

MILKING METHODOLOGIES, MILK FRACTIONS AND OXYTOCIN PROFILES IN
HOLSTEIN COWS MILKED THREE TIMES DAILY

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MILKING METHODOLOGIES AND MILK FRACTIONS IN THE HOLSTEIN COWS MILKED THREE TIMES DAILY

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The current experiments were conducted to look at the impact of differing pre-milking routines on milking time parameters, milk quality and oxytocin profiles. All experiments were performed on Holstein cows milked 3X daily and producing 13-16 Kg/milking. Lag times of 0, 60, 90, 120 and 240 s along with forestripping or not forestripping were applied to 786 Holstein cows. Cows in early to mid-lactation had the highest milk yield in the first 2 min when lag time was 60 s and forestripping was included; however, lag time or forestripping had no impact on unit on-time for early to mid-lactation cows. The combination of forestripping with lag times of 90 and 120 s increased the amount of milk harvested in the first 2 min to >60% of the total and of milk and lag times beyond 60 s reduced milking unit on-time for late-lactation cows independent of forestripping.

The quantity and quality of foremilk was analyzed to evaluate the relationship between foremilk and harvested milk plate loop count (PLC) and somatic cell count (SCC). The SCC and PLC of foremilk were not good predictors of the SCC and PLC in the harvested milk fraction. The foremilk represents 0.12% of the total milk harvested and the somatic cells and bacteria found in the foremilk represent <0.28% and 1.44% of the total somatic cells and bacteria in milk.

Manual stimulation, no stimulation and mechanical stimulation by increased cycle pulsation (300 cycles/min) with low vacuum were included as treatments to analyze their

impact on oxytocin profiles and milking parameters. Increased cycle pulsation elicits a similar oxytocin profile to that of cows which were subjected to manual stimulation. Proper cleaning of the teats and detection of mastitis during mechanical stimulation needs further investigation in regard to identification of abnormal milk in real-time and by mechanical means.

BIOGRAPHICAL SKETCH

Rick is the youngest of three children born to Richard and Joann Watters. He was raised in Fall River, Wisconsin where his desire for the dairy industry began. When he was little all he wanted to do was go to his Uncles Dave and Rog's dairy farm in Columbus. During the summer he would work for his uncles by repairing pallets in the morning and then go to the farm to unload hay in the afternoon. When he turned 14 he began working for the Ladwig family who gave him many opportunities to fulfill his desire to work with dairy cattle. Upon completion of high school he attended the University of Wisconsin-Madison where he graduated with degrees in Dairy Science and Agronomy. He then entered the work force for a few years before returning to the University of Wisconsin-Madison to work on a Master's degree with Dr. Ric Grummer. His masters focused on the impact of shortening the dry period and how this influenced animal health and reproduction in the subsequent lactation. In September of 2006 he began his trek to Ithaca, New York to begin a doctoral program under the guidance of Dr. David Galton. His doctoral program focused on milking techniques in Holstein cows milked three times daily. Upon completion of his doctoral degree he will begin working in the dairy industry.

DEDICATION

I would like to dedicate my doctoral degree to my family and the late Dr. Roger Palmer.

To my family for your dedication to teaching me the values of life. You instilled in me that success comes with hard work and dedication. You picked me up during lows points and reassured me that failure is ok if I learn from it. You taught me to appreciate today and not to worry about tomorrow. I therefore, dedicate my doctor of philosophy to my family who are my foundation.

Dr. Roger Palmer took me under his wings as a young undergraduate student and was a mentor during my master's research at Madison. Dr. Palmer was the first person to ask me if I would consider graduate school. Without him I would not have considered the path that has led me to my doctoral degree. Dr. Palmer was the ultimate scientist, educator and mentor. He stood for family and friends and invited me into his home for a holiday dinner and to his ranch in Menomonie many times. It is therefore, that I dedicate my doctor of philosophy to the memory of Dr. Roger Palmer, a gentleman, friend and mentor with a vision and passion for the dairy industry.

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TABLE OF CONTENTS

BIOGRAPHICAL SKETCH	iii
DEDICATION	iv
ACKNOWLEDGMENTS	v
LIST OF FIGURES	x
LIST OF TABLES	xii
LIST OF ABBREVIATIONS.....	xiii
REVIEW OF THE LITERATURE	1
Anatomy of the mammary gland	2
Physiology of milk ejection	5
Methodologies for milking cows	10
Machine milking of cows.....	16
Alternative forms of stimulation.....	18
Summary and conclusions	19
References.....	22
THE IMPACT OF PRE-MILKING UDDER PREPARATION ON HOLSTEIN COWS MILKED THREE TIMES DAILY.....	33
Abstract.....	34
Introduction.....	36
Materials and Methods.....	37
Cows and Treatments.....	37
Milking Equipment	39
Data Acquisition	39
Results and Discussion	41
Conclusions.....	44
Acknowledgements.....	44
References.....	45
DETERMINATION OF FOREMILK QUANTITY AND QUALITY AND ITS OVERALL IMPACT ON TOTAL MILK QUALITY IN HOLSTEIN DAIRY CATTLE.....	57
Abstract.....	58
Introduction.....	60
Materials and Methods.....	62

Cows and Treatments.....	62
Milking Equipment.....	63
Sampling and Analysis.....	63
Statistical Analysis.....	64
Results and Discussion.....	65
Conclusions.....	70
Acknowledgements.....	71
References.....	72
THE EFFECT OF MANUAL AND MECHANICAL STIMULATION ON MILKING CHARACTERISTICS AND OXYTOCIN PROFILES IN HOLSTEIN COWS MILKED THREE TIMES DAILY.....	80
Abstract.....	81
Introduction.....	83
Materials and Methods.....	85
Cows and Treatments.....	85
Milking Equipment.....	86
Data Acquisition.....	87
Statistical Analysis.....	89
Results and Discussion.....	90
Conclusions.....	97
Acknowledgements.....	98
References.....	99

LIST OF FIGURES

Figure 1. Udder cross-section indicating cisternal and alveolar milk fractions, blood flow, teat anatomy and lymphatics (Frandsen, 1986).....	30
Figure 2. Alveolus with inner epithelial lining, myoepithelial cells and milk ducts(Turner, 1962), Reprinted with permission of GEA Farm Technologies.	31
Figure 3. Normal milk curve with identification of increasing, peak, decline and low flow phases and bimodal milk curve.....	32
Figure 4. Example of a bimodal milk curve and how a milk curve is evaluated for bimodality	52
Figure 5. Milking unit on-time (s) for cows following a preparation procedure that involved pre-dipping plus drying D (▲) or pre-dipping plus forestripping and drying DF (○) with the comparison treatment (a preparation procedure involving pre-dipping plus forestripping and drying with a lag time of 90 s DF90) (●) for: EM (early- to mid-lactation) cows; and LATE (late- lactation) cows. Data are presented as lsmeans with standard errors of the mean. Asterisks (*) indicate $P < 0.01$	53
Figure 6. Milk flow rate (Kg/min) for cows following a preparation procedure that involved pre-dipping plus drying D (▲) or pre-dipping plus forestripping and drying DF (○) with the comparison treatment (a preparation procedure involving pre-dipping plus forestripping and drying with a lag time of 90 s DF90) (●) for: EM (early- to mid-lactation) cows; and LATE (late- lactation) cows. Data are presented as lsmeans with standard errors of the mean. Asterisks (*) indicate $P < 0.01$	54
Figure 7. Milking unit on-time (s) for cows analyzed solely on lag time (independent of stimulation) and for cows analyzed solely for form of stimulation (independent of lag time). Early- to mid-lactation cows analyzed for lag time only (●) as compared to a lag of 90 s. Early to mid-lactation cows and pre-dipping plus drying (–) as compared to early- to mid-lactation cows and pre-dipping plus forestripping and drying (⋯). Late-lactation cows analyzed for lag time only (●) as compared to a lag of 90 s. Late-lactation cows and pre-dipping plus drying (–) as compared to late-lactation cows and pre-dipping plus forestripping and drying (⋯). Data are presented as lsmeans with standard errors of the mean. Asterisks (*) indicate $P < 0.05$	55
Figure 8. Foremilk SCC minus harvested milk SCC by harvested milk SCC category.....	78
Figure 9. Foremilk PLC minus harvested milk PLC by harvested milk PLC category.....	79
Figure 10. Bimodal milk curve.....	105
Figure 11. Oxytocin concentration (pg/mL) prior to, during and after milking for 5 treatments. Treatments were immediate attachment of the milking machine T0 (●); dip plus forestrip and drying with 30 s lag time M30(○); dip plus forestrip and drying with 90 s lag time M90 (▼); stimulation pulsation for 30 s S30 (Δ); and stimulation pulsation for 90 s S90(■). Lines with arrow depict time point when stimulation began with treatment listed above.	106

Figure 12. Oxytocin concentration (pg/mL) prior to, during and after milking for 5 treatments. At 8 minutes after milking unit attachment an injection of oxytocin was administered. Treatments were immediate attachment of the milking machine T0 (●); dip plus forestrip and drying with 30 s lag time M30(○); dip plus forestrip and drying with 90 s lag time M90 (▼); stimulation pulsation for 30 s S30 (Δ); and stimulation pulsation for 90 s S90(■). Lines with arrow depict time point when stimulation began with treatment listed above. 107

LIST OF TABLES

Table 1. Summary of pre-milking routine studies comparing milk yield, average milk flow rate and milking unit on-time.....	29
Table 2. Within-trial treatments.....	47
Table 3. Pretrial data for cows assigned to treatments (lsmeans \pm standard errors of the mean).....	48
Table 4. Within trial milk yield/milking.....	49
Table 5. Incidence of bimodal milk curves in early-to mid (EM) and late-lactation (LATE) cows	50
Table 6. Percent of total milk harvested during the first 2 min of milking unit on-time	51
Table 7. Pretrial data for the 107 Holstein cows assigned to treatments.	74
Table 8. Within-trial milk-quality data for cows analyzed based on the somatic cell count of the harvested milk fraction. Data for milk quality presented as 2.5, 50 and 97.5 percentiles.....	75
Table 9. Within-trial milk-quality data for cows analyzed based on the plate loop count of the harvested milk fraction. Data for milk quality presented as 2.5, 50 and 97.5 percentiles	76
Table 10. Foremilk and harvested milk SCC with 200,000 and 500,000 cell/mL as cut-offs....	77
Table 11. Pretrial data for 30 cows assigned to treatments.....	101
Table 12. Within-trial milk yield, milking unit on time, milk flow rates and milk quality by treatments. Manual stimulation involves forestripping and stimulation by pulsation involves 300 cycles/min with a chamber vacuum of 20 kPa.....	102
Table 13. Incidence of bimodal milk curves by treatment. Manual stimulation involves forestripping and stimulation by pulsation involves 300 cycles/min with a chamber vacuum of 20 kPa.....	104

LIST OF ABBREVIATIONS

3X = three times daily milking	D = dip + drying
d = day	DF = dip + forestrip and drying
DIM = days in milk	LS = linear score
ECM = energy corrected milk	PLS = previous linear score
EM = early-to mid-lactation	PLC = plate loop count
LATE = late lactation	FPLC = foremilk plate loop count
Kg = kilogram	TPLC = total milk plate loop count
kPa = kilopascal	SCC = somatic cell count
ME = 305 day mature equivalent	FSCC = foremilk somatic cell count
min = minutes	TSCC = total milk somatic cell count
mL = milliliters	SPC = standard plate count
n = number of samples	P5 = PLC < 10 x 10 ³ cfu/mL
OT = oxytocin	P15 = PLC 10-20 x 10 ³ cfu/mL
P = probability	P25 = PLC > 20 x 10 ³ cfu/mL
pg = picograms	S25 = SCC < 50 x 10 ³ cells/mL
PMN = polymorphonuclear neutrophils	S75 = SCC 50-100 x 10 ³ cells/mL
PP = posterior pituitary	S225 = SCC 100-350 x 10 ³ cells/mL
prep = preparation	S500 = SCC >350 x 10 ³ cells/mL
PVN = paraventricular nuclei	M30 = dip + forestripping and drying with a lag of 30 s
s = seconds	M90 = dip + forestripping and drying with a lag of 90 s
SAS = Statistical Analysis System	S30 = 30 s of high vibration stimulation
SON = supraoptic nuclei	S90 = 90 s of high vibration stimulation
wk = week	TO = immediate unit attachment
FIL = feedback inhibitor of lactation	

CHAPTER 1

REVIEW OF THE LITERATURE

ANATOMY OF THE MAMMARY GLAND

The mammalian mammary gland is a complex organ that is developed with the intent of nourishing the neonate; however, through genetic selection of today's dairy species the mammary gland produces more milk than the neonate can consume. The development of the mammary gland is in an extension of the reproductive cycle and is crucial to the survival of mammals. Without the onset of lactation and the copious secretion of milk most mammalian species would fail to exist. The mammary gland is the only external tissue that develops after birth and is common to all female mammals. Externally the mammary gland differs greatly from species to species but the internal cellular network is very similar from one gland to the next. The developed mammary gland typically consists of a teat or nipple, duct system for transport of milk, alveoli or a secretory system, and a support system.

The mammary gland is a large skin gland that can weigh as much as 50 Kg, including milk and blood. The udder has to have a very sound attachment to the external inguinal region of the body because of the extreme weight of the tissue and the fact that it is located external to the body. The udder of the bovine is comprised of four glands with the left and right halves separated by the median ligament and the front and rear glands separated by a thin membrane (a tissue septum). The median ligament makes each half almost completely independent of each other from circulatory and nervous system points of view. The median ligament is composed of an elastic fiber, whereas the lateral ligaments are composed of less elastic fibers. The rear quarters of the bovine mammary gland account for approximately 60% of the total milk yield, whereas milk yield from the left and right halves are similar.

Teats are a protuberance through which milk is removed from the mammary gland. Teats are hairless and contain a vast circuitry of arteries, veins and lymphatic vessels. Cattle and buffalo

have four quarters and one teat per quarter and sheep, goats and mares have two quarters also with one teat per quarter. Of these species only the mare has more than one teat orifice per teat, meaning that two teat openings drain from one teat; however, each teat orifice does drain a separate gland. The pig on the other hand can have from 10 to 20 teats and the number of teats on a newborn gilt is based on the number of teats on the dam and the number of males in the same litter as the gilt (Drickamer et al., 1999).

In the cow there are four separate teats, one for each quarter with each teat approximately 5 to 7 cm in length and 2 to 3 cm in diameter (Rogers and Spencer, 1991). The front teats are typically longer than the rear teats in the bovine species. The streak canal (which is approximately 11 mm long) is the first line of defense between the mammary gland and the outer environment (Weiss et al., 2004). The physical defense that the streak canal provides is dependent upon the sphincter muscle, which is under smooth muscle control. Sphincter muscles with poor tone between milkings are thought to increase the risk for an intramammary infection. Cows that leak milk are at increased risk of getting mastitis (Schukken et al., 1990, Waage et al., 1998, Barkema et al., 1999) and the cause of leaking milk might be increased cisternal milk pressure, which may occur in the absence of milk ejection (Rovai et al., 2007). Located just above the teat end and part of the internal anatomy of the teat is Furstenberg's rosette which is involved in the local defense against invading pathogens. The teat canal contains keratin which traps bacteria and seals off the teat from the external environment (Paulrud and Rasmussen, 2004). The skin around the teat is also a barrier which protects the underlying tissue from injury and temperature changes. The shape and size of the teat also play important roles in the protection of the mammary gland. The teat serves many functions from expulsion of milk to the first line of defense against invading pathogens encountered in the environment.

The nervous system of the bovine mammary gland is present in both the gland and the teat. There are sensory nerves in the teat and tissue; the nerves lining the arterial walls and the teat canal are sympathetic in nature. The glands contain nerves that lead to the spinal column and the brain. There are also many pressure-sensitive (tactile) nerve endings located in the teat. The nervous system is not involved in the synthesis of milk; however, it is integral in the initiation of the milk ejection reflex which is required to remove the majority of milk from the bovine mammary gland.

The circulatory system leading to and within the mammary gland is very extensive and provides nutrients for the millions of milk-synthesizing cells (Figure 1; (Frandsen, 1986)). The arterial system functions to supply constituents for milk synthesis to the epithelial cells. It takes between 400 and 500 volumes of blood passing through the mammary gland to produce one volume of milk. The arteries that pass from the heart and into the mammary gland will eventually become the papillary arteries within the bovine teat. After the constituents of the blood are removed for milk synthesis the blood is then transported out of the mammary gland via the venous system with the majority of the blood exiting via the external pudic and subcutaneous abdominal veins.

The mammary parenchyma is made up of alveoli (milk synthesis centers), ducts (transport) and connective tissue (Figure 2; (Turner, 1962)). The four glands of the bovine udder produce milk independent of each other. Milk synthesis takes place in the alveolar region of the mammary gland which is located closest to the inguinal region of the body. There are thousands of alveoli producing milk and they are surrounded by epithelial cells. Milk is secreted into the lumen of the alveolus by the epithelial cells. The epithelial cells are a single layer of cuboidal or columnar cells that take up nutrients from the blood during the synthesis of milk. The nutrients

taken up by the epithelial cells are precursors for lactose, protein, and fat. Milk is continuously secreted into the lumen of the alveolus; as the pressure increases, the epithelial cells begin to flatten. The flattening of the epithelial cells also flattens the blood capillaries bringing nutrients to the cells and reduces the rate of milk synthesis.

The process known as “lactation” has many intricate pathways working in unison to produce milk and an understanding of the physiological and biochemical processes taking place to produce the alveolar secretions is necessary. At the onset of lactation there is great physiological demand placed on the mother to begin lactating and to continue the development of the mammary gland. The development of the mammary gland during gestation is ultimately what determines the milk production potential of the bovine when selected for milk production. The amount of secretory tissue and the number and activity of the secreting cells are some of the limiting factors related to milk yield.

PHYSIOLOGY OF MILK EJECTION

Milk is constantly being synthesized and secreted into the lumen of the alveolus; the removal of this milk involves both the nervous and endocrine systems. There is milk that is readily available for harvest (located in the teat and gland cistern) and then there is milk that is located in the alveolar region (that cannot be harvested until the milk ejection reflex has been achieved). The supraoptic and paraventricular pathways are involved in the neuroendocrine reflex that is initiated by a form of tactile stimulation. Oxytocin (OT) is the main hormone involved in harvesting of milk from the bovine mammary gland.

