Abstract: Three related experiments were conducted to gain a better understanding of the physiological responses of teat tissue to machine milking. In the first experiment changes in peak milk flow rate were used as an indicator of congestion of teat end tissues that occur during the milking phase of each pulsation cycle. Teat end congestion was increased by increasing both the b phase of pulsation and the milking vacuum level and was reduced by the application of increasing liner compression. Ultrasound measurements were used to measure changes in teat wall thickness and indicated that increasing vacuum level increased teat wall thickness and that at some critical level of liner compression the recovery rate of teat wall congestion may be reduced. The development of teat end hyperkeratosis was studied for liners with differing compression levels. This experiment confirmed that increasing liner compression increased the development of teat end hyperkeratosis.

INTRODUCTION

Milking vacuum level and the percentage of time the liner is open during one pulsation cycle are the primary machine factors influencing peak milk flow rate (PMF) and milking speed (Smith and Petersen, 1946; Clough, 1972; Spencer et al. 2007). Increasing milking vacuum level and increasing duration of the b-phase of pulsation have also been shown to increase teat congestion as reflected by changes in teat wall thickness after milking, measured using skin-fold calipers (Hamann et al. 1993), radiographic techniques (McDonald, 1975) or ultrasonic imaging (Gleeson et al. 2004; Neijenhuis et al. 2001a; Vinitchaikul and Suriyasathaporn, 2007; Worstorff et al. 1986). The role of liner compression (LC) in increasing milking speed by reducing teat tissue congestion during milking has become clearer in the last 20 years (Davis et al. 2000; Gleeson et al. 2004; Mein et al. 2003b;). When teats are congested after milking, the defense mechanism of the teat canal to resist invasion and removal of mastitis causing organisms from the canal is compromised (Mein et al., 1987; Hamann, 1990; Zecconi et al., 1992; Gleeson et al., 2004; Vinitchaikul and Suriyasathaporn, 2007). This is probably because the teat canal closes more slowly after milking when teats are congested (Neijenhuis et al., 2001; Mein and Reinemann, 2007). Full tissue recovery after machine milking may take many hours (Gleeson et al., 2002). When teat end thickness changed by > 5%, higher infection rates of quarters and more ducts colonized in teats were observed compared with teats showing less congestion (Zecconi et al., 1992).

LC is a critical factor in reducing teat tissue congestion during milking and can also influence peak flow rate and milking speed. At the same time excessive LC contributes to the development of teat-end hyperkeratosis (HK) (Capuco et al., 1994). HK of the skin surrounding the teat canal opening is a result of the stresses applied to skin when the milking liner collapses on the
Teat ends. The duration of milking, as affected by milk production level, milking frequency, and thresholds applied to automatic cluster removal also affect HK (Rasmussen, 1999). HK is also influenced by environmental conditions (humidity and temperature) and genetics (teat shape and dimension) (Mein, et. al., 2001). A recent survey of teat-end condition on commercial farms indicated that the percentage of cows with rough or very rough teat ends averaged about 50% with some farms exceeding 70% and some farms less than 20% (Bade et al., 2007b).

Teat ends with rough surface is more difficult to clean during pre-milking preparation and provide a site for bacteria colonization. Neijenhuis et al., (2001) found a correlation between increased risk of clinical mastitis and very rough teat-ends. HK is also an undesirable condition also because it may contribute to cow discomfort during milking (Hamann, 2000). Excessive LC may also remove excessive amounts of keratin from the teat canal which makes teats more susceptible to infections. LC equal to mean arterial pressure (about 12 kPa) is thought to be sufficient to relieve congestion with additional LC providing no additional benefit for congestive relief (Mein et al., 1987). More recently it has been speculated that the LC required to relieve congestion increases as milking vacuum level increases (Mein et al., 2003a).

While the major milking machine related influences on milking speed and teat tissue condition after milking have been studied previously, most previous studies have either altered only one causal variable and measured only one response variable, or have introduced confounding into experimental designs by lack of independent control of several causal variables. The primary objective of our studies was to quantify the milking machine effects of milking vacuum level, b-phase and LC on milk flow rates and to gain a better understanding of the physiological responses of teat tissues to machine milking. Our studies were designed to control these three causal variables independently over a broad range so that both main and interactive effects could be estimated.

