is extremely effective in preventing pathogens from penetrating into the gland (Lacy-Hulbert, 1998). Proper milking machine function is necessary to maintain the integrity of the teat canal tissue. The stress associated with overmilking and improper vacuum levels and pulsation ratios reduces the effectiveness of the canal as a barrier to infection (Bramley *et al.*, 1992). Milking machine defects and faulty milking management may explain 16–45% of an inter-herd variation in udder health and confirm that the milking machine and milking management significantly influence udder health. According to Tančin *et al.* (2001b), milking techniques, routine, and the cow itself significantly affect the milking process.

Milking vacuum is one of the major factors in keeping the milking unit attached to the cow during the milking process (Spencer and Rogers, 1991; Rasmussen and Madsen, 1998). The study by Vegricht *et al.* (2005) confirmed that the vacuum level during milking is influenced by the design of the milking unit, and thus the quality of the milking process is influenced as well. Tančin *et al.* (2001a) and Kovác *et al.* (2001) reported tendencies to lower the milking vacuum below the customarily used 50 kPa. Reinemann *et al.* (2005) stated that the vacuum should not differ by more than 2 kPa in the receiver and milk line during normal milking. As milk production per cow and herd averages has increased, interest in milking frequency and intervals by dairy farm management has also increased (Armstrong, 1997). Equal milking intervals might have resulted in detectable effects both in the morning and in the evening, because the udder pressure in that case would probably be similar at both milkings (Svennersten *et al.*, 1990).

The main concern in vacuum stability is in relation to mastitis. Vacuum fluctuations generated within the cluster may lead to direct bacterial penetration. If the massage action of pulsation ceases when vacuum is continually applied, excessive congestion and edema will result as blood and interstitial fluid collect in the large veins and lymphatics, respectively (Neijenhuis and Hillerton, 2002). Recovery of teat end thickness may take from 6 to 8 hours in conventional milking. Milking at shorter intervals may lead to incomplete recovery of the teats. This can lead to an accumulation of teat trauma. Machine milking has a greater effect than calf suckling on teat swelling and teat end callosity. Negative changes in teat condition, such as severe teat end callosity and teat swelling, increase mastitis incidence (Neijenhuis *et al.*, 2001b).

The duration of milking is determined by the milk flow rate (Mačuhová and Bruckmaier, 2005) from the udder and also by the quantity of milk removed (de Koning, 2001). As the main factors controlling milking rate are teat duct anatomy and teat shape and size, it is not surprising that milking rate is highly heritable (Bramley *et al.*, 1992).

Individual cow milk production, milking machine idle time, and milking time are important factors which affect parlour efficiency (Magliaro and Kensinger, 2005). The duration of milking can be altered by changing the end-of-milking flow setting for automatic cluster removal (Stewart *et al.*, 2002; Magliaro and Kensinger, 2005). Peak flow rate increases progressively with increasing vacuum level (Ipema *et al.*, 2005), but this is accompanied by a marked rise in strip yield at higher vacuum levels, an increase in the incidence of hyperkeratosis of the external teat orifice and an increase in the degree of machine-induced congestion and edema (Bramley *et al.*, 1992).

The historic background for overmilking is mainly based on the assumption that all milk should be removed from the udder in order to maximise the milk yield. Overmilking starts when the milk flow to the teat cistern is less than the flow out of the teat canal (Rasmussen, 2004). A prolonged period of reduced flow, i.e., overmilking, is not only inefficient, but increases the probability of damage to the teat tissue: the greater the duration of overmilking, the greater the risk. Teats overmilked for 5 minutes at 16 milkings showed fewer injuries than teats overmilked for 20 minutes at four milkings (Neijenhuis *et al.*, 2000; Gleeson *et al.*, 2003). Overmilking is almost universally regarded by advisors as a key factor in machine-induced mastitis. Overmilking combined with other faults, such as vacuum fluctuations or inadequate pulsation can exacerbate problems, probably by exposing the teat to a greater deleterious effect. The widespread use of automatic cluster removers has markedly reduced overmilking (Bruckmaier *et al.*, 2001).

The objective of this work was to evaluate the influence of different milking vacuums and the level of milk flow at the time of detachment on the level of teat characteristics, i.e., teat length, teat thickness at the middle of the teat, teat canal length, teat end width, teat wall thickness, teat cistern width after milking and on differences between these characteristics measured before and immediately after milking.