The amount of milk that is available for harvest prior to the initiation of the milk ejection reflex varies by species; buffalo, cattle, sheep and goats have 5, 20, 50-70 and 60-70% of their

milk located in the cisterns (Peaker and Blatchford, 1988, Bruckmaier and Blum, 1992, Knight and Dewhurst, 1994, Pfeilsticker et al., 1996, Thomas et al., 2004). During the milking process in dairy cows, the cisternal portion of milk reaches its maximum prior to the alveolar portion reaching its maximum (Knight et al., 1994). The cisternal milk fraction begins accumulating at the end of the previous milking and increases linearly until 16 h at which point it slows (Peaker and Blatchford, 1988). The cisternal milk yield and rate at which the cisternal fraction accumulates is greatest during the peak of lactation and it decreases to its minimum in late lactation (Pfeilsticker et al., 1996, Bruckmaier and Hilger, 2001). There is a positive correlation ($r=0.90$) between lactation number and cisternal size; older cows have the largest cisternal capacity in dairy cows (Bruckmaier et al., 1994a). When the daily milking frequency increases, the amount of milk available for harvest prior to the initiation of the milk ejection reflex becomes less.

The milk stored in the alveoli is under capillary forces and requires the activation of the milk ejection reflex for the milk to be harvested. There is always milk in the alveolus as it is constantly be synthesized; however, at the end of milking there is virtually no milk present in the cisterns (Knight et al., 1994, Bruckmaier and Hilger, 2001). The milk ejection reflex is initiated when tactile stimulation in the form of pressure, stretching, or a suckling offspring takes place and causes the release of OT. Along with the release of OT an increase in mammary pressure is required to completely harvest all milk.

Oxytocin is a nonapeptide (nine amino acids) that is synthesized in both the supraoptic (SON) and paraventricular nuclei (PVN) regions of the hypothalamus. Oxytocin is involved in the milk ejection reflex, uterine smooth muscle contraction at birth, and establishment of maternal behavior. Recently OT has received focus on its relationship with social interactions (mainly in

the areas of autism and schizophrenia) (Guastella et al., 2010).

Oxytocin that has been synthesized is then transported down nerve cell axons for storage in the posterior pituitary (PP) (Crowley and Armstrong, 1992). Oxytocin, during transport to the PP and while being stored in the neurosecretory terminals of the PP, is attached to neurophysin I, which is a carrier protein for OT. Oxytocin that is stored in the PP is in an inactive state when it is bonded to neurophysins; upon tactile stimulation the neurophysins are cleaved, thus activating OT (Acher, 1960, Legros et al., 1974). The activation of OT for transport in the blood stream is achieved through the depolarization of secretory terminals by Ca^{2+} (Bruckmaier, 2001). The basal concentration of OT in blood is between 1 and 3 (picograms/milliliter) pg/mL. The reported half-life of OT is between 1 and 3.5 min (Momongan and Schmidt, 1970, Gorewit, 1979, Schams et al., 1979). An increase in blood OT concentration and the activation of the milk ejection reflex is required for the removal of the alveolar milk (which accounts for 80% of the milk in the bovine mammary gland) (Pfeilsticker et al., 1996).

The movement of milk from the alveolar compartment for milk harvest requires the activation of the neuroendocrine reflex. The milk ejection reflex is an innate reflex that is not under conscious control of the cow; a neuroendocrine reflex is necessary to begin the secretion of milk (Lincoln and Paisley, 1982, Crowley and Armstrong, 1992). The neuroendocrine reflex is typically initiated in dairy species via a form of tactile stimulation taking place at the teat. The teat contains extensive neural receptors that are pressure sensitive (Findlay, 1966). The signal that was created by tactile stimulation is then relayed to the SON and PVN portions of the hypothalamus through the inguinal canal and along the lumbar nerves (Bruckmaier and Blum, 1998). The elevation of OT in the blood and subsequent binding to the OT receptors on the myoepithelial cells in the mammary gland causes contraction of the myoepithelial cells (Soloff et

al., 1980). The contraction of the myoepithelial cells is what moves milk from the alveolar region of the mammary gland into the milk ducts which terminate at the gland cistern. The time required for OT to travel from the PP to the receptors on myoepithelial cells varies by species and can take 16.9, 24.3 and 16-29 s for sheep, goats and cattle (Labussie.J and Durand, 1970, Labussiere et al., 1999).

Oxytocin in blood is at a basal concentration between 1 and 3 pg/mL and to achieve the milk ejection reflex the concentration of OT needs to remain elevated above the threshold of 5 pg/mL throughout the milking process (Schams et al., 1984, Bruckmaier and Blum, 1996). The second part of the milk ejection reflex is the degree of udder filling. Stage of lactation and number of daily milking times effect the extent of udder filling; milk ejection may not be achieved until 1 or 2 min after the release of OT (Bruckmaier et al., 1994b, Bruckmaier and Hilger, 2001). The release of OT after stimulation leads to the ejection of milk before milk harvesting begins. The release of OT leads to an increase in intramammary pressure and is necessary for fast and complete milk removal (Schams et al., 1984, Bruckmaier et al., 1994b). The increase in intramammary pressure may result in higher milk flow rates and shorter unit on-time. Once the threshold of OT concentration is achieved along with increased mammary pressure the only way to remove additional milk in the alveolus is with an injection of exogenous OT. Higher levels of milk yield and milk flow rates are not related to higher concentrations of endogenous OT in the blood (Schams et al., 1984).

It is important that OT remain elevated during the entire milking process. The interaction between the liner and teat mimics the stimulatory effects, thus causing a continuous release of OT (Bruckmaier and Blum, 1996). This increased concentration of OT in blood during milking is important because blocking the release of OT during the milking process or blocking the

binding of OT to its receptors will inhibit milk removal (Bruckmaier et al., 1994b, Bruckmaier et al., 1997). This was again reiterated in a study that milked cows in unfamiliar settings and the OT release was inhibited--and only 50% of the total milk was harvested prior to the administration of exogenous OT (Bruckmaier et al., 1994b). This indicates that a continuous release of OT even after the milking unit is attached is required to harvest the maximum amount of the available milk.

Disturbance of milk ejection can take place at both a central and a peripheral level. Peripheral inhibition impacts the flow of OT to the mammary gland but does not impact the release of OT. Peripheral inhibition is caused by the release of catecholamines like epinephrine and norepinephrine that act on α -adrenergic receptors; however, this does not play a role in milking practices today because pharmacological doses of catecholamines are required to inhibit milk ejection. The “flight or fight” response does not inhibit the release of oxytocin but causes vasoconstriction of the vascular system and slows the flow of oxytocin to the mammary gland. Gorewit and Aromando (1985) found that peripheral inhibition reduces mammary blood flow and that inhibition of OT release was only effected with supraphysiological doses of epinephrine. Central inhibition blocks the release of OT and can be caused by milking in unfamiliar settings or by injections of endogenous opioids. Milking in unfamiliar settings reduced the amount of available milk for harvest from 79 to 9% when compared to milking in familiar settings (Bruckmaier et al., 1993). Milking in unfamiliar settings causes an increase in both cortisol and β -endorphins and it is thought that the elevated concentrations of β -endorphin causes the blockage of OT release (Bruckmaier et al., 1991). One possible pathway for the blockage by β -endorphin is that during milking in unfamiliar settings propiomelanocortin (POMC) is released as a pre-cursor to adrenocortiotropic hormone (ACTH) cleavage. Propiomelanocortin is used to

cleave ACTH and one product of ACTH cleavage is β -endorphin (Bruckmaier and Wellnitz, 2009).

Exogenous OT can be used to cause contractions of the myoepithelial cells surrounding the milk-producing alveolus and aid in the release of alveolar milk. The use of pharmacologic OT in dairy cattle for milk harvest should be used sparingly and appropriately. The continuous administration of OT over an entire lactation increased milk yield by 800 Kg (Nostrand et al., 1991). However, continuous OT injection will reduce the amount of available milk that can be harvested without the administration of OT (Graf et al., 1973). Administration of OT intramuscularly led to elevated OT concentration in blood for up to 2 hr after milking (Macuhova et al., 2004). Intramammary pressure in the udder cistern was still above basal levels 3 hr after milking in cows that were given OT intramuscularly (Macuhova et al., 2004). Belo and Bruckmaier (2010) concluded that desensitization of the mammary gland to OT occurs with extended administration of exogenous OT, which means that the same dose of OT does not elicit the same response as it had previous to the treatment. The routine of injecting exogenous OT intramuscularly may lead to a continuous release of milk from the alveolar fraction and an overall reduction in milk yield, noting that concentrations of OT were higher longer after milking.

METHODOLOGIES FOR MILKING COWS

The pre-milking routine is typically performed manually; variation in the pre-milking routine from person-to-person and day-to-day is commonplace. The pre-milking routine consists of many components designed to improve milk quality, proper milk letdown, mammary health, and milking efficiency. The pre-milking routine can involve sanitation of the teat, forestripping,

drying, and timing of milking-unit attachment. There are many factors (e.g., breed of cow, stimulation method, and timing of milking-unit attachment) that affect milking-time variables. Immediately attaching the milking unit will allow for the harvest of the cisternal milk fraction which amounts to 20% of the milk in the udder of the cow; the remaining 80% is alveolar milk that is not readily available for milk harvest until the activation of the milk-ejection reflex (1996, Bruckmaier and Blum, 1998). A form of tactile stimulation and proper preparation (prep)-lag times are required to harvest the alveolar milk fraction (which is under the control of a neuroendocrine mechanism involving the release of OT) (Bruckmaier and Blum, 1996). Prep-lag time is defined as the time from when the first form of tactile stimulation (either forestripping or drying) is administered until milking-unit attachment.

The pre-milking routine is important to initiate the milk ejection reflex prior to the removal of the cisternal milk. If the cisternal milk is removed before OT is released and mammary pressure increases then a cascade of unfortunate events may take place. These events may be observed as bimodal milk curves, hyperkeratosis, or increased milking unit on-time. Milking without proper stimulation prior to milking-unit attachment is not recommended. If milking takes place for a long time without milk flow the potential for causing teat damage increases. Situations in which removal of cisternal milk is more likely to occur prior to the release of alveolar milk are when the cistern size is small or under situations of low extents of udder filling. Low udder filling will occur more often in late-lactation cows or in situations where cows are milked more frequently (Bruckmaier and Hilger, 2001). The start of milk ejection from the alveolar fraction is not different between high and low yielding cows if the cows are in the same stage of lactation (Wellnitz et al., 1999). Milk ejection occurs sooner in cows that have longer milking intervals and are in early lactation (Bruckmaier and Hilger, 2001).

Bruckmaier and Hilger (2001) reported that the start of milk ejection following teat stimulation is related to the level of udder filling. Level of udder filling and the delay from the start of milking until milk ejection occurred followed a linear relationship (Bruckmaier and Hilger, 2001).

There have been numerous studies dating back as far as the 1950s that have looked at pre-milking routines and the effect on OT release, milk flow rates and milking unit on-time ((Bilek and Zuda, 1959, Sagi et al., 1980a, Sagi et al., 1980b, Mayer et al., 1984, Gorewit and Gassman, 1985, Rasmussen et al., 1992, Reneau et al., 1994, Wagner and Ruegg, 2002); Table 1). These studies used cattle that were milked twice daily and produced less milk than Holstein cows milked 3x daily. Degree of udder filling, which decreases with advancing stages of lactation will require additional time until maximal mammary pressure is achieved, thus increasing the time required until the milk ejection reflex is reached (Bruckmaier and Hilger, 2001). Increasing the milking frequency beyond twice daily reduces udder fill similar to what is seen in later lactation cows. Weiss and Bruckmaier (2005) indicated that a short pre-stimulation time would increase the cows per milking stall if full udders were milked and that prolonged stimulation might be beneficial when milking udders that are not full. Lengthening the lag time lowers milking unit on-time and improves milk-flow characteristics like average milk-flow rate (Bruckmaier et al., 1995, Weiss and Bruckmaier, 2005). Increasing the preparation time from 0.5 to 3 min (which includes stimulation time, sanitizing the teat and lag time) increased milk yield in Danish Jersey cows; however, it had no impact on American or Danish Holstein cows (Rasmussen et al., 1992). In a more recent study on high-producing Holstein cows there was no impact on milk yield, milking unit attachment time and/or milk flow rate when comparing animals that were forestripped or not forestripped (Wagner and Ruegg, 2002). Decreasing the

time spent on the pre-milking routine improves cow throughput but also increases capital investment due to additional cows and housing facilities. (Smith et al., 2005). However, this approach spreads the investment out over more cows and increases the return on investment. The intensity and duration of stimulation may have an impact on the amount of OT released; however, this rarely influences milk ejection if the OT threshold has been exceeded (Schams, 1983, Bruckmaier and Blum, 1996, Weiss et al., 2003). Increasing the time spent stimulating the cow by udder massage from 0 s to 120 s did not impact milk yield; however, as the stimulation time increased the average flow rate increased and the milking unit on-time decreased (Gorewit and Gassman, 1985).

The use of pre-milking disinfectants and cleaning methods has been studied for over 30 years. The use of a pre-milking disinfectants may reduce the bacteria that are both on the teat and found in milk (Kesler et al., 1948, Hoare and Roberts, 1972, Galton et al., 1986); however, other studies do not indicate a significant reduction in the bacterial count (Newbould, 1965, Edwards and Smith, 1970, Sheldrake and Hoare, 1980). The use of disinfectants such as iodophors, sodium hypochlorite, and dodecyl benzene sulfonic acid significantly reduce the bacterial count in milk (Galton et al., 1986). The use of a towel to remove organic matter and the disinfectant and drying the teat reduce the bacteria count (Galton et al., 1986) and the somatic cell count (SCC) (Skrzypek et al., 2003). A challenge study with *Streptococcus uberis* showed that the use of a wet towel plus drying and the use of a pre-milking teat disinfectant plus drying significantly reduced the number of new intramammary teat infections (Galton et al., 1988). Overall, the use of a pre-milking teat disinfectant along with drying may reduce the bacterial count of milk and reduce the risk of a new intramammary infection by reducing the bacteria count on the teat surface prior to attachment of the milking unit.

The process of forestripping (defined as removing the first two to three streams of milk from each teat prior to the initiation of the milk ejection reflex) is a method that is used to observe milk for abnormalities prior to attachment of the milking unit (Bruckmaier and Hilger, 2001, Sarikaya and Bruckmaier, 2006). The Pasteurized Milk Ordinance of the United States requires that lactating animals which show evidence of abnormal milk secretions must have their abnormal milk secretions discarded (Grade 'A' Pasteurized Milk Ordinance, 2006). The evidence for abnormal milk may be obtained based on microbiological standards, chemical procedures and/or physical observations (Grade 'A' Pasteurized Milk Ordinance, 2006). Forestripping not only allows one to inspect milk visually for abnormalities such as clots or flakes but it also is a form of tactile stimulation, which will initiate the milk ejection reflex. There has been a long-standing theory that if forestripping is omitted the risk of a cow getting mastitis increases because infectious bacteria from the foremilk will infect other animals. This may be true; however, it is also possible that a cow may become infected via bacteria that are in the milk harvested after the foremilk. It is also thought that forestripping will reduce the overall bacteria count in the harvested milk. It has been determined that the foremilk fraction represents 0.1 to 0.2% of the total milk, but it should be noted that the SCC may be 2- to 3-fold greater in the foremilk than in the harvested milk (Sarikaya and Bruckmaier, 2006). If the SCC of the harvested milk was $< 100 \times 10^3/\text{mL}$ the foremilk portion was very similar to the overall portion, however when the harvested milk portion had a SCC of $> 100 \times 10^3/\text{mL}$ the foremilk portion was significantly higher than the alveolar portions (Sarikaya and Bruckmaier, 2006). When the foremilk portion had a SCC $> 300 \times 10^3/\text{mL}$ this was not representative of the harvested milk SCC and when the foremilk SCC was between $50\text{-}300 \times 10^3/\text{mL}$ there was no difference in SCC in the foremilk and harvested milk fractions (Wellnitz et al., 2009). Wellnitz et al. (2009) found

that when the foremilk SCC was $< 50 \times 10^3/\text{mL}$ that the remaining milk harvested had a higher SCC than the foremilk. Several studies indicated that forestripping is a risk factor for mastitis or that forestripping had no influence on SCC (Elbers et al., 1998, Koester et al., 2006); however, others have found that forestripping as well as the order of forestripping within the pre-milking routine reduced the SCC in milk (Skrzypek et al., 2003). It should be stated that the documentation of forestripping alone is not the same as observing milk for abnormalities and discarding the milk. It may be that the process of observing the milk for abnormalities and removing the cow from the milk string has greater influence on mastitis risk factors than just forestripping to remove the milk at that given time.

Mastitis is the most expensive disease that occurs on today's modern dairy operation when one considers the cost of lost milk, treatments and increased risk for culling (Gill et al., 1990, Degraives and Fetrow, 1993, Seegers et al., 2003). Proper sanitation methods prior to attachment of the milking unit reduce bacteria counts and sediment in milk. One of the reasons for the pre-milking routine is to clean or disinfect the teat and observe the milk for abnormalities prior to the harvest of milk. The process of sanitizing the teat and observing the foremilk can also act as the tactile stimulation that is necessary to harvest the majority of milk. Sanitizing teats can take two different forms. The first is the use of a pre-milking disinfectant or germicide to kill any bacteria present on the teat. The second part of sanitizing the teat is the physical process, which may involve a towel or some sort of absorbent material used to remove organic matter and the disinfectant from the teat. These two processes combined are designed to provide a clean, dry, and well stimulated teat for milking. The second part of the pre-milking process is the visual observation of foremilk.

MACHINE MILKING OF COWS

The goal of removing milk from a cow is to completely remove all available milk in the fastest and most economical manner without jeopardizing the health of the mammary gland or quality of the milk (Bruckmaier and Blum, 1998). The milking routine may impact the completeness of milking because a cow that is not properly stimulated or is being milked in a stressful manner will not let down all the alveolar milk. Once the milk ejection reflex threshold concentration for OT has been reached there is no benefit to having a higher OT concentration (Schams et al., 1984). This means that the timing of the OT release in relation to milking-unit attachment is more important than the amount of OT that is released above the threshold. If the cow is properly stimulated (causing the OT concentration to exceed the threshold) and the milking unit is attached at the right time in relation to mammary pressure then 90% of the milk within the mammary gland should be harvested (Knight, 1994, Knight et al., 1994, Bruckmaier, 2003).

There are many ways to analyze the milking process e.g., flow rates, incidence of bimodal milk curves, milking unit on-time, and milk flow curves. The milk flow curve can be broken down into 4 different phases (Figure 3A). The first is the incline phase (the time required from milking unit attachment until the milk flow curve reaches the maximum sustained flow rate). The second stage is the plateau (peak) phase which is the time spent at the maximum milk flow rate. The third phase is the decline phase represented by the time from the end of the plateau until low flow. The final phase is the low-flow phase which is the time spent below the milk flow threshold for automatic detach. Each of these phases are based on time and milk flow. The goal is to have a rapid incline phase of < 45 s and then a well maintained plateau of at least 3 Kg/min for a period of time longer than the incline phase and then a rapid decline phase of less

than 1 min.