**MATERIALS AND METHODS**

**Experiment I. Effects of milking vacuum, b-phase and LC on PMF and teat end congestion:**
The main and interactive effects of vacuum level, b-phase duration, and LC on PMF of 88 Holstein cows were studied by independently controlling these causal variables over a wide range of settings (42 to 53 kPa system vacuum, 220 to 800 milliseconds of b-phase, and LC from 8 to 14 kPa) using an inscribed central composite experimental design (Bade, 2007a). Pulsation rate and ratio were adjusted so that the d-phase of pulsation was maintained at 250 ms for all treatments. Automatic cluster removers were set at a flow threshold of 0.6 kg/min and a detachment delay of 3 seconds. PMF was defined as the maximum milk yield from all four teats in any 11.2 s interval during the milking session. Average milk flow (AMF) was defined as the total milk yield divided by the total cups-on time for the milking session.

**Experiment II. Ultrasonic measurement of teat wall thickness:** Ultrasonic scans of teat wall thickness were performed on six Holstein cows using the method described by Spanu et al. (2008). Scans were performed immediately prior to milking, immediately after milking, one hour after milking, two hours after milking and four hours after milking. Measurements of teat-wall thickness 1 cm above the top of the teat canal were taken from each scan. Teat wall thickness was expressed as percentage change compared to the pre-milking values. The following four treatments selected as a subset from the 15 treatments from experiment I were applied to each cow [A: Vacuum = 44.2 kPa, b-phase = 322 ms, LC = 9 kPa; B: Vacuum = 47.5 kPa, b-phase = 500 ms,
LC = 11 kPa; C: Vacuum = 50.8 kPa, b-phase = 678 ms, LC = 9 kPa; D: Vacuum = 50.8 kPa, b-phase = 678 ms, LC = 13 kPa.

Experiment III. Effect of LC on HK: This study was conducted on 75 Holstein cows, milked twice per day. A quarter-udder experiment was performed by installing four different types of liners on each of the four clusters in the milking parlor so that each quarter of every cow was milked with the same liner for a period of one month (Zucali, et al, 2008). Liners L1, L2, and L3 were round Nitrile rubber that had similar dimensions but varying wall thickness. Liner L4 was a silicon liner that is round in the open position and triangular shaped when collapsed, achieved by fixing the outer walls of the liner to the inner walls of the shell at three points. Liner L2 was used on all clusters for several months prior to the start of this experiment. LC was measured using the Start-of-Milk-Flow Method as described by Mein et al. (2003a) [L1 = 18 kPa, L2 = 15 kPa, L3 = 13 kPa, L4 = 9 kPa]. All teats were visually scored using the N, S, R, VR method recommended by the Teat Club International (Mein et al, 2001) as well as photographed before the experiment began and once each week for the 4 week duration of the study.

RESULTS

Experiment I. Effects of milking vacuum, b-phase and LC on PMF and teat end congestion:
A physical model was used as the basis for statistical analysis of PMF data. The Bernoulli equation for incompressible fluid flow through a tube (neglecting static pressure) can be written as: \(u^2 = C_1 + C_2 V\), where \(u\) is the velocity of fluid flow and \(V\) is negative pressure – or vacuum difference across the tube (in this model the average claw vacuum at the peak milk flow rate) and the \(C_2\) accounts for fluid density. The volumetric flow of milk when the liner is open can be calculated using the cross sectional average fluid velocity and effective area diameter of the teat canal. The volumetric flow rate of milk, PMF, is further proportional to the fraction of the pulsation cycle in which the liner is open \((F, \text{or milk fraction})\): \(PMF = F * A * (C_1 + C_2 V)^{1/2}\).

The teat canal opens at some critical vacuum difference across it and continues to open further as this vacuum level is increased until the canal is fully unfolded and the skin has reached its elastic limit. Congestion of tissue surrounding the canal will act to decrease its effective diameter while LC will act to increase canal diameter by reducing congestion. Increasing the b-phase \((B)\) may also act to increase congestion but is likely interactive with milking vacuum (e.g. the effect of b-phase on congestion is likely to be greater as the milking vacuum increases). The following physically based model is assumed for the cross sectional area of the teat canal as a function of milking vacuum, LC and b-phase: \(A = C_6 + C_7 V + C_8 LC + C_9 B + C_{10} V*LC + C_{11} V*B + C_{12} V^{1/2} + C_{13} LC*V^{1/2} + C_{14} B*V^{1/2} + C_{15} B*LC*V^{1/2}\). This expression was substituted into the PMF equation and fitted to the data using the SAS GLM procedure eliminating insignificant terms \((p>0.05)\) to yield the following final model: \(PMF = 1.499 + (0.5202 * F * V^{1/2}) + (0.02826 * F * V^{1/2} * LC) + (0.001025 * F * V^{1/2} * B) - (0.00019 * F * V * B)\). Response surfaces for LC = 8, 11 and 14 kPa are shown in Figures 1, 2, and 3.