MATERIAL AND METHODS

Animals

A total of 51 cows (26 Danish Red and 25 Holstein) were included in all experiments. All the cows had clinically healthy udders. Some cows were involved in two or more experiments (23 cows were included in all treatments, 41 cows in at least 3 treatments, 48 cows in at least 2 experiments and 51 in least one experiment). Finally, 330 teat measurements of 165 cows were taken and statistically processed, 165 front (162 right and 3 left) and 165 rear teats (154 right and 11 left). The left teats were measured only in the case that the right ones were not milked at that time.

Primiparous cows included in these experiments represented 30%; 34% of the cows were in the second lactation, and 36% in the third and subsequent lactations. For the statistical processing the cows were divided into three groups according to their milking interval. In the first group there were cows with a milking interval shorter than or equal to 480 minutes (24% of all cows); in the second group there were cows with a milking interval longer than 481 minutes and shorter than or equal to 960 minutes; and in the third group there were cows with a milking interval longer than 961 minutes. Other characteristics such as lactation stage (days in milking) and parity (lactation number) used in the statistical model were obtained from the automatic milking machine as well. According to the number of days in milking, the cows were divided into three groups as follows: less than 99 days, from 100 to 199 days, and more than 200 days.

The influence of milking vacuum and the level of milk flow at the time of the milk machine detachment from the udder – detachment levels on selected milking performance was monitored in four separate experiments. All these experiments were carried out in a free-stall barn with an automatic milking system (AMS) at Kvægbrugets Forsøgscenter Forskningscenter Foulum, Tjele, Denmark. The DeLaval automatic milking system was used in this experiment. In all there were 3 AMSs in the barn. There was a group of 50 to 60 cows per AMS. The AMS that was used for the experiments was chosen at random. Each experiment took one day (from 6 am to 4 pm). If the cow entered the AMS more than once during the measurement period, only its first visit was taken into account for the experiment. Particular experiments were carried out over a week interval. Each day immediately before taking measurements a certain milking vacuum and detachment level were adjusted in the proper automatic milking machine. The vacuum level was adjusted manually using valve the designated for this purpose. The detachment level was adjusted in the computer of the automated milking system. When a detachment level of 100 g.min^{-1} was applied, it was regarded as overmilking.

Measured parameters

All cows which entered the AMS and were accepted for milking during the measuring period were measured. In the observed cows, the following teat characteristics were measured:

Teat length was measured via Vernier calliper in centimetres with an accuracy of one-tenth. It was measured as the distance between the base of the udder and top of the teat.

Teat diameter at the base was measured via Vernier calliper in centimetres with an accuracy of one-tenth. It was measured at the base of the udder.

Teat diameter in the middle of the teat was measured via Vernier calliper in centimetres with an accuracy of one-tenth.

In accordance with the above-mentioned parameters, ultrasound scans were taken. The teats were scanned in B-mode using a portable ultrasonograph. To avoid direct contact between the probe and teat and consequently deformation of the teat canal, the teats were dipped into a rubber cup filled with warm water (37 ± 1 °C). The probe was placed in the wall of the rubber cup and held lateral to the teat. The ultrasound images obtained were recorded by a digital video camcorder. Using Pinnacle Studio software, the proper images were chosen for measurements, and subsequently certain teat parameters were measured using Lucia software.

Teat canal length was measured in millimetres as the distance between the distal and proximal end. Teat end width was measured in millimetres as a perpendicular to the axis of the teat canal at its proximal end. Teat wall thickness was measured in millimetres 5 millimetres above the proximal end of the teat canal in the narrowest area. Teat cistern width was measured in millimetres 5 millimetres above the proximal end of the teat canal perpendicular to the axis of the teat canal.

Apparaturs

Technical instruments used during observation and data collection were as follows: ultrasonograph ALOKA SSD-500 with linear probe UST-5561 (7.5 MHz) (ALOKA Co. – Ltd., Tokyo, Japan) – used for scanning teats; digital video camcorder CANON DM-MV600i (CANON, Japan) – used for recording scannings; rubber cup for the probe to be placed in during the scanning; vernier calliper – used for taking measurements of the teat; Pinnacle Studio software (Version 8, Pinnacle Systems, Inc. – Mountain View, California, USA) – used for choosing the best image of a scanned teat; Lucia software (Version 4.1, Laboratory Imaging, s. r. o. – Prague, Czech Republic) – used for measurements of the teat parameters.