Historically milk yield, milk quality, and milking unit on-time were the key variables of interest when analyzing milking routines. Recently with advancements in milk flow-meter technology the ability to measure milk-flow curves has changed. The incidence of bimodal milk curves and percent of milk harvested in the first 2 minutes are newer parameters to evaluate milking routines. Bimodal milk curves are now analyzed as a way to determine if proper stimulation and timing are part of the pre-milking routine. A bimodal milk curve is characterized by the presence of at least one peak followed by a decrease to a nadir and a second increase after the decrease occurring prior to a specified amount of time (Figure 3B). A bimodal milk curve indicates that the cisternal milk fraction was removed prior to the alveolar milk fraction reaching the gland and teat cistern. A bimodal milk curve may mean no stimulation took or place or that the milking unit was attached too soon after stimulation. A recent study of Italian Holstein-Friesian cattle found that 35% of milk-flow curves were bimodal (which suggests poor pre-milking routines) (Sandrucci et al., 2007). Dodenhoff et al. (1999) reported higher conductivity of milk (6.7 vs. 6.3 mS/cm) and linear score (LS) (3.5 vs. 3.2) were associated ($P < 0.001$) with a higher incidence of bimodal milk curves. Determining if the change in conductivity or the incidence of a bimodal milk curve occurs first has not been determined. It is not known if over milking and/or poor teat health lead to a high LS and then bimodality. On the other hand a high LS could mean the presence of flakes or clots, which in turn could plug the teat end or cause physical damage the teat upon removal of the clot and lead to increased bimodal milk curves.

Milk that is left in the alveolar fraction after the completion of milking is referred to as “alveolar milk” and can represent 8-25% of the total milk. Residual milk can only be obtained via an injection of exogenous OT. Total milk can be determined by taking the current milk yield

and adding the residual milk to it. The breed of cow, stage of lactation, and age of cow all can influence the amount of residual milk. First-lactation cows have less residual milk than older cows; cows with lower milk yield will have a greater percentage of residual milk when compared to higher yielding cows (Schmidt, 1971). Dutch Friesian and Russian Red Steppe cows had 9 and 25% residual milk respectively (Brandsma, 1978). A more recent study indicated that cows with < 8% residual milk had elicited a normal milk ejection whereas those cows with > 8% residual milk had an interrupted milk ejection (Negrao and Marnet, 2006). A study with robots and the use of brushes, vibration stimulation and the combination of both reported similar levels (14.8-15.9%) of residual milk across all treatments (Macuhova et al., 2003).

ALTERNATIVE FORMS OF STIMULATION

The initiation of the milk ejection reflex has historically been achieved via contact between the human hand and the teat. Stimulation can also be achieved through the use of the milking equipment as witnessed by the constant release of OT once the milking unit is attached. Even without proper pre-stimulation prior to attachment of the milking unit one can still harvest the alveolar fraction of milk; however, an interruption in milk flow may be seen as the cisternal fraction is removed prior to the alveolar milk being ejected (defined above as bimodal milk flow). The attachment of the milking unit in the milking phase is a form of stimulation to which the pressure-sensitive neural receptors on the teat will respond. With the advancement of milking technology and the introduction of robots, forms of stimulation other than the human hand have been used. Forms of mechanical stimulation that have been used are rotating brushes, different forms of pulsation, and simply the attachment of the milking unit in the milk mode. One of these mechanical stimulation methods is the use of an alternative form of pulsation that cycles 300/min with a maximum vacuum level of 20-22 kilopascals (kPa), whereas standard

pulsation would cycle at 60/min with a vacuum level of 40-46 kPa. Worstorff et al. (1987) investigated the use of a high-cycle and low-vacuum pulsation (300 cycles/min at 20-22 kPa; “vibration stimulation”) as compared to manual stimulation and determined that vibration stimulation was an adequate method to cause the ejection of milk from the alveolar region. The use of high-vibration pulsation was applied for 0, 20, 40, 60 or 90s and no difference was detected in milk yield; however, as the vibration time increased so too did the average milk flow rate (Weiss and Bruckmaier, 2005). Positive pressure and high-speed pulsation were two other forms that Sagi et al.(1980b) investigated as forms of stimulation. Sagi et al. (1980b) reported no difference in milk yield when comparing manual and mechanical stimulation. High-speed pulsation had the highest unit on-time when compared to manual stimulation; however, it was not stated if time spent in stimulation phase was included in the unit on-time (Sagi et al., 1980b). The difference in unit on-time between manual stimulation and high-speed pulsation was 60 s and high-speed pulsation was turned on for 60 s, therefore it is possible that unit on-time was similar between mechanical and manual stimulation (Sagi et al., 1980b).

SUMMARY AND CONCLUSIONS

The harvest of 80% of the milk depends on the release of OT and the activation of the milk ejection reflex. An increase in mammary pressure is required along with the increase in OT to harvest the alveolar fraction of milk. There is a longstanding belief that if the prep-lag time is extended beyond 60 s or if forestripping is omitted as part of the pre-milking routine, completeness of milking will not be achieved. Incomplete milking or an increase in residual milk which may be caused by incomplete milk ejection leads to an increase in feedback inhibitor of lactation (FIL). Feedback inhibitor of lactation is a milk protein that is synthesized in secretory cells and its action reduces milk secretion by secretory cells (Wilde and Peaker, 1990).

Extending the prep-lag time beyond 60 s is thought to exceed the half-life of OT and therefore the effects of OT on the mammary gland will be diminished. The complete omission of forestripping from the pre-milking routine is thought to reduce the amount of OT released and reduce milk quality. The data do suggest that increasing the lag time (time between stimulation and unit attachment with effective milking vacuum and pulsation) has no effect on milk yield and increases the average flow rate while decreasing the milking unit on time (Gorewit and Gassman, 1985, Bruckmaier et al., 1995, Weiss and Bruckmaier, 2005). Extending the lag time from 30 s to 180 s increased milk yield in the Jersey breed but had no effect on the Holstein breed (Rasmussen et al., 1992). Increasing the milking frequency reduces udder filling and milking udders with less mammary pressure may require additional prep-lag time (Weiss and Bruckmaier, 2005). However, the majority of data to date consists of cows that are not entirely Holstein, milked 2x daily and/or have milk production levels < 10,000 Kg/lactation.

The release of OT and activation of the milk ejection reflex is an innate response. Udder filling is the other components that is effected by stage of lactation and milking intervals. The lag time chosen may be influenced by the stage of udder filling. It is not that the form of stimulation is important, but rather the timing of the tactile stimulation prior to the attachment of the milking unit. The attachment time may vary based on the degree of udder filling--which is influenced by stage of lactation and frequency of milking. Increasing the prep-lag time beyond 60 s in Holstein cows milked 3x daily may improve milking efficiency. Extending the lag-time is hypothesized to improve milking efficiency by: 1) increasing mammary pressure prior to milking unit attachment; 2) increasing average milk flow rate; 3) reducing the incidence of bimodal milk curves; and 4) reducing milking unit on-time.

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Table 1. Summary of pre-milking routine studies comparing milk yield, average milk flow rate and milking unit on-time

Author/yr	Comparison	Difference
Sagi et al. 1980 (mechanical)		
Sagi et al. 1980 (prep and oxy)		
Mayer et al. 1984	No stimulation vs. manual stimulation + 60 s lag time	Increased milk flow rate for manual stimulation + 60 s lag Decreased unit on-time for manual stimulation + 60 s lag
Gorewit and Gassman 1985		
Reneau and Farnsworth 1994		
Rasmussen et al. 1992	Increasing lag time from 0.5 to 3.0 min	Decreased milk yield in Danish Jersey cows
Bruckmaier and Blum 1996	No stimulation vs. 60 s of manual stimulation	No difference in milk yield Increased unit on-time for no stimulation
Wagner et al. 2002	Forestripping compared to not forestripping	No difference in average milk flow rate No difference in milk yield No difference in average milk flow rate
Weiss and Bruckmaier 2005	Vibration stimulation (300 cycles/min) for 0, 20, 40, 60 or 90 s	No difference in milk yield across all treatments Increasing vibration time increased average milk flow rate
Kaskous and Bruckmaier 2011	Manual stimulation for 15, 30 or 45 s followed by latency times of 0, 30, 45 or 60 s within different degrees of udder filling	No difference in milk yield across all treatments Milking unit on-time longest when udder filling < 40% and no latency period allowed independent of length of stimulation

Figure 1. Udder cross-section indicating cisternal and alveolar milk fractions, blood flow, teat anatomy and lymphatics (Frandsen, 1986)

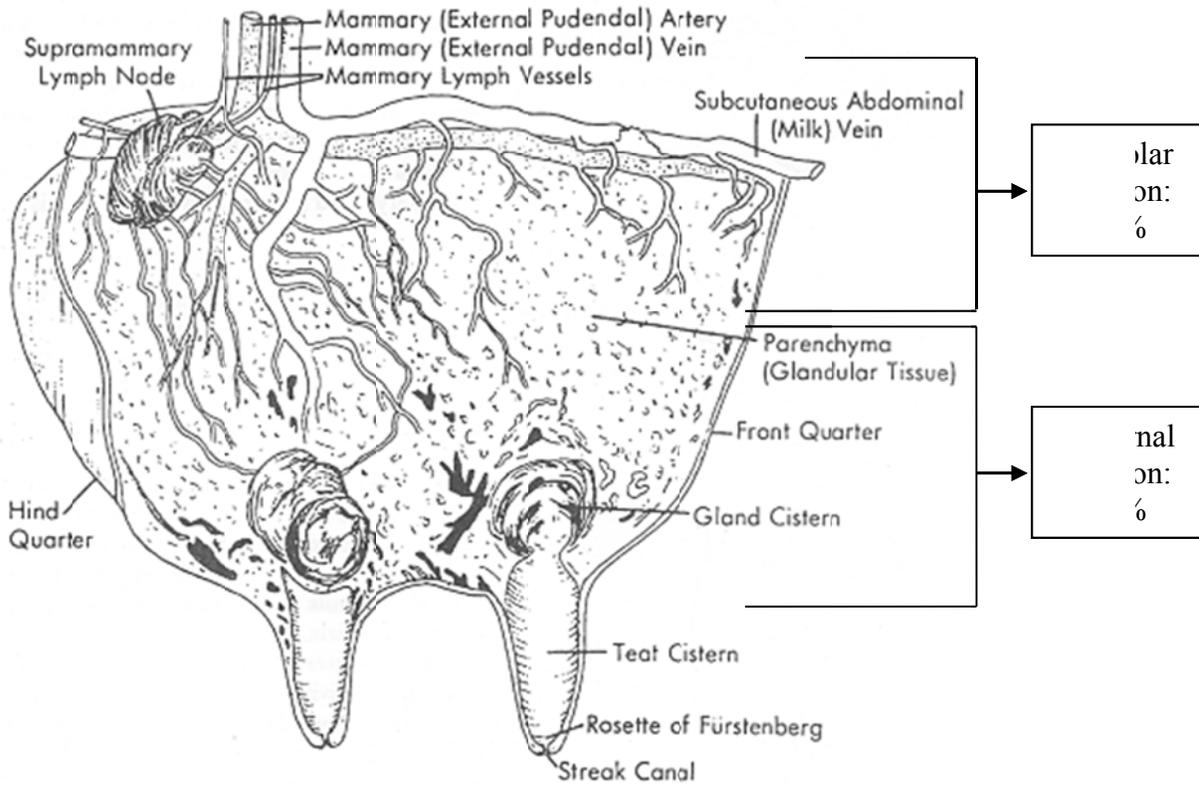


Figure 2. Alveolus with inner epithelial lining, myoepithelial cells and milk ducts(Turner, 1962), Reprinted with permission of GEA Farm Technologies.

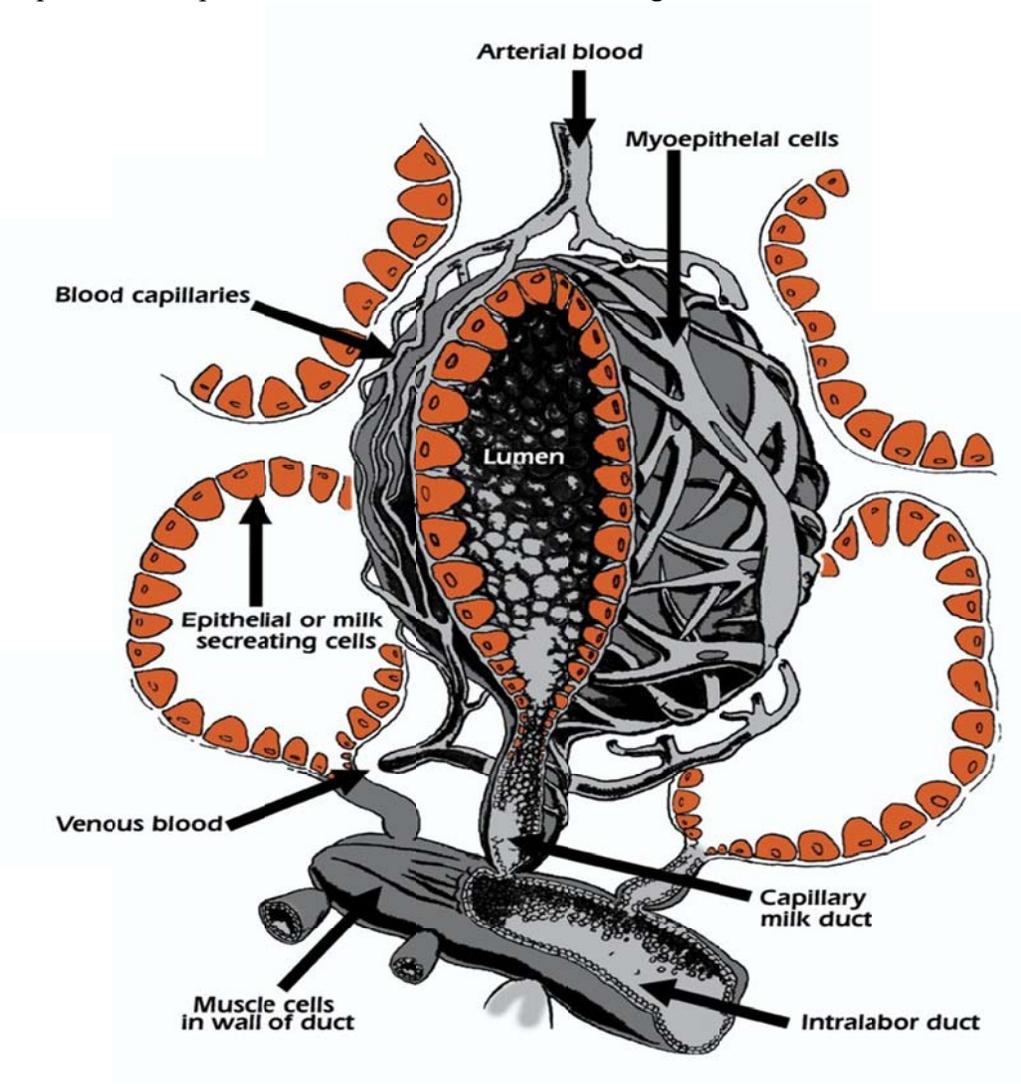
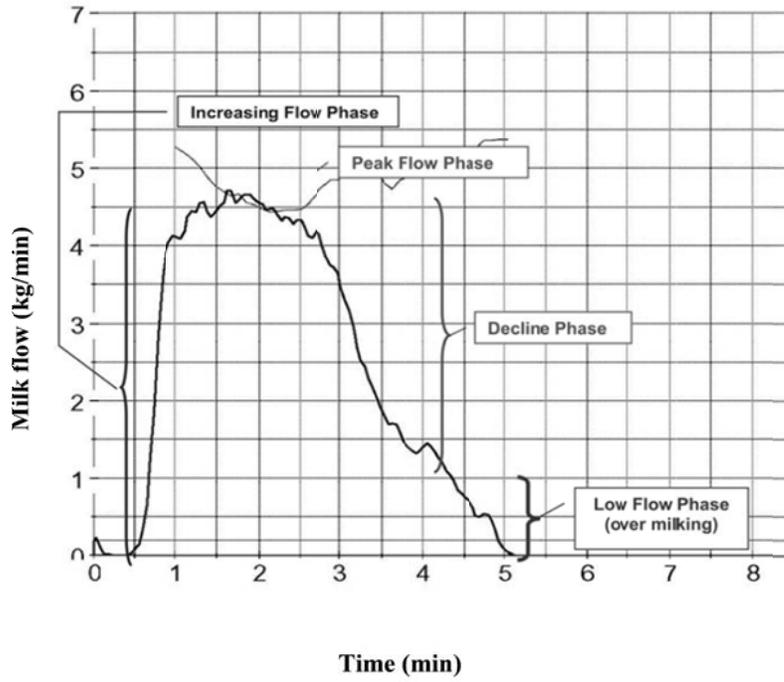
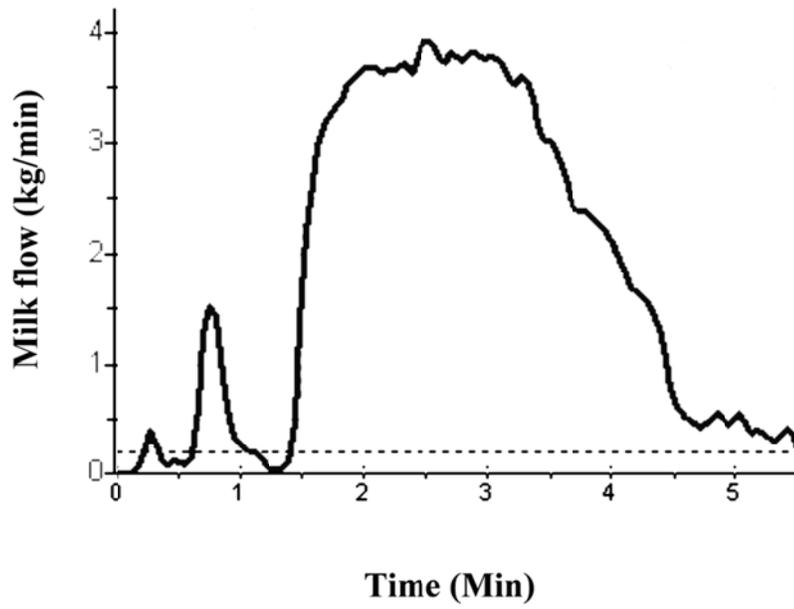


Figure 3. Normal milk curve with identification of increasing, peak, decline and low flow phases and bimodal milk curve

A)



B)



CHAPTER 2

THE IMPACT OF PRE-MILKING UDDER PREPARATION ON HOLSTEIN COWS MILKED THREE TIMES DAILY

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ABSTRACT

Pre-milking udder preparation (including forestripping and duration of lag time—the time between first tactile stimulation and milking unit attachment) might influence milking measures such as milking unit on-time, incidence of bimodality and milk flow rates in Holstein cows milked three-times daily (3X). Holstein cows (n=786) from an 1,800-cow commercial dairy herd were enrolled under a restricted randomized design to determine the impact of 9 different pre-milking routines on milk yield, milking unit on-time, incidence of bimodal milk curves, percentage of milk in the first 2 min and flow rates. Lag times were: 0, 60, 90, 120 and 240 s and included forestripping or no forestripping for a total of 9 treatments conducted from February to November 2008. All cow-treatment combinations were compared to the control: pre-dipping plus forestripping and drying with 90 s of lag time (DF90; used as the control because previous data suggested that a prolonged stimulation time was beneficial when milking cows with less udder pressure (Weiss and Bruckmaier, 2005)). Cows were initially assigned to one of three treatments for a period of 7 d and upon completion of the first 7-d period were re-assigned to a different treatment until all treatments had been completed. Early- to mid-lactation (EM) and late-lactation (LATE) cows were housed in two different pens. Milk yield was significantly different from DF90 for two of the treatments for EM cows; however, this was not thought to be due to treatments because the only significant lag times were so different (60 and 240 s) and yet not both extremes. There was no difference in milk yield for the LATE cows. There was no difference in milking unit on-time when comparing all treatments for EM to DF90; however, when lag time was 60 s or less for LATE cows there was an increase in milking unit on-time. The highest incidence of bimodal milk curves was when lag time = 0 and this was independent of stage of lactation; a lag time of 240 s had the second-highest incidence rate of

bimodal milk curves for EM and LATE cows. Milk harvested in the first 2 min was lower than DF90 when lag time = 0 or 240 s. Increasing the lag time for all cows appeared to improve overall milking time efficiency (noting that lag time had no impact on EM cows).