At low B-phase durations PMF increases with increasing claw vacuum, however at higher levels of B-phase duration PMF decreases with increasing claw vacuum. LC increases PMF at all levels of B-phase and claw vacuum, however the influence of LC is much larger at higher levels of both b-phase and claw vacuum. The effect of LC on PMF is an indication of the degree of teat end tissue congestion occurring in each pulsation cycle as increasing LC reduces this congestion and allows the canal to open more fully in the next pulsation cycle. It is interesting to note that increasing the b-phase has more influence on PMF than does increasing claw vacuum over the
ranges tested in this experiment. It is important to put these PMF results into the broader perspective. The range of milking conditions applied in this experiment is much wider than in current practice and resulted in an overall increase in PMF from about 3.2 to 5.0 Kg/min (just over 50% increase). Over the same wide range of milking conditions the average milk flow rate increased from about 2.4 to 3.2 kg/min (just over 30%) so that these increases in PMF would not reflect commensurate reduction in milking duration. Cows also showed noticeable discomfort at the more aggressive milking conditions.

Experiment II. Ultrasonic measurement of teat wall thickness: The combination of milking vacuum level and b-phase duration had an effect on the degree of teat wall thickness after milking with an increase of about 25% at a milking vacuum level of 44 kPa and B-phase of 322 ms and an increase of about 35% at milking vacuum levels of 47 and 50 kPa and B-phase of 500 ms or more (Figure 4). The teat wall may have been approaching its maximum possible thickness increase under the more aggressive milking conditions. The steeper recovery slope for treatment D indicates that liner compression may have had an influence on the recovery rate of teat tissues, possibly because of the limiting of edema occurring during milking.

Experiment III. Effect of LC on HK: The results of the multiple correspondence analyses showed that HK scores R and VR were most closely related to long milking duration and liners L1 and L2. While HK score S (smooth rings) was most closely related to short milking duration and Liner L4. The results of the logistic regression analysis indicated that the initial teat-end score and the duration of milking (as influenced by milk yield and other cow factors) had a large influence on the risk of developing a poor HK score (R or VR). Teats that started with a score of N were less likely to become R and VR than teats starting with a score of S. Not surprisingly, teats that began the experiment with a score of R or VR were much more likely to end the experiment with a score of R or VR than teats starting the experiment with a score of S. This is probably an indication of the importance of teat size and shape on the risk of developing HK as well as the difficulty for teats to ‘recover’ from the development of rough teat ends. Cows (teats) with milking duration <4.3 min were much less likely to develop HK than cows (teats) with a milking duration > 5.3 minutes, probably due to the decreased number of times the liner collapsed on these teats during milking. While liner type also had an influence on the risk of HK, it was not as large as the influence of milking duration. The risk of developing HK score R or VR was maximized with liner L2 and minimized with liner L3. Logistic Regression Comparisons across other liners were not significant.

Milking duration and initial teat-end HK scores had a larger influence on final teat-end HK scores than liner type and liner compression. However, there are indications that liner compression does contribute to the development of teat-end HK. Each form of analysis had at least one indication that increased LC was associated with increased HK score; however the results of the different statistical analyses were not entirely consistent. These results differ from the field study reported by Bade et al. (2007b). Reasons for this difference could include the low level of teat-end HK at the start of this study (15% of the teats scoring R or VR) were considerably lower that than on the commercial farms studied by Bade et al. The milking conditions used in this study also used moderate vacuum levels and moderately aggressive settings for automatic cluster removal resulting in gentle milking and minimal over-milking. In addition milking frequency of these cows was twice per day as compared to the majority of commercial farms reported in the field study by Bade et al., (2007b) which milked three times per day.
CONCLUSIONS

The worldwide trend to increase milking speed has often resulted in substantial increases in teat tissue stress and possible reduction in the efficacy of teat canal defense mechanisms. Teat tissue congestion during milking resulting in edema and open teat canals after milking, and excessive hyperkeratosis are two teat tissue responses that are thought to increase the risk of mastitis infections. Three related experiments used a similar range of milking conditions to examine the effect of machine milking on milk flow rates, teat tissue congestion, and teat end hyperkeratosis. This is the first series of experiments that we know of that has used independent control of milking vacuum level, b-phase duration and liner compression. The results of these experiments provide a quantitative measure of the effects of the major machine related factors influencing both milking speed and teat tissue reactions. These studies provide guidance for choosing milking settings and teatcup liners to provide an optimal balance between milking quickly, gently and completely.
REFERENCES