Design of measurement

Two different vacuum (39 vs 45 kPa) as well as two detachment level (100 g.min^{-1} vs 400 g.min^{-1}) was used in 4 separate experiments. When a detachment level of 100 g.min^{-1} was applied, it was regarded as overmilking.

Experiment 1 – (39/100)

The vacuum level was adjusted at 39 kPa and the detachment level at 100 g.min^{-1}. Thirty-seven cows (16 Danish Red and 21 Holstein) were included in this experiment, i.e., 74 teats were measured (36 right and 1 left front teat, 34 right and 3 left rear teats). The average DIM was 161.6 (ranging from 11 to 488). Of these cows, 10 were in their first lactation, 12 in the second and 15 in the third or subsequent lactation.

Experiment 2 – (39/400)

The vacuum level was adjusted at 39 kPa and the detachment level at 400 g.min^{-1}. Thirty-seven cows (21 Danish Red and 16 Holstein) were included in this experiment, i.e., 74 teats were measured (37 right front teats, 35 right and 2 left rear teats). The average number of days in milking (DIM) was 149.3 (ranging from 4 to 481 days). Of these cows, 11 were in the first lactation, 11 in the second and 15 in the third or subsequent lactation.

Experiment 3 – (45/100)

The vacuum level was adjusted at 45 kPa and the detachment level at 100 $g.min^{-1}$. Forty-six cows (22 Danish Red and 24 Holstein) were included in this experiment, i.e., 92 teats were measured (45 right and 1 left teat, 42 right and 4 left rear teats). The average DIM was 174.5 (ranging from 8 to 622). Of these cows, 15 were in their first lactation, 17 in the second and 14 in the third or subsequent lactation.

Experiment 4 – (45/400)

The vacuum level was adjusted at 45 kPa and the detachment level at 400 $g.min^{-1}$. Forty-five cows (23 Danish Red and 22 Holstein) were included in this experiment, i.e., 90 teats were measured (44 right and 1 left front teat, 43 right and 2 left rear teats). The average DIM was 161.4 (ranging form 13 to 495). Of these cows, 14 were in the first lactation, 16 in the second and 15 in the third or subsequent lactation.

Statistical analysis

Basic statistical data were determined for all the monitored characteristics and parameters: minimal (min) and maximal (max) value during each experiment, average value (mean), divergence (s) and median (med). The number of measurements was recorded as well.

The MIXED procedure of SAS software (SAS INSTITUTE, 2001) was used to test the effect of the experiment conditions on teat length, teat thickness in the middle of the teat, teat canal length, teat end width, teat wall thickness, teat cistern width and differences between teat characteristics measured before and immediately after milking. The effects of independent class variables: milking vacuum (KPA), detachment level (DETACH), interaction between milking vacuum and detachment level (KPA x DETACH), milking interval and quarter position (QUARTER) on dependent variables (Y) were tested.

Teat characteristics measured with a Vernier calliper and the difference between the values measured before and immediately after milking were evaluated in accordance with the following model (1) using the independent variable quarter position (QUARTER):

(1) Y = KPA + DETACH + KPA × DETACH + BREED + + LACTATIONSTAGE + PARITY + MILKING INTERVAL + QUARTER

For teat canal length measured via ultrasound, teat length was added as an effect to the model based on correlation between teat length and teat canal length. The following model (2) was used:

(2) Y = KPA + DETACH + KPA × DETACH + BREED + + LACTATIONSTAGE + PARITY + MILKING INTERVAL + QUARTER + TL

For teat canal length, the difference instead of teat length (TL), teat length difference (TLDIF) was used in the previous model (2).

Teat end width was tested by the following model (3) using teat diameter in the middle (TDM). Teat diameter in the middle was added as an effect to the model based on correlation between teat end width and teat diameter in the middle of the teat:

(3) Y = KPA + DETACH + KPA × DETACH + + QUARTER + PARITY + BREED + + LACTATIONSTAGE + TDM

For teat end width difference, instead of teat diameter in the middle (TDM), teat diameter in the middle difference (TDMDIF) was used in the previous model (3).