(Key words: pre-milking routine, udder preparation, lag time, milking unit on-time, bimodality)

INTRODUCTION

The pre-milking routine is typically performed manually; variation in the pre-milking routine from person-to-person and day-to-day is commonplace. The pre-milking routine consists of many components designed to improve overall milk quality, proper milk letdown, mammary health, and milking-time efficiency. The pre-milking routine can involve sanitation of the teat, forestripping, drying, and timing of milking-unit attachment. There are many factors (e.g., breed of cow, stimulation method, and timing of milking-unit attachment) that affect milking-time variables. Immediately attaching the milking unit will allow for the harvest of the cisternal milk fraction which amounts to 20% of the milk volume in the udder; the remaining 80% is alveolar milk that is not readily available for milk harvest until the activation of the milk-ejection reflex (1996, Bruckmaier and Blum, 1998). A form of tactile stimulation and proper prep-lag times are required to harvest the alveolar milk fraction (which is under the control of a neuroendocrine mechanism involving the release of oxytocin) (Bruckmaier and Blum, 1996). Prep-lag time is defined as the time from when the first form of tactile stimulation (either forestripping or drying) is administered until milking-unit attachment. The release of oxytocin and its subsequent binding to receptors on myoepithelial cells leads to the expulsion of the alveolar milk fraction.

A summary of studies from the past 30 yr indicated that stimulation of at least 20 s and a total prep-lag time of 60 s reduced milking unit on-time and increased the average flow rate when compared to no stimulation (Reneau and Chastain, 1995). Those studies were performed on cows that were either milked twice daily, were crossbred cattle, or had levels of milk production that were importantly lower than today's high producing cows. Weiss and Bruckmaier (2005) indicated that a short pre-stimulation time would increase the cows per milking stall if full udders were milked and that prolonged stimulation might be beneficial when

milking udders that are not full. Lengthening the lag time lowers milking unit on-time and improves milk-flow characteristics like average milk-flow rate (Bruckmaier et al., 1995, Weiss and Bruckmaier, 2005). Decreasing the time spent on the pre-milking routine improves cow throughput and has an impact on farm financials. Increasing cow throughput may also increase capital investment due to the need of additional cows and housing facilities (Smith et al., 2005). However, increasing cow throughput and the addition of more cows spreads the investment out over more cows and increases the return on investment.

Much of the focus historically has been placed on milk yield and milking unit on-time as the key variables of interest. More recently (with advancements in milk flow-meter technology) the ability to measure milk-flow curve characteristics more accurately has increased. The incidence of bimodal milk curves and percent of milk harvested in the first 2 min are newer parameters to evaluate milking routines. A bimodal milk curve is defined as a milk flow curve having a peak in milk flow followed by a decrease and then increasing again within a specified period of time. A recent study of Italian Holstein-Friesian cattle found that 35% of milk-flow curves were bimodal (which suggests poor pre-milking routines) (Sandrucci et al., 2007).

Our objective was to determine the impact of the forestripping, lag time and their interaction on milking characteristics of Holstein cows with varying DIM and milked 3X. We hypothesized that increasing the lag time to greater than 60 s would reduce milking unit on-time. The milking characteristics of interest were milk yield, milking unit on-time and milk-flow rates.

MATERIALS AND METHODS

Cows and Treatments

Holstein cows (n=786) from a 1,800-cow commercial dairy herd were enrolled in a restricted randomized design, noting that cows had to switch method of stimulation from one

period to the next, but not lag time. Cows were housed in a 6-row freestall barn and bedded with a combination of kiln-dried sawdust and drywall gypsum. Cows were fed a total mixed ration that met or exceeded the NRC requirements. The mature equivalent (305ME) of the herd was 13378 Kg/lactation with a median lactation number of 2. The 305ME is a predicted value for milk yield that is adjusted for age and stage of lactation. Cows were milked 3X daily on a 50-bail rotary parlor. The experiment was conducted from February through November of 2008. Early- to mid-lactation cows (**EM**; 17-167 DIM) and late-lactation cows (**LATE**; 174-428 DIM) were housed in separate pens for the study. Pen size was 200 and 220 cows for EM and LATE cows, respectively. All cows had to be ≤ 400 DIM at the time of enrollment in the study. All cows had to have four functioning quarters and could not have had a case of clinical mastitis during the current lactation. The treatments involved two forms of stimulation and five lag times. The stimulation methods were predipping plus drying (**D**) and predipping plus forestripping and drying (**DF**). Lag time was timed from first form of tactile stimulation (either forestripping or drying) and continued until milking-unit attachment and was 0, 60, 90, 120, or 240 s. This resulted in 9 total treatments (there is no DF0 because there is no manual stimulation with immediate attachment; Table 2). Cows were randomly assigned via a computer generated list to one of three treatments initially. Cows were balanced for milk yield, unit on-time, milk yield in the first 2 min, DIM and parity. A treatment lasted for 7 d with the first 3 d (9 milkings representing an adaption period) and the last 4 d (12 milkings representing the data-collection period), which took place between February and November, 2008. A maximum of three treatments were administered during any period. Upon completion of a 7-d period, cows were reassigned to another treatment but had to switch method of stimulation from one period to the next. There was no restriction on lag time other than that a cow never repeated the same

treatment combination. A mutual agreement was reached prior to the initiation of the experiment as to the level of participation required by the producer; thus, an informed-consent agreement was reached between the dairy producer and Cornell University. Cornell University's Institutional Animal Care and Use Committee approved the experimental protocol.

Cows were identified by leg bands as to which lag time and stimulation method they were to receive. Consistent lag time was achieved by starting a stopwatch at first tactile stimulation of the cow and then attaching it to the stall that the cow occupied on the rotary parlor. Parlor operators pressed the start button for milking-unit attachment when the stop watch read 5 s less than the indicated lag time for the given treatment. Pre-milking routine was performed by laborers on the farm and milking unit attachment was completed by undergraduate research assistants.

Milking Equipment

Cows were milked 3x daily on a 50-bail rotary parlor (GEA Farm Technologies, Bönen, Germany). The milking system had a vacuum setting of 50 kPa and pulsation rate of 60 cycles/min and ratio of 65:35. The milking claw had a volume of 300 mL (Classic 300, GEA Farm Technologies, Bönen, Germany) and was used with a silicone liner that had a collapse force of 33 kPa (Tri-Circle Silicone Liner, Lauren AgriSystems, New Philadelphia, OH).

Data Acquisition

During both the adaptation and data-collection phases, cow and milking data were gathered on-farm by herd-management software (DairyComp 305, Valley Agricultural Software, Tulare, California; DairyPlan C21, GEA Farm Technologies, Bönen, Germany).

Bimodal milk curves were determined by DairyPlan software (DairyPlan C21, GEA Farm Technologies, Bönen, Germany). A bimodal milk could only be calculated once 500 g of

milk had been harvested (Figure 4). Upon the harvest of 500 g of milk there was 60 s allowed for a bimodal milk curve to occur. An increase in milk flow from 500 g followed by a decrease and then 2 increases had to occur within 60 s in order for a bimodal calculation to occur. The bimodal calculation divides the lowest flow rate by the highest flow rate prior to the lowest and multiplies it by 100. If the lowest values divided by the highest value was < 72% then a bimodal milk curve occurred.

Statistical Analysis

Separate statistical analyses were performed for EM and LATE cows. Milking parameters measured were: milk yield, milking unit on-time, incidence of bimodal milk curves, percentage of total milk yield harvested in the first 2 min of unit on-time, and average flow rate. Results are reported as least-square means \pm standard error of the means (LSM \pm SEM; from SAS (SAS, 1999)) unless otherwise noted. Data were analyzed as repeated measures utilizing the Proc Mixed procedure. The model used to analyze the experiment was:

$$Y_{ijk} = \mu + M + D_i + S_j + L_k + S_j \times L_k + Re_{ijk}$$

where Y_{ijk} = variable of interest, μ = overall mean, M_i = milk yield, D_i = days in milk (1 to 2), S_j = method of stimulation (1 to 2), L_k = lag time (1 to 5), $S_j \times L_k$ = interaction of stimulation and lag time, R is the correlation matrix in the error term, correcting for repeated effect of cow within treatment and e_{ijk} = random error. The correlation structure in the repeated measures effect that was used in the model was compound symmetry. The variables of interest were milking unit on-time, percent of milk harvested in the first 2 min and average milk flow rate. Bimodal milk curves were analyzed using Proc Freq (chi-squared) methodology in SAS (SAS, 1999). Significant differences were declared at $P < 0.05$.

RESULTS AND DISCUSSION

Pretrial data were gathered for milk yield, milking unit on-time, milk yield in the first 2 min, average flow rate, DIM and lactation number (Table 3). A total of 817 cows were assigned to the trial and 786 were used for analysis. Of the 31 cows that left the study, 16 cows were from the EM group and 15 were from the LATE group. Cows were removed for mastitis (n=14; EM=9 and LATE=5), feet and legs (n=5; EM=2 and LATE=3), ketosis (n=3; EM=3), teat injury (n=2; EM=1 and LATE=1), loss of electronic ID (n=2; LATE=2), oxytocin injection (n=1; EM=1) and other (n=4; LATE=4).

Mean milk production per milking for DF90 was 16.7 ± 0.3 Kg for EM and 10.4 ± 0.3 Kg for LATE during the trial (Table 4). The differences in milk yield are not thought to be due to treatments because the lag time was so different (60 and 240 s) between the treatments that differed from DF90—and yet were not seemingly part of consistent trends. Sagi et al. (1980) found no difference in milk yield when comparing no stimulation, manual stimulation, manual stimulation with lag time of 30 min, and a treatment involving the injection of oxytocin. Our data for EM cows agree with their study--noting that all cows on Sagi's experiment were between the third and sixth mo of lactation. Wagner and Ruegg (2002) found no difference in milk yield for Holstein cows milked twice daily when comparing cows that were forestripped or not forestripped and independent of stage of lactation. In contrast to our results, Rasmussen et al. (1992) found a tendency for a decrease in milk yield for Holstein cows when lag time was 3 min as compared to a lag time ≤ 1.3 min, regardless of stage of lactation. Tancin et al. (2007) determined that a prep-lag time totaling 60 s had numerically, but not significantly higher milk yield (2.8 ± 0.9 kg) when compared to a prep-lag time of 10 s

The mean milking unit on-time for DF90 was 279 ± 4.7 s and 236 ± 3.8 s for EM and

LATE. There was no difference in unit on-time for EM ($P = 0.22$) cows when compared to DF90; however, on-time for LATE was longer ($P < 0.01$) than DF90 for lag times of ≤ 60 s (Figure 5). In contrast, Sandrucci et al. (2007) found the shortest on-time when lag times were between 1 and 60 s. Gorewit and Gassman (1985) found that on-time decreased when stimulation time was increased from no stimulation to 15, 30, 60 and 120 s of stimulation; however, what they did not account for was the interaction between increasing stimulation time and the fact that it also increases lag time. As the lactation progressed beyond 24 wk, Merrill et al. (1987) indicated that milking unit on-time decreased when comparing a full pre-milking routine (60 s) to a minimal pre-milking routine (15 s), which agrees with our data for EM cows.

The incidence of bimodal milk curves differed from DF90 for all but two treatments ($P < 0.05$) for EM cows and for all but three treatments for LATE cows (Table 5). A recent field study analyzing $> 2,400$ milk-flow curves from 82 Italian Holstein-Friesian cows indicated that 35.1% of milk curves were bimodal and found a significant decrease in the incidence of bimodal milk curves as the lag time was increased (Sandrucci et al., 2007). A bimodal milk curve is an indicator of an improper milk routine; however, the impact of bimodal milk curves on udder health is unknown. Dodenhoff et al. (1999) found that a higher electrical conductivity of milk (6.7 vs. 6.3 mS/cm) and LS (3.5 vs. 3.2) were associated ($P < 0.001$) with a higher incidence of bimodal milk curves but the time sequence (which came first—the change in conductivity or in incidence) is unknown.

The percentage of milk harvested in the first 2 min for DF90 was $51.7 \pm 1.4\%$ and $61.5 \pm 1.8\%$ for EM and LATE cows, respectively. For EM cows most treatments didn't differ from DF90; however, all but two treatments differed from DF90 for LATE (Table 6). Reid and Stewart (2001) indicated across all cows that 57% of milk was harvested in the first 2 min on an

800-cow dairy; we found that 50-55% of milk was harvested in the first 2 min if lag time was ≥ 60 s and < 240 s for EM and 53-61% of milk was harvested if lag time was ≥ 60 s for LATE. The cistern fraction of milk decreases from 18% to 13% when comparing mo 2 and 3 of lactation to mo 10 and 11 (Bruckmaier et al., 1994) and this may explain why the longer lag time showed a higher percentage of milk harvested in the first 2 min for LATE cows.

The mean average flow (Kg/min) for DF90 was 3.9 ± 0.07 and 3.0 ± 0.05 for EM and LATE (Figure 6). Average flow only differed for LATE cows and only for them if the lag time was < 90 s. Merrill et al. (1987) found that the average flow rate increased beyond wk 24 of lactation when a full pre-milking routine (60 s) was used instead of a minimal pre-milking routine (15 s). Wagner and Ruegg (2002) found that the milk flow rate was 0.36-Kg/min higher for high-producing cows when compared to low-producing cows; the increase in flow rate with high-producing cows is in agreement with our study (the EM cows had a flow rate that was 0.9 Kg/min higher than LATE cows). In contrast to our study, Wellnitz et al. (1999) found no difference between high-producing cows and low-producing cows (2.8 and 2.5 Kg/min). The EM cows on our experiment produced > 45 Kg/d whereas the high-producing cows in the previously mentioned study produced 24 Kg/d (and even our low-producing LATE cows produced >30 Kg/d); this higher production level may explain why we (and not the other authors) saw differences in the flow rates.

When analyzing the data for milking unit on-time and based solely on form of stimulation (thus independent of lag time), the amount of time the milking unit is on for complete milkout is virtually the same: 283 (D) vs. 279 (DF) and 245 (D) vs. 242 (DF) for EM and LATE (Figure 7). When analyzing all data for milking unit on-time (and based solely on lag time, thus independent of form of stimulation), there was no difference in treatments when comparing to DF90 for EM;

however, when lag time was < 90 sec there was a significant difference ($P < 0.05$ for all treatments) compared to DF90) for LATE (Figure 7). One can see that as lag time increases the amount of time it takes for complete milkout decreases for LATE.

CONCLUSIONS

The interaction of forestripping with lag time had the greatest impact as indicated by the shortest milking unit on-times and the highest amount of milk harvested in the first 2 min of milk. Late-lactation cows showed an increased benefit from a longer lag time as indicated by shorter unit on-time. Increasing lag time beyond 60 s did not have a negative effect on EM cows, however decreased milking unit on-time and increased milk flow rates were seen for LATE cows when lag time was increased beyond 60 s for cows milked 3X daily.

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Table 2. Within-trial treatments

Lag (s)	Dip + Dry (D)	Dip + Forestrip (DF)
0	D0	
60	D60	DF60
90	D90	DF90
120	D120	DF120
240	D240	DF240

Table 3. Pretrial data for cows assigned to treatments (lsmeans \pm standard errors of the mean)

Variable	EM ¹		LATE ¹	
	Mean	SEM	Mean	SEM
N (analysis)	397		389	
N (assigned)	413		404	
Milk yield/milking (Kg)	16.3	0.04	12.4	0.03
Milking unit on-time (s)	284	0.6	247	0.8
Milk first 2 min of unit on-time (%)	50.5	0.2	55.4	0.3
Average flow (Kg/min)	3.8	0.02	3.2	0.03
Parity	2.9	0.03	2.9	0.04
DIM	80	0.4	270	0.7

¹Stage of lactation are: EM = early to mid-lactation; LATE = late-lactation

Table 4. Within trial milk yield/milking

Lag (s)	EM ¹ (Kg/milking) (<i>P</i> < 0.001)		LATE ¹ (Kg/milking) (<i>P</i> = 0.21)	
	Dip + Dry (D)	Dip + Forestrip (DF)	Dip + Dry (D)	Dip + Forestrip (DF)
0	16.7		10.7	
60	16.0	15.1 ^a	10.6	10.6
90	16.9	16.7	10.7	10.4
120	16.0	15.9	10.2	10.7
240	16.3	15.2 ^a	10.2	10.9

¹Stage of lactation are: EM = early-to mid-lactation; LATE = late lactation; comparisons were made against dip + forestrip and 90 (DF90) s of lag time as indicated by a checkered box and within stage of lactation.