Teat wall thickness and teat cistern width were tested in the following model (4) using teat diameter base (TDB) and teat diameter middle (TDM). Both these diameters were added to the model as effects based on correlations between teat wall thickness and these two diameters:

(4) Y = KPA + DETACH + KPA × DETACH + QUARTER + PARITY + BREED + + LACTATIONSTAGE + TDB + TDM

For teat wall thickness and teat cistern width differences, instead of teat diameter base (TDB) and teat diameter middle (TDM) teat diameter base difference (TDBDIF) and teat diameter middle difference (TDMDIF) were used in the previous model (4).

Spearman's correlation coefficients were assessed among the monitored characteristics and parameters, and the significance of this relationship was evaluated.

RESULTS AND DISCUSSION

Selected traits of the udder belong among the functional traits affecting the length of productive life (Mészáros *et al.*, 2008). In accordance with this, it is important to observe and evaluate the effect of milking conditions on trait parameters. Tab. I shows the influence of interaction between milking vacuum and milking with or without overmilking on external teat parameters. The teats were statistically longer in experiment 1 (39/100) compared to experiment 2 (39/400; $P < 0.01$, Tab. I), and experiment 4 (45/400, $P < 0.05$, Tab. I). In experiment 1, where a milking vacuum of 39 kPa and overmilking were applied, the teat length after milking was 4.70 cm, which was 0.24 cm longer compared to experiment 2 and 0.14 cm longer compared to experiment 4. A statistically highly significant difference (0.21, $P < 0.001$, Tab. I) was found between experiments 2 and 3. Teat length

I: *Influence of interaction between vacuum and detachment level on external teat parameters*

Experiment	1	2	3	4	P
Vacuum (kPa)	39		45		
Detachment (g.min^{-1})	100	400	100	400	
Teat length after milking (cm)	4.70	4.46	4.67	4.56	a** c* d*** f*
Teat diameter base after milking (cm)	2.48	2.55	2.41	2.45	a* b* d*** e***
Teat diameter middle after milking (cm)	2.28	2.33	2.21	2.26	b** d*** e** f*

* P < 0.05; ** P < 0.01; *** P < 0.001

II: *Influence of interaction between vacuum and detachment level on differences in external teat parameters measured before and after milking*

Experiment	1	2	3	4	P
Vacuum (kPa)	39		45		
Detachment (g.min^{-1})	100	400	100	400	
Teat length difference (cm)	0.26	0.10	0.32	0.29	d** f*
Teat diameter base difference (cm)	–0.28	–0.34	–0.24	–0.31	d**
Teat diameter middle difference (cm)	–0.28	–0.27	–0.28	–0.24	

* P < 0.05; ** P < 0.01; *** P < 0.001
a – difference between 39 kPa * 100 g.min^{-1} and 39 kPa * 400 g.min^{-1} (experiment 1 and 2), b – difference between 39 kPa * 100 g.min^{-1} and 45 kPa * 100 g.min^{-1} (experiment 1 and 3), c – difference between 39 kPa * 100 g.min^{-1} and 45 kPa * 400 g.min^{-1} (experiment 1 and 4), d – difference between 39 kPa * 400 g.min^{-1} and 45 kPa * 100 g.min^{-1} (experiment 2 and 3), e – difference between 39 kPa * 400 g.min^{-1} and 45 kPa * 400 g.min^{-1} (experiment 2 and 4), f – difference between 45 kPa * 100 g.min^{-1} and 45 kPa * 400 g.min^{-1} (experiment 3 and 4)

measured immediately after milking was longer with higher vacuum and overmilking compared to lower vacuum without overmilking (45/100, 4.67 vs. 39/400, 4.46 cm, Tab. I). The teats were also longer with the use of overmilking within the same vacuum level. When milking under a vacuum of 45 kPa was applied, the overmilked teats were 4.67 cm long and the not overmilked teats were 4.56 cm long. The difference of 0.11 cm was statistically significant (P < 0.05, Tab. I).

Teat diameter measured at the base became narrower with higher vacuum and overmilking compared to a detachment level of 400 g.min^{-1} (Tab. I). In experiment 1 (39/100) the teat diameter measured at the base was 2.48 cm, which was significantly narrower (P < 0.05) than the diameter in experiment 2 (39/400, 2.55 cm) and significantly larger (P < 0.05) than in experiment 3 (45/100, 2.41 cm). A significantly larger (P < 0.001) teat diameter at the base was found in experiment 2 compared to experiment 3 (39/400, 2.55 cm vs 45/100, 2.41 cm, Tab. I). A significant influence (P < 0.001) of higher vacuum was evident in teat diameter measured at the base in experiment 4 (45/400, 2.45 cm) compared to experiment 2 (39/400, 2.55 cm, Tab. I).

The same trends as mentioned above were found in teat diameter measured in the middle of the teat (Tab. I). The difference between overmilked teats milked at 39 and 45 kPa was statistically significant (P < 0.01); the diameters measured in teats milked with a higher vacuum were narrower (experimen 3, 45/100, 2.21 vs. experiment 1, 39/100, 2.28 cm, Tab. I). The same influence of milking vacuum (P < 0.01) was evident between the diameters measured in teats milked with a different vacuum, but with the same detachment level of 400 g.min^{-1} (experiment 4, 45/400, 2.26 vs. experiment 2, 39/400, 2.33 cm, Tab. I). The influence of overmilking on teat diameter measured in the middle of the teat within the same milking vacuum was also statisticaly significant. Within the same vacuum (45 kPa) there was an evident influence of the detachment level; overmilked teats were narrower (P < 0.05) by 0.05 cm compared to non-overmilked teats.

In general, it can be summarized that overmilking (Tab. I) as well as machine milking (Tab. II) increased teat length and decreased both teat diameters which is inconsistent with the statement of Gleeson *et al.* (2002), who reported that milking increased teat length and teat thickness. On the contrary, our results confirmed the findigs of Stádník *et al.* (2010). The greatest difference in teat length (0.32 cm) measured before and after milking was found in experiment 3, when the teats were milked under a vacuum of 45 kPa and were overmilked (Tab. II). Prolongation of teats were significantly higher (P < 0.01) in experiment 3 compared to those of experiment 2 (45/100, +0.32 vs. 39/400, +0.10 cm). Within the same milking vacuum (45 kPa) there was evident influence of overmilking; the difference of teats length before and after milking was higher in overmilked teats in experiment 3 (P < 0.05) compared to non-overmilked teats in experiment 4 (+0.32 vs +0.29 cm, Tab. II). The smallest difference in teat length before and after milking was found in experiment 2 (39/400, +0.10 cm), where a lower milking vacuum and no overmilking were applied (Tab. II). It can be assumed that these milking conditions are the mildest from this point of view.

Hamann and Stanitzke (1990) reported that different milking methods lead to changes in teat length in the range of ± 1 mm, which is consistent with our results, while Stádník *et al.* (2010) found prolongation from 14 to 15 mm.

The largest difference in teat diameter at the base before and after milking was found in experiment 2 (39/400, –0.34 cm), which significantly differed (P < 0.01) from experiment 3 (45/100, –0.24, Tab. II). Other differences were not significant, but it could be seen that differences in teat diameter at the base before and after milking were greater in non-overmilked teats compared to overmilked teats (experiments 2 and 4 vs experiments 1 and 3, Tab. II).

The opposite trend was evident in teat diameter measured in the middle of the teat length before and after milking (Tab. II). A greater difference in teat diameter before and after milking was found in experiments 1 and 3 (39/100 and 45/100), i.e., in overmilked teats, but the differences found were not significant. This can be assumed because the teat tissue is more consistent at the teat base than in the middle of the teat length, and therefore the middle part of the teat undergoes greater changes and is more strained during milking. Neijenhuis *et al.* (2004) published results that change in teat thickness is influenced by milking vacuum, pulsation rate, milk yield, overmilking and the liner. Stádník *et al.* (2010) added that teat parameters differed according to the different time after milking, and Sawa and Krężel-Czepek (2009) similarly described different teat parameters during the entire lactation. A difference in thickness of about 2 mm or more in teat diameter before and after milking (Tab. II) corresponds with the results of Hamann and Mein (1990), who suggested that machine milking should not increase or decrease teat thickness by more than 5%. The IDF machine milking and mastitis group has recommended the use of a 5% limit as a reference point, when determining physiological teat reactions induced by the milking process. Neijenhuis *et al.* (2004) also reported that an increase in the teat thickness of more than 5% is associated with the prevalence of infection. In our study the minimal difference from before to immediately after milking in the middle of the teat diameter was in experiment 4 (45/400, 9.72%), and the greatest difference was found in experiment 3 (45/100, 11.29%). At the teat diameter base the difference before and after milking was even greater; the minimal difference was determined in experiment 3 (45/100, 9.13%) and the greatest one in experiment 2 (39/400, 11.72%). Neijenhuis *et al.* (2001a) reported that a relative increase of teat thickness measured by a cutimeter of more than 5% increases the rate of new infection rate and teat duct colonization. Hamann and Mein (1990) reported that changes in thickness may be affected by reduction in intramammary pressure, changes in the geometry and conformation of the teat, changes in the degree of contraction and/or the tone of smooth muscles in the teat or the degree of machine-induced edema in the tissue of the teat apex or by a combination of two or more of these conditions. According to Gleeson *et al.* (2002), reactions of the teat tissue may decrease the efficiency of the teat defence mechanism and lead to increased risk of new infection. They further reported that changes in teat thickness can be interpreted as impairment of the local circulatory system of the teat tissue due to congestion or edema. Hamann and Mein (1990) defined edema as an extravascular accumulation of fluid. The extent and persistence of edema depend on the milking conditions and milking duration. According to Hamann and Mein (1990), the consequence of edema includes reduced blood supply to the tissue and therefore a reduction in O_2 tension. The resulting metabolic changes may impair the normal functioning of defence mechanisms such as phagocythosis. The results of a series of experiments using an artificially high level of bacterial challenge indicate that the risk of new infections significantly correlated with increased teat thickness.