^aTreatments differ from DF90 within the same column at *P* < 0.01 (Bonferroni correction)

²Common SE for the data set is 0.04

Table 5. Incidence of bimodal milk curves in early-to mid (EM) and late-lactation (LATE) cows

	Lag	Dip + Dry (D)		Dip + Forestrip (DF)	
		Total	Bimodal (%)	Total	Bimodal (%)
EM ¹	0	1261	22 ^{ab}		
	60	496	9 ^{ac}	487	9 ^{ab}
	90	560	3 ^{ad}	658	5 ^c
	120	1142	8 ^{ac}	1114	6 ^{bc}
	240	585	13 ^{ae}	662	13 ^{ac}
LATE ¹	0	1035	29 ^{ab}		
	60	422	9 ^c	493	7 ^b
	90*	516	18 ^{ad}	457	6 ^b
	120*	994	15 ^{ad}	995	8 ^b
	240	487	26 ^{ab}	604	20 ^{ac}

^a $P < 0.01$ when compared to dip + forestrip and 90 s of lag time (DF90) as indicated by the checkered box (Bonferroni correction)

^{bcd} Comparisons made within row and within stage of lactation differ at $P < 0.01$

*Comparisons made within row differ at $P < 0.05$

¹EM and LATE differ at $P < 0.001$ when compared to DF90

Table 6. Percent of total milk harvested during the first 2 min of milking unit on-time

Lag	EM ¹		LATE ¹	
	Dip + Dry (D), %	Dip + Forestrip (DF), %	Dip + Dry (D), %	Dip + Forestrip (DF), %
0	44.0 ^{ab}		44.7 ^{ab}	
60	52.5 ^c	55.8 ^{ab}	55.2 ^{ac}	53.1 ^{ab}
90	50.3 ^{cd}	51.0 ^c	55.9 ^{ac}	61.6 ^c
120	51.7 ^c	52.8 ^c	57.3 ^{ac}	61.3 ^c
240	48.0 ^d	51.2 ^c	57.2 ^{ac}	57.4 ^{ab}

¹Stage of lactation are: EM = early-to mid-lactation; LATE = late lactation; comparisons were made against dip + forestrip and 90 (DF90) s of lag time as indicated by a checkered box and within stage of lactation.

^aTreatments differ from (DF90) at P < 0.01.

^{bcd}Comparisons made within row and within stage of lactation differ at P < 0.05

Figure 4. Example of a bimodal milk curve and how a milk curve is evaluated for bimodality

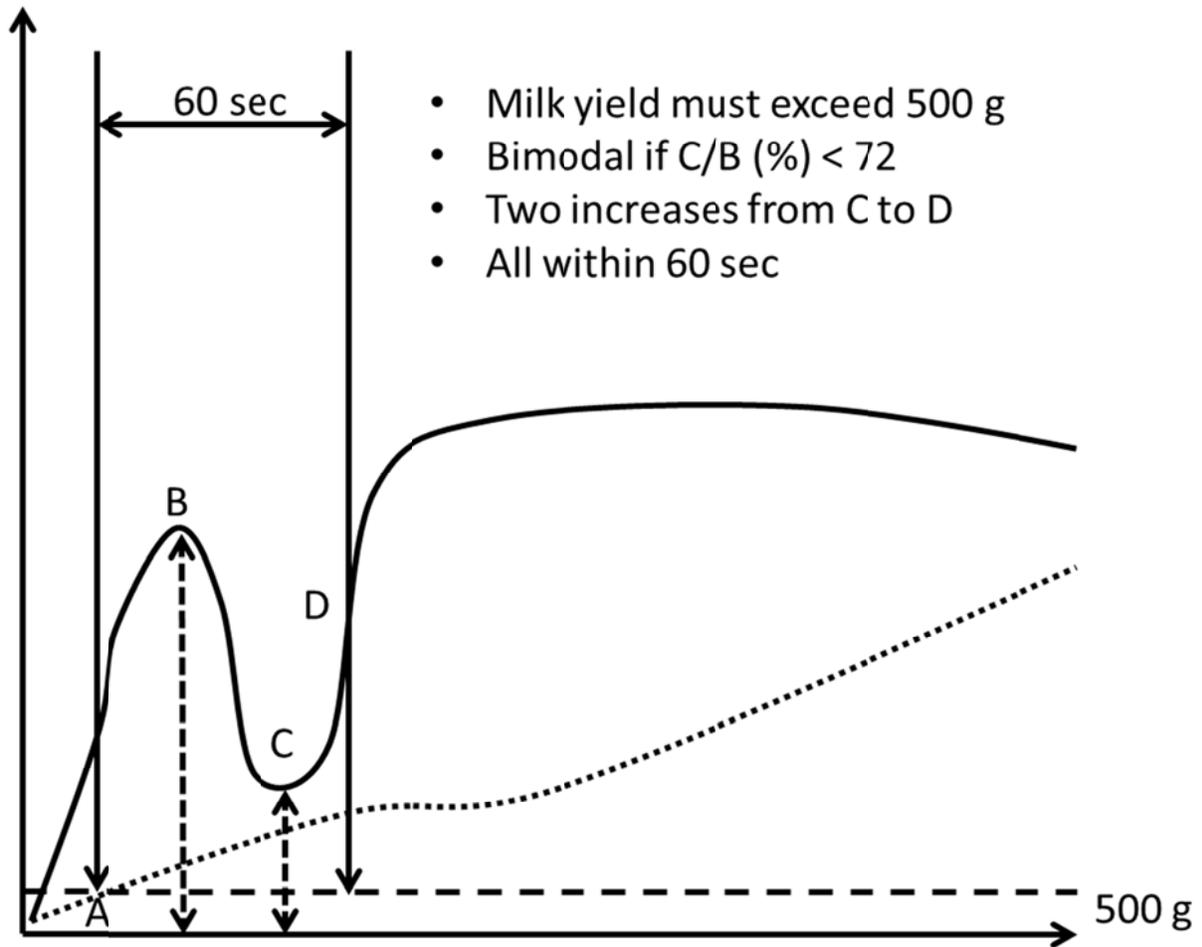


Figure 5. Milking unit on-time (s) for cows following a preparation procedure that involved pre-dipping plus drying D (▲) or pre-dipping plus forestripping and drying DF (○) with the comparison treatment (a preparation procedure involving pre-dipping plus forestripping and drying with a lag time of 90 s DF90) (●) for: EM (early- to mid-lactation) cows; and LATE (late-lactation) cows. Data are presented as lsmeans with standard errors of the mean. Asterisks (*) indicate $P < 0.01$.

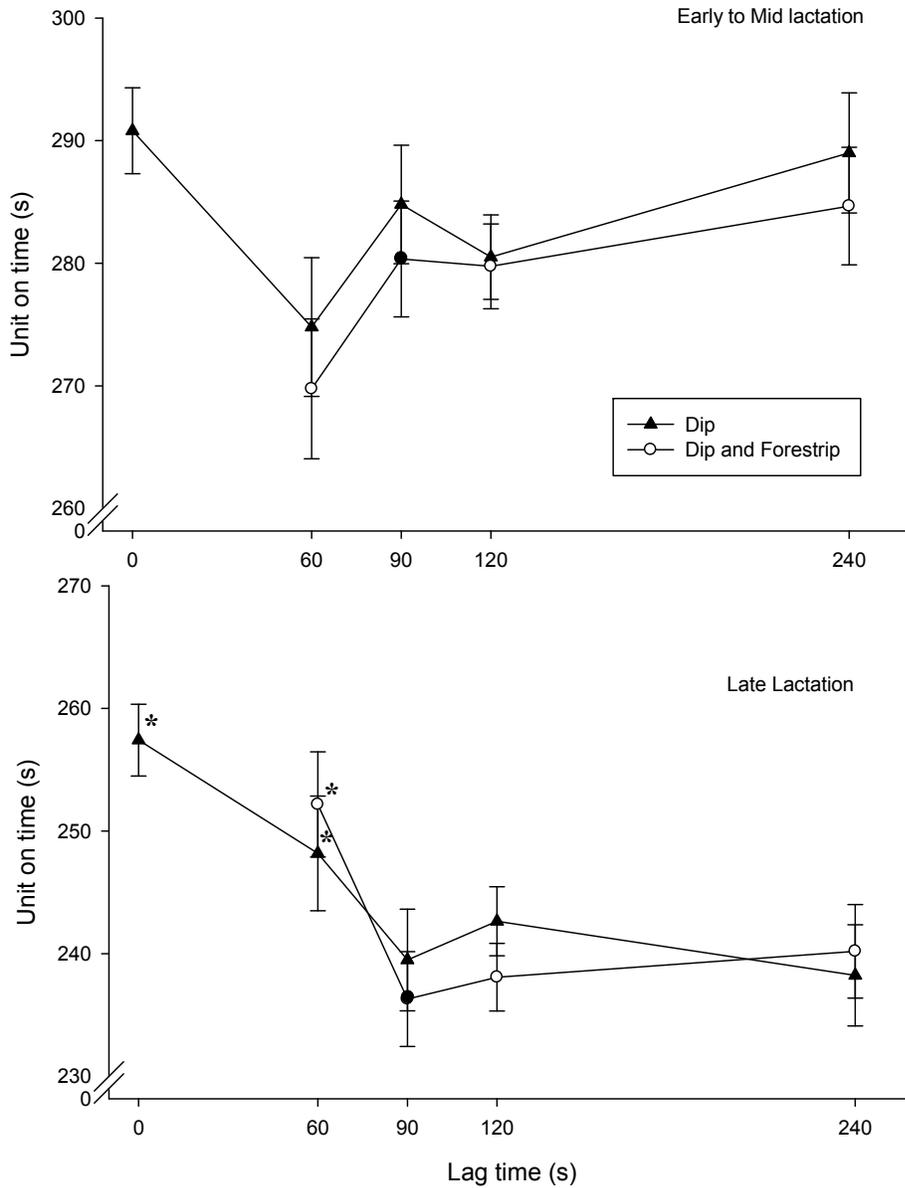


Figure 6. Milk flow rate (Kg/min) for cows following a preparation procedure that involved pre-dipping plus drying D (▲) or pre-dipping plus forestripping and drying DF (○) with the comparison treatment (a preparation procedure involving pre-dipping plus forestripping and drying with a lag time of 90 s DF90) (●) for: EM (early- to mid-lactation) cows; and LATE (late-lactation) cows. Data are presented as lsmeans with standard errors of the mean. Asterisks (*) indicate $P < 0.01$.

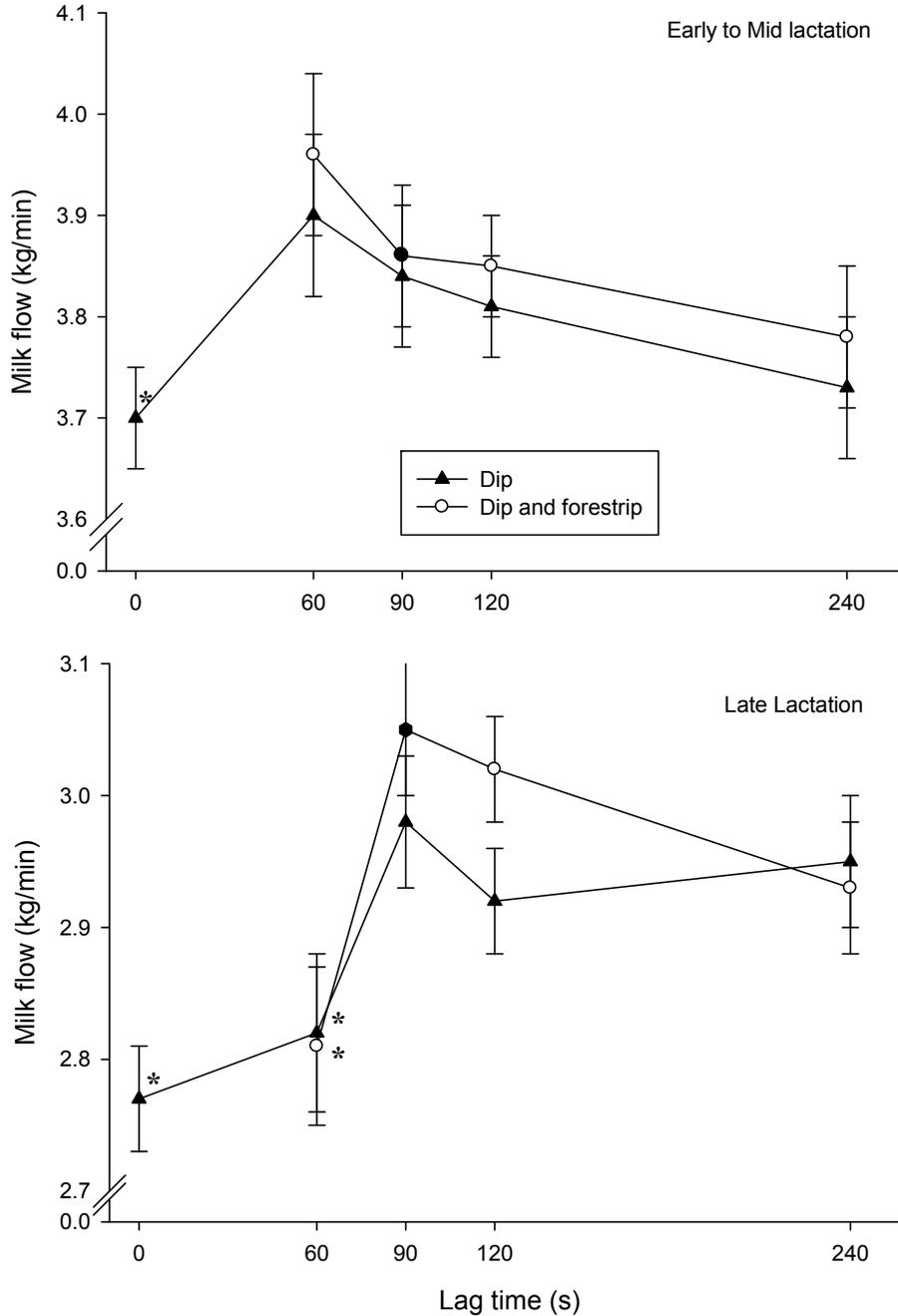
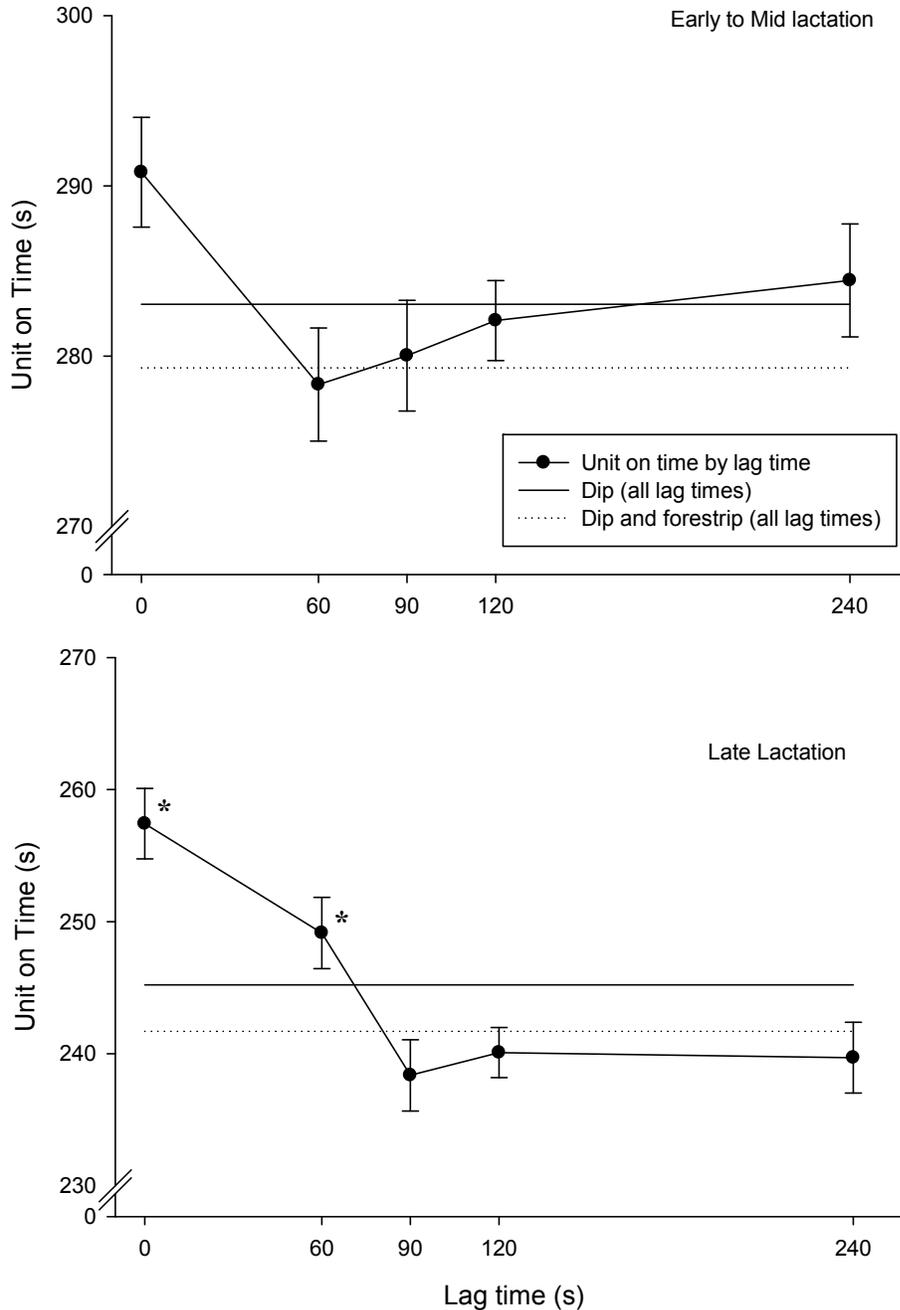


Figure 7. Milking unit on-time (s) for cows analyzed solely on lag time (independent of stimulation) and for cows analyzed solely for form of stimulation (independent of lag time). Early- to mid-lactation cows analyzed for lag time only (●) as compared to a lag of 90 s. Early to mid-lactation cows and pre-dipping plus drying (–) as compared to early- to mid-lactation cows and pre-dipping plus forestripping and drying (⋯). Late-lactation cows analyzed for lag time only (●) as compared to a lag of 90 s. Late-lactation cows and pre-dipping plus drying (–) as compared to late-lactation cows and pre-dipping plus forestripping and drying (⋯). Data are presented as lsmeans with standard errors of the mean. Asterisks (*) indicate $P < 0.05$.