Teat canal length was affected by the interaction of milking vacuum and detachment level. There was a longer teat canal in experiment 1 compared to experiment 2 (39/100, 12.45 vs. 39/400, 11.48 mm, P < 0.001, Tab. III). The teat canal in experiment 3 was also significantly longer compared to experiment 2 (45/100, 12.11 vs. 39/400, 11.48 mm, Tab. III). A highly significant difference in teat canal length (P < 0.001) was found between experiments 2 and 4 (39/400 vs 45/400), where no overmilking was applied. It can be seen that under milking conditions with higher vacuum, teat canal was 0.95 mm longer compared to lower vacuum applied (45/400, 12.43 vs. 39/400, 11.48 mm, Tab. III).

Teat canal length increased relatively more than teat end width, which indicates that the teat tip is more pliable or more under stress during milking

III: *Influence of interaction between vacuum and detachment level on internal teat parameters*

Experiment	**1**	**2**	**3**	**4**	**P**
Vacuum (kPa)	**39**		**45**		
Detachment ($g.min^{-1}$)	**100**	**400**	**100**	**400**	
Teat canal length after milking (mm)	12.45	11.48	12.11	12.43	a*** d** e***
Teat end width after milking (mm)	21.27	20.93	21.61	21.33	a* b* d*** e* f*
Teat wall thickness after milking (mm)	7.58	7.50	7.73	7.34	f*
Teat cistern width after milking (mm)	6.79	6.85	6.94	7.49	c** e* f*

* P < 0.05; ** P < 0.01; *** P < 0.001

in length than in width, which is consistent with Neijenhuis *et al.* (2001a). A wider teat end was found when overmilking was applied both at vacuum levels of 39 kPa and 45 kPa (Tab. III). There was a significant difference in teat end width between experiments 1 and 2 (39/100, 21.27 vs 39/400, 20.93; $P < 0.05$), 1 and 3 (39/100, 21.27 vs 45/100, 21.61; $P < 0.05$), 2 and 3 (39/400, 20.93 vs 45/100, 21.61; $P < 0.001$), 2 and 4 (39/400, 20.93 vs 45/400, 21.33; $P < 0.05$) and 3 and 4 (45/100, 21.61 vs 45/400, 21.33; $P < 0.05$).

Overmilked teats had wider teat ends because they were more strained during overmilking. On the other hand, teats milked under higher milking vacuum had wider teat ends when the same detachment levels were compared (Tab. III). Neijenhuis *et al.* (2001b) reported that changes in teat end width were relatively small compared with other teat parameters. The average teat end width increased in their study from 21.2 mm before milking to 21.7 mm after milking. While Stádník *et al.* (2010) mentioned significant and the highest differences (+18.4–24.1%) in the area of the teat end compared to other measured traits of the teat, Hamann and Stanitzke (1990) reported that after calf suckling and hand milking teat thickness values were markedly reduced compared with premilking measurements, whereas in all other cases teat end thickness increased markedly after machine milking. According to Hamann and Stanitzke (1990), the milking machines caused marked increases in teat end diameter after their first 3 days of use. After use of the machine for several weeks, this increase in teat end diameter declined. A possible explanation could be that the tissue might have adapted to machine milking. In their study they found an increase of teat end thickness after machine milking between 26.9 and 17.9%. The mean values were in the range of 12 mm. In our experiment teat end width became thicker after milking as well. In the study by Spencer *et al.* (1996) teat apex thickness increased by 0.35 cm from pre- to post-milking as measured via ultrasonography. Stádník *et al.* (2010) measured the area of the teat end and determined and increase from 0.35 cm^2 to 0.49 cm^2 in relation to milking.