CHAPTER 3

DETERMINATION OF FOREMILK QUANTITY AND QUALITY AND ITS OVERALL IMPACT ON TOTAL MILK QUALITY IN HOLSTEIN DAIRY CATTLE

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ABSTRACT

An experiment was designed to investigate the effect the somatic cell count (SCC) and plate loop count (PLC) in foremilk had on the SCC and PLC of the harvested milk fraction from Holstein cows milked 3X daily. Two squirts of milk were harvested from each teat and collected as a composite sample from each cow. A milk sample weight, SCC and PLC were obtained for the foremilk and harvested milk fractions. Foremilk was defined as the first 2 squirts of milk harvested from each teat after cleaning and sanitizing the teat. The cleaning of each teat may elicit the milk ejection reflex; therefore, it is possible that the cisternal and alveolar milk fractions may have mixed prior to the foremilk being harvested. The total foremilk removed from each cow was 15 mL which represented 0.12% of the total milk. Total milk was defined as the foremilk fraction plus the harvested milk (total milk = foremilk + harvested milk). The SCC of the harvested milk was used to classify cows into 4 groups: SCC ($\times 10^3$ cells/mL) < 50 (S25), 50-100 (S75), 100-350 (S225) and >350 (S500). The foremilk SCC (FSCC) for S25, S75, S225 and S500 medians with minimum and maximum were 41 (10-160), 130 (69-400), 380 (110-970) and 1200 (140-5,000) $\times 10^3$ cells/mL and the harvested milk SCC was 19.5 (6-49), 74 (58-93), 180 (100-320) and 738 (385-2650) $\times 10^3$ cells/mL. The foremilk PLC (FPLC) for S25, S75, S225 and S500 were 2 (0-81), 6 (0-110), 10 (0-90) and 5 (0-720) cfu/mL whereas the harvest milk PLC was 1 (0-101), 1 (0-7), 1.5 (0-85) and 1.3 (0-34) $\times 10^3$ cfu/mL. Forestripping lowered the PLC of all categories to ≤ 1.5 cfu/mL; however, the SCC remained $>180 \times 10^3$ cells/mL in the harvested milk for S225 and S500 after forestripping occurred. The foremilk represented < 0.28% of the SCC in total milk whereas the FPLC represented up to 1.44% of the bacteria in total milk. Foremilk SCC was significantly different from the SCC for all categories and the FPLC was significantly different from PLC for all categories except S25. The fraction of milk

being analyzed is important when trying to predict the health of the udder using SCC and quality of the harvested milk.

(Key words: foremilk, somatic cell count, milk quality)

INTRODUCTION

The SCC and PLC are used as predictors of udder health and quality of raw milk. Worldwide, SCC has been the main focus for milk quality. A visual observation of milk can be made prior to milk harvest via forestripping and this visual observation has been implemented to look for abnormalities in milk. Foremilk, cisternal, and alveolar milk fractions may have different SCC and bacterial counts; therefore, relating one fraction to another may not be sensible. Historically, milk from a healthy quarter is reported as having a SCC of $< 200 \times 10^3$ cells/mL (Smith, 1995); however, a SCC of $< 100 \times 10^3$ cells/mL represents a quarter that is uninfected (Hillerton, 1999). The SCC of quarters that were classified as healthy were compared to quarters that had subclinical mastitis and the SCC were 84,000 vs. 293,000 cells/mL (Urech et al., 1999). Even though the foremilk fraction only represents 0.2% of the total milk harvested the SCC in foremilk may be 2- to 3-times greater than the alveolar fraction (Sarikaya and Bruckmaier, 2006).

An increase in somatic cells in milk implies that the immune system has been activated and is functioning; however, it also may mean that pathogenic microorganisms have invaded the teat and are present in the mammary gland. A change in SCC can be seen from one milking to the next; therefore, the use of the SCC as an indicator of udder health and even milk quality has been worldwide (Harmon, 1994). There are always somatic cells present, but when microorganisms invade the teat canal, leukocytes are recruited to the site of invasion and therefore an increase in the SCC is observed. The cells that are measured and referred to as “somatic cells” in the mammary gland are mainly lymphocytes, macrophages, polymorphonuclear neutrophils (PMN), and epithelial cells. The cisternal milk from healthy quarters will be highest in macrophages, whereas immediately after an udder infection an

increase in PMN will be seen (Sordillo et al., 1997, Rainard and Riollet, 2006, Sarikaya and Bruckmaier, 2006). Mastitis occurs when white blood cells (mainly leukocytes) are released into the milk to combat the presence of pathogenic microorganisms (Sordillo et al., 1997, O'Brien et al., 1999). Pathogenic microorganisms that invade the teat canal are the primary cause for mastitis in cattle. Bacteria are part of the normal microflora of the udder and healthy udders had between 46 and 138 cfu/mL in milk that was machine harvested (Kleter, 1974, Kleter and Devries, 1974).

When the SCC of the harvested milk was $< 100 \times 10^3$ cells/mL, the foremilk fraction was similar to the harvest milk; however, when the harvested milk had a SCC of $> 100 \times 10^3$ /mL, the foremilk portion was significantly higher than the alveolar portions (Sarikaya and Bruckmaier, 2006). Foremilk fractions that had a SCC $> 300 \times 10^3$ /mL were not representative of the harvested milk SCC; however, when the foremilk SCC was between $50\text{-}300 \times 10^3$ cells/mL there was no difference in the foremilk and harvested milk fractions SCC (Wellnitz et al., 2009). Wellnitz et al. (2009) also found that when the foremilk SCC was $< 50 \times 10^3$ cells/mL, the remaining milk harvested had a higher SCC than the foremilk. Some studies have indicated that forestripping is a risk factor for mastitis or that forestripping had no influence on SCC (Elbers et al., 1998, Koester et al., 2006); however, others have found that forestripping as well as the order of forestripping within the pre-milking routine reduced the SCC (Skrzypek et al., 2003).

Studies have observed the SCC of the foremilk fractions and measured the relationship between these fractions and the SCC of the harvested milk (Paape and Tucker, 1966, Urech et al., 1999, Vangroenweghe et al., 2002, Sarikaya and Bruckmaier, 2006, Wellnitz et al., 2009), but there is little information relating the SCC or PLC of different milk fractions to the bacterial load of the harvested milk. Studies reported the effect of increasing or decreasing the milk

frequency and how this affects milk yield, milk composition, and even SCC (Davis et al., 1999, Smith et al., 2002, Dahl et al., 2004), but there are little analogous data on the relationship for Holstein cows (not crosses) milked 3X daily.

Our objectives were to determine the quantity and quality of foremilk from Holstein cows milked 3X daily and whether forestripping and removal of forestripped milk from the harvested milk affected the SCC or PLC of the harvested milk fraction. Our hypothesis was that omitting forestripping would not reduce the quality of the harvested milk fraction as measured by PLC and SCC.

MATERIALS AND METHODS

Cows and Treatments

Holstein cows (n=107) selected from the 500-cow herd at the Cornell University Teaching and Research Dairy were enrolled in this study. Pretrial data were gathered for the cows enrolled in the experiment were: mature-equivalent 305-d milk yield, milk yield per milking, DIM, parity, last test-day SCC, last test-day linear score (LS), and previous test-day linear score (PLS) (Table 7). Mature-equivalent represents an estimate of mature production and therefore was defined as the lactational milk-production average for all cows in the herd for the previous 365 d. The LS and PLS are log-transformed values of the SCC ($LS \text{ or } PLS = \log_2 (SCC/100) + 3$). The 305ME of the Holstein cows enrolled in study was 13281 Kg. The cows were in their first to sixth lactation; DIM ranged from 28-425 d for the enrolled animals. Cows were milked 3X daily; cows were housed 30/pen in a 4-row freestall barn and fed a total mixed ration that either met or exceeded NRC requirements. The experiment was conducted from February through June of 2009. All cows assigned to the study had to have 4 functioning quarters to qualify for the study. Cornell University's Institutional Animal Care and Use

Committee approved the experimental protocol.

Milking Equipment

Cows were milked 3X daily in a double-10 parallel parlor with Flo-Master Pro Milk Meter (Delaval, Tumba, Sweden). The milking system had a vacuum setting of 44 kPa. The pulsation rate was 60 cycles/min and the pulsation ratio was 65% milk phase and 35% rest phase. The milking claw was a Superflow Lite and the liner was a WC-01 (Delaval, Tumba, Sweden).

Sampling and Analysis

Upon entering the milking parlor, each teat was cleaned and sanitized with alcohol swabs. After sanitizing the teat and teat end, two squirts of foremilk were harvested from each teat and directed into the vial labeled for the given cow. The same individual collected all foremilk samples and if more than 2 squirts were removed the sample was discarded. The milk vial containing the foremilk sample was then placed on ice. The milking unit was then attached to harvest the remaining milk. During the harvest of the remaining milk, a sampling device collected a milk sample. A duplicate sample was taken from each milk sampler after the milking unit detached. The milk vials containing the foremilk and duplicates of the harvested milk samples were weighed and then put on ice. The same method used for pre-weighing and labeling the milk vials was used for weighing the filled milk vials.

Raw milk samples were taken on ice to a laboratory (Dairy One, Ithaca, New York) and analysis began within 24 h of the sample being harvested. Samples were held at 0-4.4 C until they were heated in a water bath. Samples were heated to 37 to 38 C and then analyzed by the Fossomatic 5000 (Foss, Hillerød, Denmark). An hourly control sample with a known SCC of between 500 and 800 cells/mL was analyzed. The known samples were prepared at the laboratory and the reference method was used to determine the SCC of these samples through

direct microscopic SCC. The instrument was then calibrated to these reference samples (of raw bovine milk) and the samples were used to ensure the Fossomatic 5000 was functioning properly.

Raw milk samples were analyzed for bacterial load via the plate loop count (PLC) methodology. Briefly, the milk sample is diluted and then incubated 3M™ Petrifilm™ Aerobic Count Plate (3M, St. Paul, Minnesota, USA). The plate is a culture medium which contains Standards Methods nutrients, cold-water-soluble gelling agent and tetrazolium indicator to facilitate colony enumeration. The culture is held at 32 ± 1 C for 48 ± 3 h at which time the colonies are counted. The PLC is determined in the same manner as a standard plate count (SPC) except the PLC uses a different apparatus as outlined in section 21 of the FDA-2400, Updated official laboratory evaluation forms under the section Standard plate count, coliform and simplified count methods (revised 2005) (Food and Drug Administration, Silver Spring, Maryland, United States).

Statistical Analysis

Statistical analyses were performed for all cows assigned to the experiment. The cows were divided into 4 categories based on the SCC of the harvested milk. The 4 categories were as follows: 1) SCC $< 50 \times 10^3$ cells/mL (**S25**); 2) SCC $50-100 \times 10^3$ cells/mL (**S75**); 3) SCC $100-350 \times 10^3$ cells/mL (**S225**) and 4) SCC $> 350 \times 10^3$ cells/mL (**S500**). Cows were divided into 3 categories based on the PLC of the harvested milk. The PLC groups were as follows: 1) PLC $< 10 \times 10^3$ cfu/mL (**P5**); 2) PLC $10-20 \times 10^3$ cfu/mL (**P15**) and 3) PLC $> 20 \times 10^3$ cfu/mL (**P25**). Somatic cell count and PLC data were log transformed for normalization. A \log_{10} transformation was chosen and normality was determined by use of the Shapiro-Wilk statistic. Log-transformed data were used for statistical comparisons; however, the actual (raw) values are presented.

Values are reported as means \pm standard deviation or means (means \pm SD) or 2.5th, 50th and 97.5th percentiles unless otherwise noted. Analyses were carried out with linear models in PROC GLM of SAS (SAS, 1999). Milk yield was included in the model as a confounder and lactation and DIM were included in the full model but removed for lack of significance. Somatic cell count and PLC category were included as independent variables in the model. Dependent variables were the following milk quality measurements: foremilk SCC (FSCC), harvested milk SCC (SCC), total milk SCC (TSCC), foremilk PLC (FPLC), harvested milk PLC (PLC) and total milk PLC (TPLC). Multivariate linear regression was also used to determine what proportion of variation FSCC and FPLC represented in predicting the TSCC and TPLC. The dependent variable in the regression equation was either TSCC or TPLC and in both cases the predictors variables were FSCC and FPLC. Multivariate regression was performed by using the Proc GLM procedure (SAS, 1999). Significant differences were declared at $P < 0.05$ for main effects without interaction terms, using 2-sided P-values.

RESULTS AND DISCUSSION

The total foremilk harvested from each cow (which represented a composite sample of 2 squirts from each of the 4 teats) was 15.16 ± 5.6 mL. The amount of milk harvested on each individual forestripping was 1.9 ± 0.7 mL. The volume of foremilk removed did not significantly differ between SCC categories ($P > 0.81$). Foremilk represented 0.12% of the total milk (total milk = foremilk + harvested milk). Sarikaya and Bruckmaier (2006) determined that the first 2 squirts of foremilk from Brown Swiss cattle was 9.4 mL and that this represented 0.3% of the total milk harvested. Differences seen in the amount of foremilk harvested may be related to the breed of cow used for the experiment.

The range in SCC and PLC for foremilk were 10 to 5000 x10³ cells/mL and 0 to 720 x 10³ cfu/mL and for harvested milk were 6 to 2650 x10³ cells/mL and 0 to 101 x 10³ cfu/mL (Table 8). Foremilk SCC was lowest for S25 and highest for S500 and all comparisons of FSCC between harvested SCC categories were significantly different (P < 0.001; Table 8). Our study was in agreement with Sarikaya and Bruckmaier (2006) who determined that the foremilk SCC was lowest in the lowest SCC category and that the foremilk SCC increased with each increasing SCC category. The PLC was lowest for S25 and highest for S500 and all comparisons of FPLC between harvested SCC categories were significantly different (P < 0.001; Table 8). Forestripping removed between 7.80 x 10⁵ to 23.48 x 10⁶ total somatic cells from the harvested milk, which could be calculated from the milk yield and the SCC and PLC concentrations of the foremilk and harvested milk fractions. The somatic cells in foremilk represented 0.2 to 0.27 % of the total somatic cells in milk if not removed. Forestripping removed 12.7 x 10⁴ to 86.7 x 10⁴ bacterial colonies from the harvested milk. The bacteria in foremilk accounted for 0.18 to 1.44 % of the bacteria in milk if not removed. Of the SCC categories, 2 of them had foremilk that accounted for > 1.35 % of the total bacteria in milk. Removal of the foremilk had a bigger impact on the bacterial count of the harvested milk as compared to the SCC. It should be noted that the SCC and PLC are lowered by forestripping; however, the resulting bacteria count for all SCC categories was < 10.9 x 10³ cfu/mL (mean) and < 1.9 x 10³ cfu/mL (median). After forestripping the maximum bacteria count when looking at all categories was 5.3 cfu/mL whereas the SCC was >193 x 10³ cells/mL in the harvested milk for S225 and S500. The FSCC was significantly different from the SCC and TSCC within each category (P < 0.05). Wellnitz et al. (2009) also found that when the harvested milk had a SCC < 50 x 10³ the FSCC was lower than the harvested milk SCC. The FPLC was significantly different (P < 0.05) from the PLC and

TPLC within all categories except S25 where the FPLC and PLC did not differ ($P = 0.17$).

The bacterial load of milk was separated into 3 different categories based on the PLC of the harvested milk. There was no difference in milk yield per milking or DIM between the 3 categories for PLC (Table 9). However, a difference ($P < 0.05$) in parity was detected when comparing P5 to P15 (Table 9). Mean values for PLC of the harvested milk were 1.5, 13.9 and 51.5 for P5, P15 and P25 (Table 9). Foremilk PLC was highest for P25 (FPLC: 38.6×10^3 cfu/mL); however, the harvested PLC for P25 (PLC: 51.1×10^3 cfu/mL), was higher than the FPLC. The FSCC was significantly different ($P < 0.05$) from SCC and TSCC within P5. The FPLC was significantly different ($P < 0.05$) from PLC and TPLC for P5 and P25. Even when PLC was $< 10 \times 10^3$ cfu/mL, the FSCC was still elevated ($> 450,000$ cells/mL). Recently a German experiment determined that when the SCC was $< 10 \times 10^3$ cell/mL the prevalence of mastitis pathogens was 8.5%--and of this 65% of the pathogens isolated were minor and $< 25\%$ were major (Schwarz et al., 2010). Schwarz et al. (2010) also reported that when SCC was $> 400 \times 10^3$ cell/mL, *Staphylococcus aureus* was frequently isolated and when the SCC was $> 800 \times 10^3$ cell/mL mainly coliforms were isolated. The low PLC and high FSCC that is seen in P5 may be explained by the presence of specific pathogens that elicit a stronger immune response rather than the quantity of bacteria present.

Raw and log-10 transformation of FSCC, FPLC and SCC were analyzed by multivariate regression analysis to see if FSCC and FPLC were predictors for SCC. The R^2 of the log 10-transformed values when FSCC and FPLC were predictors of SCC were 0.54, 0.09, 0.20 and 0.04 for S25, S75, S225 and S500. Therefore, FSCC and FPLC were not strong predictors of TSCC. This is in agreement with Wellnitz et al. (2009) who only found similarities between FSCC and TSCC when the FSCC was between $50-300 \times 10^3$ cell/mL. In contrast, Paape and

Tucker (1966) indicated that any milk fraction from foremilk to the strippings at the end of milking could be used to predict the SCC of the harvest milk. The same regression analysis was done for the PLC categories with both raw and log₁₀ transformed values to determine if FPLC and FSCC alone or combined were correlated to PLC. The R² of the log 10 transformed values when FPLC and FSCC were predictors of PLC were 0.02, 0.13 and 0.16 for P5, P15 and P25. We nevertheless note that FSCC did account for >50% of the variation in S50; when the SCC is low in the harvested milk, the FSCC accounts for a larger portion of the variation.

Harvested milk SCC was subtracted from the FSCC for each of the harvested milk SCC categories to determine if FSCC was a good indicator of the harvested milk SCC (Figure 8). When the harvested milk SCC category was < 500 x 10³ cells/mL the difference was near zero indicating that the foremilk SCC was similar to the harvested milk SCC; however, when the harvest milk SCC category was > 500 x 10³ cells/mL the median difference was near 500 indicating that the FSCC was substantially greater than the SCC of the harvested milk fraction. Harvested milk PLC was subtracted from the FPLC for each of the harvested milk PLC categories to determine if FPLC was a good indicator of the harvested milk PLC (Figure 9). When the harvested milk PLC category was <20 x 10³ cfu/mL the difference was near zero--indicating that the foremilk PLC was similar to the harvested milk PLC; however, when the harvest milk PLC was >20 x 10³ cfu/mL the median difference was less than zero--the harvested PLC was greater than the FPLC. In summary, FSCC and FPLC generally are not good predictors of TSCC and TPLC.

Cut points for SCC of 200,000 and 500,000 cell/mL for foremilk and harvested milk were used to create a 2X2 table (Table 10). The table was created to compare the cut-points of 200,000 and 500,000 cells/mL were chosen because they represent the cut-points for new

infection in DairyComp305 and the California Mastitis Test (CMT). When the SCC of foremilk is $< 200,000$ cells/mL the positive predictive value for the harvested milk having a SCC $< 200,000$ cells/mL is 95% (sensitivity is 0.82). However, if the SCC of the foremilk is $> 200,000$ cells/mL, 30% of the harvested milk samples will have a SCC $< 200,000$ cells/mL. When the SCC of foremilk is $< 500,000$ cells/mL the positive predictive value for the harvested milk having a SCC $< 500,000$ cells/mL is 99% (sensitivity is 0.89). However, if the SCC of the foremilk is $> 500,000$ cells/mL, 39% of the harvested milk samples will have a SCC $< 500,000$ cells/mL. When the SCC of the foremilk was $< 200,000$ and $500,000$ cells/mL there was a strong positive predictive value that the harvested milk was $< 200,000$ and $500,000$ cells/mL for each cut-point..

Mean milk production per milking ranged from 11.2 to 14.1 kg/milking for the 4 SCC categories (Table 8). Milk production was highest (14.1 kg/milking) when the SCC was $< 50 \times 10^3$ cells/mL. Milk production per milking was significantly higher when comparing SCC $< 50 \times 10^3$ cells/mL to SCC categories with $> 100 \times 10^3$ cells/mL ($P < 0.05$). This is in agreement with previous data which indicated that even at a SCC of 100,000 when compared to 50,000, there was a decrease in milk yield (Hortet et al., 1999). In contrast, Green et al. (2006) found that the SCC peaked between 10-20 l/d and decreased when milk yield was > 20 l/d suggesting a dilution effect. Bennedsgaard et al. (2003) reported that when the SCC was between 100,000 to 1,500,000 a twofold increase in SCC reduced ECM by 0.2 Kg/d in Danish cows managed organically. Days in milk were highest (285 d) for S75 and lowest (188 d) for S25 and a significant difference was found when comparing S75 to S25 and S500 (Table 8). There was no difference for parity between any of the comparison categories ($P = 0.57$). Recently, Hagnestam-Nielsen et al. (2009) reported that when the SCC was $> 500,000$ cells/mL, the loss in milk yield

per/d was from 0.7 to 2.0 and 1.1 to 3.7 Kg for primiparous and multiparous cows.

Care should be taken when comparing the SCC of the so-called “foremilk fraction” from one study to another. Paape and Tucker (1966) describe the foremilk fraction as the first milk harvested by the milking machine after the first streams of milk were discarded.

Vangroenweghe et al. (2002) classified the foremilk as the first 60 mL harvested from each quarter. Wellnitz et al. (2009) obtained the foremilk fraction after the udder was massaged for 1 min and removal of 2 to 3 streams of milk. Sarikaya and Bruckmaier (2006) classified foremilk as the first 2 streams of milk removed from the quarter prior to the initiation of the milk ejection reflex. In our study the foremilk fraction represented the first two squirts of milk after cleaning of the teats. This means that the milk ejection reflex was initiated and that the alveolar and cisternal milk fractions could have mixed. This approach was taken because the teat needs to be properly sanitized to harvest a milk sample for a bacteria count. Simply on definition of foremilk one can see why experiments may not be in agreement on the prediction of the SCC or PLC in harvested milk by the analysis of the foremilk.

CONCLUSIONS

In conclusion, the SCC and PLC of foremilk are not good predictors of udder health or the quality of the harvested milk fraction. The foremilk represents a small portion of the total milk harvested and the somatic cells and bacteria found in the foremilk represent a small portion of the total somatic cells and bacteria in milk. When the harvested milk fraction SCC is < 200,000 cells/mL the foremilk has a similar SCC 95% of the time. Forestripping to remove somatic cells and bacteria from the harvested milk fraction may not be as important as the value of forestripping as a visual tool for evaluating the foremilk for abnormalities. The process of observing the milk for abnormalities and removing the cow from the milk string (if abnormality

is detected) may have greater influence on other risk factors such as transmission of mastitis-causing organisms, culling and decreased milk yield than just forestripping to remove the milk from the harvested fraction.

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Table 7. Pretrial data for the 107 Holstein cows assigned to treatments.

Variable	Mean	Standard Deviation
305ME (Kg/305 d)	13281	1932
Milk yield/milking (Kg)	13.4	3.7
Parity	2.3	0.9
DIM	216	93
Somatic cell count (x 1,000/mL)	255	503
Linear score (LS) ¹	2.7	2.1
Previous linear score (PLS) ¹	2.7	2.0

¹LS = $\log_2 (\text{SCC}/100) + 3$

¹PLS = $\log_2 (\text{SCC}/100) + 3$

Table 8. Within-trial milk-quality data for cows analyzed based on the somatic cell count of the harvested milk fraction. Data for milk quality presented as 2.5, 50 and 97.5 percentiles

SCC (x 10 ³ cells/mL)	<50 (S25) (N = 43)			50-100 (S75) (N = 15)			100-350 (S225) (N = 25)			>350 (S500) (N = 24)		
	Mean	SD		Mean	SD		Mean	SD		Mean	SD	
Milk (Kg/milking)	14.1 ^a	3.4		12.9 ^{ab}	4.3		11.2 ^b	3.6		11.7 ^b	3.1	
DIM	188 ^a	102		285 ^b	58		229 ^{ab}	93		208 ^a	72	
Parity	2.2	0.8		2.4	1		2.4	0.9		2.5	1.2	
Percentiles	2.5	50	97.5	2.5	50	97.5	2.5	50	97.5	2.5	50	97.5
Foremilk SCC ¹	15	41 ^{ax}	140	69	130 ^b	400	110	380 ^c	970	140	1200 ^d	5000
Harvested SCC ¹	6	19.5 ^a	45	58	74 ^b	93	100	180 ^c	320	385	734 ^d	2650
Total SCC ¹	6	19.6 ^a	45.1	58.1	74 ^b	93	100.1	180 ^c	321	386.3	738 ^d	2650
Foremilk PLC ²	0	2 ^{ax}	51	0	6 ^{abx}	110	0	10 ^{bx}	90	0	5 ^{bx}	720
Harvested PLC ²	0	1 ^y	24.5	0	1 ^y	7	0	1.5 ^y	85	0	1.3 ^y	34
Total PLC ²	0	1 ^y	24.5	0	1 ^y	7	0	1.5 ^y	85	0	1.3 ^y	34.1

¹ SCC x 10³ cells/mL

² PLC x 10³ cfu/mL

^{abcd} Values within row and with different superscripts indicate subgroups in which log₁₀ transformed data are significantly different at (P<0.05)

^{xy} Values within column and with different superscripts indicate subgroups in which log₁₀ transformed data are significantly different for SCC at (P<0.05)

^{wz} Values within column and with different superscripts indicate subgroups in which log₁₀ transformed data are significantly different for PLC at (P<0.05)

Table 9. Within-trial milk-quality data for cows analyzed based on the plate loop count of the harvested milk fraction. Data for milk quality presented as 2.5, 50 and 97.5 percentiles

PLC (x 10 ³ cfu/mL)	<10 (P5) (N = 92)			10-20 (P15) (N = 7)			>20 (P25) (N = 8)		
Variable	Mean	SD		Mean	SD		Mean	SD	
Milk (Kg/milking)	12.8	3.9		12	2.1		13.2	2.3	
DIM	218	94		200	97		209	97	
Parity	2.4 ^a	0.9		1.6 ^b	0.8		2.3 ^{ab}	0.9	
Percentiles	2.5	50	97.5	2.5	50	97.5	2.5	50	97.5
Foremilk SCC ¹	20	130 ^x	3100	17	200	3100	20	290	1800
Harvested SCC ¹	6.5	74 ^y	2000	7.5	100	1900	12.5	162	1200
Total SCC ¹	6.5	74 ^y	2000	7.5	100	1901	12.5	163	1200
Foremilk PLC ²	0	4 ^w	110	0	5	90	0	12 ^w	150
Harvested PLC ²	0	1 ^{az}	7	11	14.5 ^b	16.5	24.5	32.5 ^{cz}	101
Total PLC ²	0	1 ^{az}	7	11	14.5 ^b	16.6	24.5	32.6 ^z	101

¹ SCC x 10³ cells/mL

² PLC x 10³ cfu/mL

^{abcd}Values within row and with different superscripts indicate subgroups in which log₁₀ transformed data are significantly different at (P<0.05)

^{xy}Values within column and with different superscripts indicate subgroups in which log₁₀ transformed data are significantly different for SCC at (P<0.05)

^{wz}Values within column and with different superscripts indicate subgroups in which log₁₀ transformed data are significantly different for PLC at (P<0.05)

Table 10. Foremilk and harvested milk SCC with 200,000 and 500,000 cell/mL as cut-offs

		Harvested SCC	
		< 200,000	>200,000
Foremilk	< 200,000	60	3
	>200,000	13	31

		Harvested SCC	
		<500,000	>500,000
Foremilk	< 500,000	80	1
	>500,000	10	16

Figure 8. Foremilk SCC minus harvested milk SCC by harvested milk SCC category

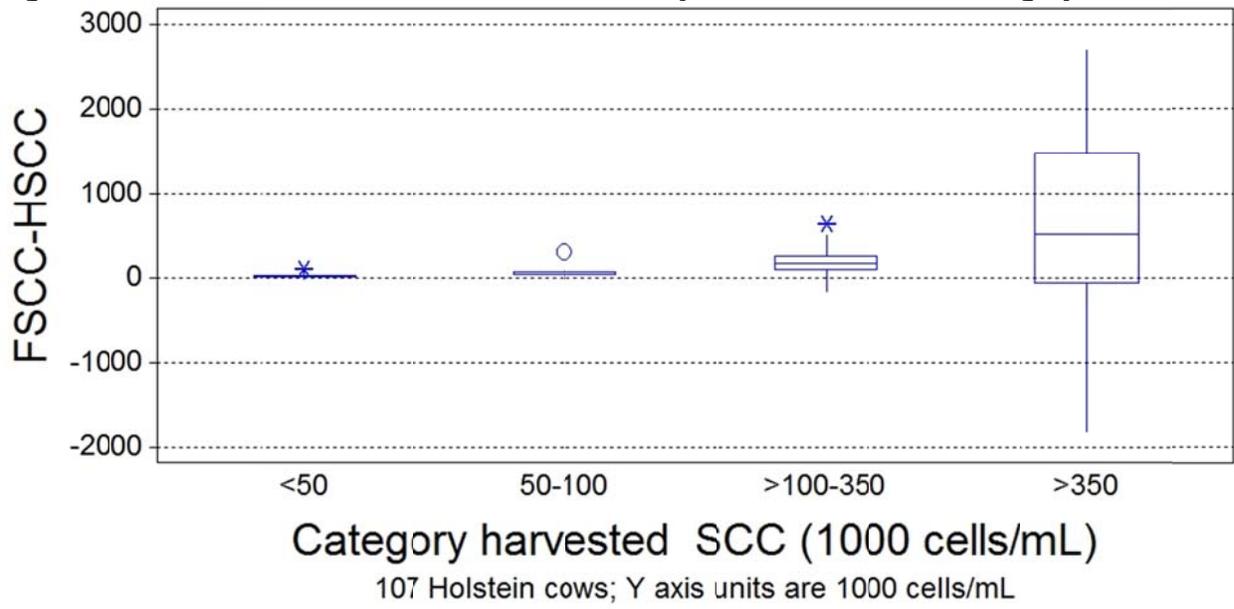
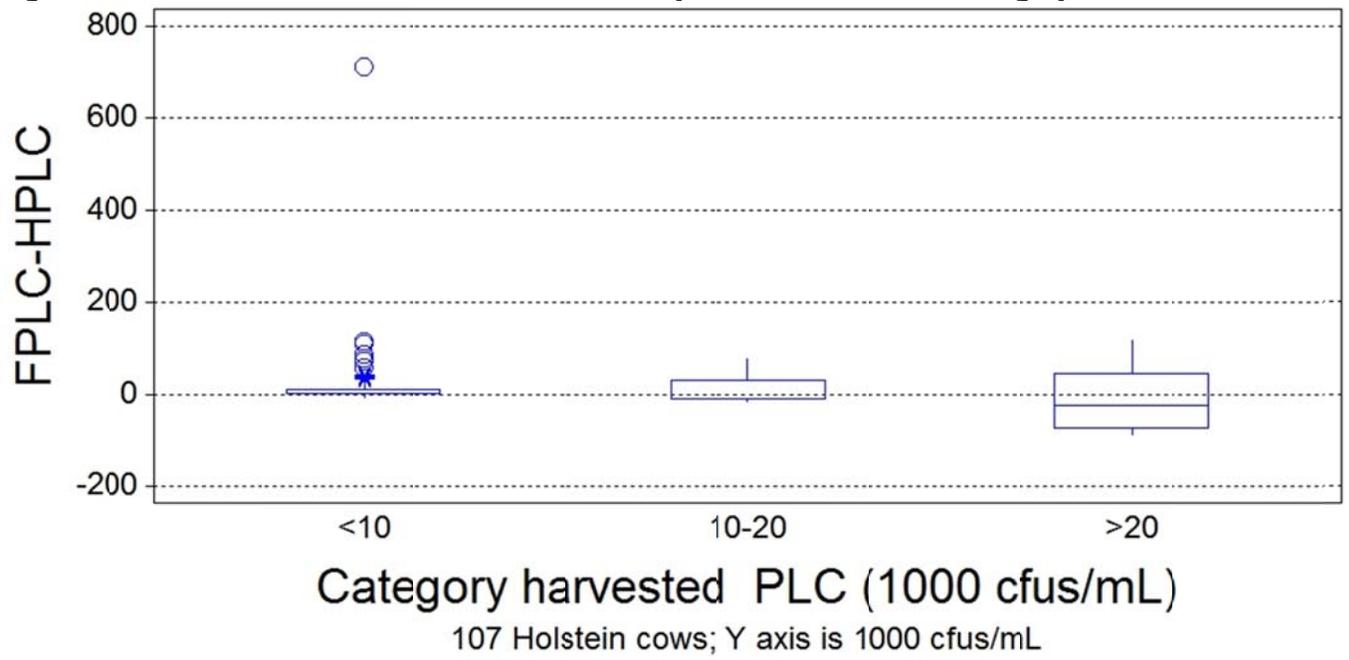


Figure 9. Foremilk PLC minus harvested milk PLC by harvested milk PLC category



CHAPTER 4
THE EFFECT OF MANUAL AND MECHANICAL STIMULATION ON MILKING
CHARACTERISTICS AND OXYTOCIN PROFILES IN HOLSTEIN COWS MILKED
THREE TIMES DAILY

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ABSTRACT

The form of stimulation that is administered to a cow prior to attachment of the milking unit is usually manual. Development of milking technology has removed manual stimulation and replaced it with mechanical forms. Holstein cows (n=30) were enrolled in a cross-over design to determine the effect of manual and mechanical stimulation on milk yield, milk flow rates, incidence of bimodal milk curves, residual milk and oxytocin profiles in cows milked 3 times daily (3X) daily. All cows were subjected to all treatments. Cows received manual or mechanical stimulation along with lag times of 0, 30 or 90 s. Machine stimulation occurred either by immediate attachment of the milking unit or by stimulation pulsation which involved increasing the pulsation cycles from 60/min to 300/min and reducing the vacuum in the chamber to 20 kPa. The 5 treatments were 1) immediate attachment of the milking machine (T0); 2) dip plus forestrip and drying with 30 s lag time (M30); 3) dip plus forestrip and drying with 90 s lag time (M90); 4) stimulation pulsation for 30 s (S30); and 5) stimulation pulsation for 90 s (S90). Milk yield/milking averaged 14.0 Kg and did differ between treatments ($P < 0.01$); the maximum difference detected was 0.8 Kg/milking. Time spent milking (not including stimulation time) ranged from 245-262 s and was shortest (245 s) for cows subjected to 90 s of mechanical stimulation (S90). During the 30 and 90 s for mechanical stimulation 0.13 and 0.32 Kg of milk were harvested for S30 and S90 indicating that minimal amounts of milk were harvested during stimulation pulsation. The median somatic cell count (SCC) was $< 45 \times 10^3$ cells/m for all treatments. The median bacterial counts were significantly ($P < 0.05$) different and highest for T0 and S30 and lowest for S90. Residual milk represented 12 to 14% of the total milk and did not differ among treatments. Oxytocin (OT) profiles peaked sooner in manually stimulated cows; however, there was no difference in OT concentration beyond 2 min after milking unit

attachment. Mechanical stimulation elicited similar OT profiles and milking unit on-time when taking the start time and form of stimulation into consideration. Bimodal milk curves were highest when no lag time was allowed (T0; 21%) and lowest for manual stimulation and 90 s of lag time (M90; 7%). In conclusion, mechanical stimulation elicits similar oxytocin profiles and reduced unit on-time when 90 s of duration was utilized as compared to manual stimulation.

(Key words: mechanical stimulation, pre-milking routine, lag time, milking unit on-time, oxytocin)

INTRODUCTION

Initiation of milk ejection from the alveolar tissue is necessary to harvest the majority (80%) of milk from dairy cows, whereas the cisternal portion of milk can be harvested without any form of stimulation (Bruckmaier et al., 1994). The pre-milking routine is the main method used to elicit the milk ejection reflex and begin the expulsion of the alveolar milk fraction. The initiation of the cascade of events involved in the milk ejection reflex has historically been achieved via contact between the calf or human hand and the teat (Bruckmaier and Blum, 1996). Another form of stimulation comes from the interaction of the milking machine liner and the teat; this stimulation is maintained throughout the milking procedure while the milking unit is attached (Bruckmaier et al., 1997). Even without proper pre-stimulation prior to attachment of the milking unit one can still harvest the alveolar fraction of milk; however, an interruption in milk flow may be seen as the cisternal fraction is removed prior to alveolar milk expulsion. With the advancement of milking technology forms of stimulation other than the human hand have been used. Such forms of mechanical stimulation include rotating brushes, different rates, ratios and levels of vacuum used for pulsation, and simply the attachment of the milking unit without a prior stimulation phase. Current technology in robotic milking machines uses mechanical stimulation (to which the pressure-sensitive neural receptors on the teat will respond) to initiate the release of oxytocin--not just maintain oxytocin above the reflex threshold (Schams et al., 1984).

The use of vibration pulsation as an alternative form of pulsation that cycles 300/min with a maximum vacuum level of 20-22 kPa in the pulsation chamber (keeping the liner closed) has been investigated. Worstorff et al. (1987) investigated the use of a high cycle and low vacuum pulsation (300 cycles/ 60 s at 20-22 kPa) as compared to manual stimulation and determined that vibration stimulation was an adequate method to cause the ejection of milk from

the alveolar region. The type of liner used in conjunction with vibration stimulation (300 cycles/60 s) affected flow rates (Karch et al., 1988).