The teat wall was thicker in teats which were overmilked at both vacuum levels. In experiment 1 the teat wall was 0.08 mm thicker compared to experiment 2 (39/100, 7.58 vs. 39/400, 7.50 mm, Tab. III), and in experiment 3 it was 0.39 mm thicker compared to experiment 4 (45/100, 7.73 vs. 45/400, 7.34 mm, Tab. III). According to Neijenhuis *et al.* (2004), milking vacuum generates strain in the teat wall, which induces dilation of the blood vessels and accumulation of interstitial fluid. Changes in the deformability of teat tissue, caused by congestion or edema, may change the resistance of the teat canal to bacterial invasion. Neijenhuis *et al.* (2001a) reported an increase in teat wall thickness from 6.8 mm before milking to 9.1 mm after milking, and Stádník *et al.* (2010) detected proportions from 7.4 mm to 9.0 mm. Both findings are similar to our results.

Teat cistern width and teat wall thickness changed during the milking process in the opposite direction, which is consistent with studies performed by Neijenhuis *et al.* (2001a) and Stádník *et al.* (2010). The milk is withdrawn from the teat cistern during milking. As the cistern decreases, the dimension of the teat wall increases. The teat cistern was wider with a higher vacuum level within the same detachment level, and on the other hand it was narrower in overmilked teats within the same vacuum level. A significant difference ($P < 0.01$) was found between experiment 1 and 4 (39/100, 6.79 vs. 45/400, 7.49 mm, Tab. III) which was caused both by the vacuum level and overmilking. The same influence of interaction between milking vacuum and detachment level can by found between experiments 2 and 4 (39/400, 6.85 vs. 45/400, 7.49 mm, $P < 0.05$, Tab. III). A strong influence of overmilking on the teat cistern width was confirmed by the significant difference within the vacuum level of 45 kPa (45/100, 6.94 vs. 45/400, 7.49 mm, $P < 0.05$, Tab. III), which is 0.55 mm.

The milking increased teat canal length, teat end width, and teat wall thickness and presently decreased teat cistern width compared to level before milking (Tab. IV), which is consistent with

IV: *Influence of interaction between vacuum and detachment level on differences in internal teat parameters measured before and after milking*

Experiment	1	2	3	4	P
Vacuum (kPa)	39		45		
Detachment ($g.min^{-1}$)	100	400	100	400	
Teat canal length difference (mm)	1.98	1.46	1.99	2.05	a* d* e*
Teat end width difference (mm)	0.02	0.14	0.21	0.05	
Teat wall thickness difference (mm)	1.70	1.51	1.76	1.61	
Teat cistern width difference (mm)	–4.88	–4.70	–4.83	–4.51	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

a – difference between 39 kPa * 100 $g.min^{-1}$ and 39 kPa * 400 $g.min^{-1}$ (experiment 1 and 2), b – difference between 39 kPa * 100 $g.min^{-1}$ and 45 kPa * 100 $g.min^{-1}$ (experiment 1 and 3), c – difference between 39 kPa * 100 $g.min^{-1}$ and 45 kPa * 400 $g.min^{-1}$ (experiment 1 and 4), d – difference between 39 kPa * 400 $g.min^{-1}$ and 45 kPa * 100 $g.min^{-1}$ (experiment 2 and 3), e – difference between 39 kPa * 400 $g.min^{-1}$ and 45 kPa * 400 $g.min^{-1}$ (experiment 2 and 4), f – difference between 45 kPa * 100 $g.min^{-1}$ and 45 kPa * 400 $g.min^{-1}$ (experiment 3 and 4)

Gleeson *et al.* (2002) and Stádník *et al.* (2010). Rákos (2005) found that teat parameters are significantly influenced by machine milking. Neijenhuis *et al.* (2001a) reported that the average relative change in teat parameters caused by milking were from 10 to 30% in teat canal length, from 2 to 10% in teat end thickness, from −50 to 3% in teat cistern width, and from 20 to 50% in teat wall thickness. Stádník *et al.* (2010) determined relative changes in teat length from 13.4 to 16.2%, from 18.4 to 24.1% in the area of the teat end, and from 14.6 to 19.7% in wall thickness. In our experiments the average relative change in teat canal length according to milking was from 15 to 20%, in teat end thickness from 0 to 1%, in teat cistern width from −37 to −42%, and in teat wall thickness from 25 to 30%. Rasmussen and Madsen (2000) reported that teat thickness decreased by 1 to 2% (0.1 to 0.2 mm) during milking in cows milked at 38 kPa.

The mildest influence of milking conditions on teat canal length was found in experiment 2 (39/400), where the lowest difference (+1.46 cm, $P < 0.05$) in teat canal lengths measured before and after milking was detected (Tab. IV). This is consistent with the difference found in teat length. A significantly ($P < 0.05$) greater difference in teat canal length before and after milking was found in overmilked teats in experiment 1 compared to experiment 2 (39/100, +1.98 vs. 39/400, +1.46 mm, Tab. IV). Teat canal lengths before and after milking obtained in experiments 2 and 3 (39/400, +1.46 vs. 45/100, +1.99 mm, Tab. IV) and in experiments 2 and 4 (39/400, +1.46 vs. 45/400, +2.05 mm, Tab. IV) also significantly differed ($P < 0.05$). Geishauser and Querengässert (2000) reported that lengthening of the teat canal by ≥ 2 mm was the best cut-off value for prediction of rupture in the teat canal area. If the teat canal was lengthened by ≥ 2 mm, the odds that rupture, and not other causes of milk flow disturbances, were present 4 times higher. Thus, teat canal lengthening by ≥ 2 mm can be used as a test for ruptures in the teat canal area. In our study (Tab. IV), the teat canal prolonged by more than 2 mm in experiment 4 (45/400); in experiment 1 (39/100) it was prolonged by 1.98 mm, and in experiment 3 (45/100) by 1.99 mm. Therefore we can assume that some teat tissue ruptures around the canal can occur in the teats. Geishauser and Querengässert (2000) further reported that with ruptures in the teat canal area there are often associated milk flow disturbances. The tissue in these teats is frequently inverted into the teat cistern. In all experiments the teat end became wider, but the differences between the experiments were not statistically significant (Tab. IV). No significant differences were found in the other two observed parameters: teat wall thickness and teat cistern width. The teat wall became thicker in overmilked teats and the teat cistern narrower in overmilked teats, which is the same trend as described above.

SUMMARY

Based on the results obtained from measurements of observed teat parameters, we can conclude that vacuum and milking with or without overmilking significantly influence the external and internal teat parameters. During milking the teats became longer and narrower.

Milking vacuum has an influence on two of three measured external teat parameters: teat diameter measured at the base of the teat and half-way between the udder base and the teat tip. The difference between these diameters measured before and after milking was not influenced by the milking vacuum. Change in teat length measured before and after milking was higher when a vacuum of 45 kPa was used. Internal teat parameters – teat canal length, teat end width and teat cistern width were larger when using a higher milking vacuum. The influence of milking vacuum on any change in the parameters mentioned was not significantly confirmed.

Detachment level has an influence on teat proportions. It was found that overmilked teats were longer and narrower in both observed diameters. In addition, it was determined that change in teat diameter measured at the base of the teat was larger in non-overmilked teats. Further, it was found that overmilked teats had longer teat canals and narrower teat ends compare to non-overmilked teats.

Interaction between milking vacuum and detachment level influences external teat parameters; the teats were longer in overmilked cows at both vacuum levels. Teat diameters were narrower in overmilked teats at both vacuum levels. The interaction of detachment level and milking vacuum affected the change in teat length and teat diameter measured at the base of the teat. The teats lengthened most during overmilking and a vacuum of 45 kPa was applied. The largest change in teat diameter measured at the base was found when using a vacuum of 39 kPa and a detachment level of 400 $g.min^{-1}$. All the monitored internal teat parameters were affected by the interaction of the vacuum and detachment level. The shortest teat canal was found in teats when a vacuum of 39 kPa and no overmilking was applied. The only change in internal teat parameters influenced by the interaction of vacuum and detachment level was change in teat canal length measured before and after milking. The smallest change was found when a milking vacuum of 39 kPa and no overmilking were applied to the teats.

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