Stimulation of the teat for 15, 30 or 45 s followed by either 30 or 45 s of latency time (time without touching) caused a similar and unchanged oxytocin release throughout milking (Kaskous and Bruckmaier, 2011). When lag times of 90 or 120 s and forestripping was involved > 60% of milk was harvested in the first 2 min of unit on-time for late lactation cows (Watters et al., 2011). When the degree of udder filling was < 40% a lag time of 45 s decreased unit on-time independent of how long tactile stimulation took place (Kaskous and Bruckmaier, 2011). Our recent data indicated that lag time > 60 s in Holstein cows milked 3X reduced milking unit on-time in late-lactation cows (Watters et al., 2011). Weiss and Bruckmaier (2005) reported that a short pre-stimulation time would increase the number of cows per milking stall if milking full udders and that prolonged stimulation might be beneficial when milking udders that are not full. Differences in the breed of cattle used for research, milking frequency, and daily milk yield could affect the fullness of the udder.

Improper milking techniques may prevent complete milkout of the mammary gland. Milk that is left in the mammary gland after removal of the milking unit may be from improper milking routines or may instead truly be residual milk, which can only be harvested by exogenous doses of OT. Milk that is left in the alveolar fraction after the completion of milking is referred to as “residual milk” and this fraction may represent 8 to 25% of the total milk. Residual milk can only completely be obtained via an injection of supraphysiological amounts of exogenous oxytocin. Total milk can be determined by adding the current milk yield and the residual milk. First-lactation cows have less residual milk when compared to older cows (Schmidt, 1971). A more recent study indicated that cows with < 8% residual milk had had

normal milk ejection whereas those cows with > 8% residual milk had had interrupted milk ejection (Negrao and Marnet, 2006). The use of brushes and/or vibration stimulation in a robotic milking system did not have effect on residual milk which was between 14.8 and 15.9% of total milk (Macuhova et al., 2003).

The objective of this experiment was to determine if mechanical stimulation without any form of manual stimulation prior to the harvest of milk is an adequate form of stimulation for Holstein cows milked 3X daily. The hypothesis was that mechanical stimulation by an alternate form of pulsation will reduce milking unit on-time and elicit a similar oxytocin profile as manual stimulation. It is our interest to observe milk flow profiles, plasma oxytocin concentration, and residual milk based upon differing manual and milking machine induced pre-milking routines for Holstein cows milked 3X.

MATERIALS AND METHODS

Cows and Treatments

Holstein cows (n=30) were selected from the 500-cow herd at the Cornell University Teaching and Research dairy facility. Cows were milked 3X in the Cornell University Research Parlor. Cows were housed in a 4-row freestall barn and fed a total mixed ration that either met or exceeded NRC requirements. A total of 30 cows were enrolled in the study. The study cows were housed together in a single 30-cow pen. The experiment was conducted from March through May of 2010. All cows assigned to the study had to have four functioning quarters, no case of mastitis during the current lactation, and be < 350 DIM at the time of enrollment. Cornell University's Institutional Animal Care and Use Committee approved the experimental protocol.

A randomized cross-over design was used with 5 treatments, 5 periods of 4 days and 6 cows per treatment phase (n=30 cows). The treatments involved 2 forms of stimulation and 3 lag

times. The stimulation methods were either forestripping or mechanical stimulation from the milking unit. Lag times were determined from first tactile stimulus until milking machine was in milk mode; the 3 lag times were 0, 30 and 90 s. The first tactile stimulus for manual stimulation was forestripping and for mechanical stimulation the first stimulus was initiated at the attachment of the milking unit. Mechanical pulsation was either performed by the milking machine under normal milking conditions or by StimoPuls pulsation (StimoPuls, GEA Farm Technologies, Bönen, Germany). StimoPuls is a pulsation process that increases the pulsation cycles from 60 cycles to 300 cycles per minute and a vacuum level of 20 kPa in the pulsation chamber at simultaneously full vacuum (42 kPa) in the milk line to keep the liner closed that is intended not to harvest milk (Karch et al., 1988). The difference between system and pulsation chamber vacuum (42 kPa minus 20 kPa) of 22 kPa minus the collapse force of 11.9 kPa keeps the liner closed during the stimulation phase. The 5 treatments were immediate attachment of milking unit with no lag time (**T0**), dip plus forestripping and drying with a lag of 30 s (**M30**), dip plus forestripping and drying with a lag of 90 s (**M90**), immediate attachment of milking unit and 30 s of StimoPuls pulsation (**S30**) and immediate attachment of milking unit and 90 s of StimoPuls pulsation (**S90**). All cows received all treatments in an order determined for each cow by a restricted randomization based on a table of random numbers.

Milking Equipment

Cows were milked 3X daily in a double-5 herringbone parlor (GEA Farm Technologies, Bönen, Germany). The milking system had a vacuum setting of 42 kPa. The pulsator had a pulsation rate of 60 cycles/min and ratio of 65:35 (StimoPuls, GEA Farm Technologies, Bönen, Germany). The milking claw had a volume of 300 mL (Classic 300, GEA Farm Technologies,

Bönen, Germany) and was used with a rubber liner that had a collapse force of 11.9 kPa (086, GEA Farm Technologies, Bönen, Germany).

Data Acquisition

During both the adaptation and data-collection phases, cow and milking data were gathered on-farm by herd-management software (DairyComp 305, Valley Agricultural Software, Tulare, California; DairyPlan C21, GEA Farm Technologies, Bönen, Germany).

Bimodal milk curves were determined by DairyPlan software (DairyPlan C21, GEA Farm Technologies, Bönen, Germany). A bimodal milk could only be calculated once 500 g of milk had been harvested. Upon the harvest of 500 g of milk there was 60 s allowed for a bimodal milk curve to occur. An increase in milk flow from 500 g followed by a decrease and then 2 increases had to occur within 60 s in order for a bimodal calculation to occur. The bimodal calculation divides the lowest flow rate by the highest flow rate prior to the lowest and multiplies it by 100. If the lowest values divided by the highest value was $< 72\%$ then a bimodal milk curve occurred.

Sampling and Analysis

When the cow entered the milking parlor each teat (if she was on a manual-treatment phase) was forestripped and dipped. At the time of the first forestrip a stopwatch was started to keep track of the lag time. At 10 s prior to the end of the desired lag time cows teats were dried and the milking unit was attached. Cows requiring manual stimulation were identified first and the stimulation and timing process was started. The cows requiring no stimulation had the milking unit attached after the timing process was initiated for the manual stimulation cows. On sample days, a milk-collection device was connected to the milking system to obtain a representative milk sample. A duplicate milk sample was taken from each milk sampler after the

milking unit detached during the last milking of each period. The milk samples were put on ice and then taken to a lab for somatic cell count (SCC) and plate loop count (PLC) analysis (Dairy One, Ithaca, New York).

Chilled raw milk samples were taken to a lab and analysis began within 24 h of harvesting the sample. Samples were held between 0-4.4 C until they were analyzed. Milk samples were heated in a water bath until they reached temperatures between 37 and 38 C at which time they were analyzed by the Fossomatic 5000 (Foss, Hillerød, Denmark). An hourly control sample with a known SCC of between 500 and 800 cells/mL was analyzed. The known samples were prepared at the laboratory and the reference method was used to determine the SCC of these samples through Direct Microscopic Somatic Cell Count. The instrument was then calibrated to these reference samples (of raw bovine milk) and the samples were used to ensure the Fossomatic 5000 was functioning properly.

Raw milk samples were analyzed for bacterial load via the plate loop count (PLC) methodology. Briefly, the milk sample is diluted and then incubated 3MTM PetrifilmTM Aerobic Count Plate (3M, St. Paul, Minnesota, USA). The plate is a culture medium which contains Standards Methods nutrients, cold-water-soluble gelling agent and tetrazolium indicator to facilitate colony enumeration. The culture is held at 32 ± 1 C for 48 ± 3 h at which time the colonies are counted. The PLC is determined in the same manner as a standard plate count (SPC) except the PLC uses a different apparatus as outlined in section 21 of the FDA-2400, Updated official laboratory evaluation forms under the section “Standard plate count, coliform and simplified count methods (revised 2005)” (Food and Drug Administration, Silver Spring, Maryland, United States).

A unilateral venous catheter (Dural 100 cm x 1.0-mm i.d. x 1.5-mm o.d.) was inserted in conscious cows by percutaneous venipuncture. Jugular catheters were inserted into two cows from each treatment (n=10) after the seventh milking after the start of the study (during the first treatment phase). During the tenth milking of each treatment phase, blood samples were taken from each of the catheterized cows. Blood samples (10 mL) were harvested from the jugular catheter at -5, 0, 1, 2, 4, 5, 8, 9, 10, 11 and 30 min from milking-unit attachment time. Whole blood was collected in EDTA tubes and immediately placed on ice until being spun in a centrifuge at 1500 g for 20 min. Plasma from each blood vial was split into duplicates and frozen at -20 C until analysis. Plasma oxytocin was determined by radioimmunoassay according to the assay developed for cattle (Schams, 1983). Standards were ran in triplicate and samples in duplicate. The samples were extracted in 8 mL glass tubes and the assays ran in 3 mL glass tubes. Extraction with SEP-PAK C18 cartridges (Waters Assoc., Inc., USA) was used. Recovery is based on the amount of plasma extracted. I¹²⁵ was used for the labeling of the oxytocin. Results are reported as pg/mL.

At 8 min after the start of milking, an intravenous injection of oxytocin (1mL, 10 IU) was administered to each cow with a jugular catheter. At exactly 60 s after administration of the oxytocin, the milking unit was reattached for 120 s. The milk weight was recorded at the time of oxytocin administration and again after the 120 s allowed for residual milk harvest. These weights were used to determine the amount of residual milk.

Statistical Analysis

Statistical analysis was performed for milk yield, milking unit on-time, incidence of bimodal milk curves, average flow rate, OT profiles, SCC and PLC. All values are reported as least-square means \pm standard error of the means (LSM \pm SEM; from SAS (SAS, 1999)) unless

otherwise noted. Data were analyzed as repeated measures using the Proc Mixed procedure. Somatic cell count and PLC data were log transformed for normalization. A \log_{10} transformation was chosen and normality was determined by the Shapiro-Wilk statistic. Log-transformed data were used for statistical comparisons; however, the actual (raw) values are presented.

The model used was as follows:

$$Y_{ijk} = \mu + M_i + A_i + S_j + L_k + S_j \times L_k + R e_{ijk}$$

where Y_{ijk} = variable of interest, μ = overall mean, M_i = milk yield, A = average milk flow rate, S_j = method of stimulation (1 to 2), L_k = lag time (1 to 3), $S_j \times L_k$ = interaction of stimulation and lag time, R is the correlation matrix in the error term, correcting for repeated effect of cow within treatment and e_{ijk} = random error. The correlation structure in the repeated measures effect that was used in the model was compound symmetry. The variables of interest were milk yield, milking unit on-time, percent of milk harvested in the first 2 min and average milk flow rate, OT concentration, PLC and SCC. Stage of lactation (DIM) was considered and analyzed for the primary outcome variables and was left out of the model because of lack of significance. Bimodal milk curves were analyzed using Proc Freq (chi-squared) methodology in SAS (SAS, 1999). Significant differences were declared at $P < 0.05$.

RESULTS AND DISCUSSION

Pretrial data were gathered for the cows enrolled in the experiment including the following: mature equivalent 305 milk (305ME), milk yield per milking, DIM, parity, milking unit on time, average flow rate and maximum flow rate (Table 11). Mature equivalent represents an estimation of mature milk production and therefore was defined as the milk production average for all cows in the herd over the previous 365 days. The 305ME of the cows enrolled in the study was $13,533 \pm 2256$ Kg (mean \pm SD). The cows were in their first to sixth lactation and

DIM ranged from 70-300 d at the time of enrollment. There was a possibility for 1,800 milkings to be analyzed during the data-collection period and of those a maximum of 1,719 milkings were used in the analysis. Milkings were removed from the analysis if the milk yield at a given milking was < 4 Kg or if the milking unit on time was < 120 s or > 420 s (settings within the data acquisition software from the farm).

Mean milk production for all treatments was 14.0 ± 0.4 Kg/milking (Table 12). The highest milk yield was 14.5 ± 0.3 Kg/milking (T0) and this was significantly different ($P < 0.05$) from the 3 lowest yielding treatments that all had milk yields of 13.7 ± 0.3 Kg/milking (M30, M90 and S90). Milk yield/milking was similar to a recent study (16.7 and 10.4 Kg/milking for early and late lactation cows) that analyzed pre-milking routines in high yielding Holstein cows milked 3X daily (Watters et al., 2011). Milk yield per milking did not differ when comparing 60 s of manual stimulation to 60 s of vibration stimulation (Karch et al., 1988). Milk production did not differ when comparing manual stimulation, no stimulation and mechanical stimulation (Sagi et al., 1980b, Weiss and Bruckmaier, 2005). Milk yield did not differ within classes of udder filling when comparing lag times of 0, 30, 45 and 60 s (Kaskous and Bruckmaier, 2011).

The mean milking unit on-time for all treatments combined was 257 ± 2.0 s (Table 12). Milking unit on time was shortest for S90 (245 s) and this was significantly different ($P < 0.05$) from all other treatments. Milking unit on-time was significantly reduced ($P < 0.05$) when 60 s of vibration stimulation was compared to 60s of manual stimulation (332 s vs. 350 s; (Karch et al., 1988)). Latency time (time without touching) had no effect on milking unit on-time when proportion of udder filling was > 40%; however, increasing the latency period from 0 to 30, 45 or 60 s independent of stimulation time reduced the unit on-time when the degree of udder filling was <40% (Kaskous and Bruckmaier, 2011). Sandrucci et al. (2007) found the shortest unit on-

time when lag times were between 1 and 60 s whereas Watters et al. (2011) reported that lag time > 60 s decreased unit on-time in late-lactation cows. High-speed pulsation had the highest unit on-time when compared to no stimulation, manual stimulation, and stimulation by positive pressure; however, it was not stated if time spent in stimulation phase was included in the unit on-time (Sagi et al., 1980b). The difference in unit on-time between manual stimulation and high-speed pulsation was 60 s and high-speed pulsation was turned on for 60 s; therefore, it is possible that unit on-time was similar between mechanical and manual stimulation (Sagi et al., 1980b). Our data are in agreement with Weiss and Bruckmaier (2005) who reported that milking unit on-time was longest for 90 s of vibration stimulation if stimulation time is counted as part of milking unit on-time and shortest if stimulation is removed from the calculation of unit on-time. One reason the milking unit on-time may be lower for S90 is that the 90 s spent in stimulation phase are not counted as part of the unit on time. Milk may have been harvested during the 90 s of stimulation; therefore, reducing the total time required to harvest the remaining milk. The amount of milk harvested during the mechanical stimulation time (30 and 90 s for S30 and S90) was 0.13 ± 0.03 and 0.32 ± 0.1 Kg, representing 1.2 and 2.2 % of the total milk harvest, which does not support the theory that milk was being harvested during the mechanical stimulation phase for S90. The time required to harvest 0.13 and 0.32 Kg of milk based on total milk yield divided by unit on-time means that 2.4 and 5.5 s of unit on-time could be reduced by the milk harvested during stimulation time. The time required for manual stimulation needs to be considered if an increase in milking unit on-time is reported to determine if the additional unit on-time is offset by the time required for manual stimulation.

Average milk flow rate was 3.6 ± 0.1 Kg/min and ranged from 3.2 to 3.7 ± 0.1 Kg/min (T0 and M90; Table 12). All treatments had significantly higher ($P < 0.05$) average milk flow

rates than T0. The average flow rate was in agreement with Watters et al. (2011) who recently reported an average milk flow rate of 3.9 Kg/min for cows milked 3x daily. Average milk flow rate was the lowest when no lag time was allowed in unison with low degrees of udder filling; however, when udder filling was > 40% lag time had no effect on the average milk flow rate (Kaskous and Bruckmaier, 2011). Average milk flow rate did increase numerically with each degree of udder filling category; however, only within the category of 20-40% udder filling were differences seen between lag times and milk flow rates (Kaskous and Bruckmaier, 2011). There was no difference in peak milk flow when comparing mechanical to manual stimulation (Sagi et al., 1980b). Average milk flow rate was lowest for high-speed pulsation when compared to manual stimulation; however, this may be because the time spent in high-speed pulsation was counted as part of total unit on-time (Sagi et al., 1980b). The use of high-vibration pulsation was applied for 0, 20, 40, 60 or 90s and as the vibration time increased so too did the average milk flow rate (Weiss and Bruckmaier, 2005). Maximum milk flow rate in this study was defined as the maximum milk flow detected at any given time during milking. Mean maximum milk flow rate for all treatments was 5.6 ± 0.2 Kg/min and ranged from 5.6 to 5.8 ± 0.2 Kg/min. Regression analysis was performed for average milk flow rate with maximum milk flow rate as the predictor for average milk flow rate ($R=0.67$). The correlation was positive; therefore it is expected that as the maximum flow rate increases so too will the average milk flow rate. The average milk-flow rate was thought to be lower for T0 because no time was allowed between the release of oxytocin and the beginning of milk harvest, thus extending the time required to harvest all the milk.

The percentage of milk harvested in the first 2 min was 48.0 ± 0.9 % across all cows. All treatments differed from each other ($P<0.05$) with T0 and S90 (40.4 ± 1.1 and 57.6 ± 1.1)

representing the highest and lowest values (Table 12). Recent data also indicated that when lag time was 0 s the lowest percentage of milk was harvested (< 45%) and when lag time was 90s in late lactation cows > 60% of milk was harvested in the first 2 min (Watters et al., 2011). Cows on S30 and S90 treatment that had stimulation time during the first 2 min had the stimulation time added to 2 min to determine milk harvested for 2 min in milk mode. Cows on S30 and S90 were analyzed for 150 and 210 s to determine milk harvested in the first 2 min, which was 45.5 ± 1.0 and 57.6 ± 1.0 for S30 and S90 (Table 12). Removing the milk harvested during mechanical stimulation (0.13 ± 0.03 and 0.32 ± 0.1 Kg; S30 and S90) from the total milk harvested during 150 and 210 s for S30 and S90 changed the percentage of milk harvested in the first 2 min to 44.3 and 55.3 for S30 and S90. Previously it was thought that during the extended stimulation phase for S90, milk harvest was taking place; however, the data indicate that on average only 0.32 Kg are harvested during these 90 s of stimulation at low vacuum. The data clearly indicate that the greatest percentage of milk is harvested when the stimulation or lag time is equal to 90 s in cows milked 3X.

The incidence of bimodal milk curves was highest for T0 (21%) and lowest for M90 (7%; $P < 0.05$; Table 13). The data indicate that the lowest (M90) and second highest (S90) incidence of bimodal milk curves involved lag or stimulation time of 90 s, which seems contradictory. It is possible that during the shift from stimulation mode into milk mode a surge of milk creates a peak and then valley, thus creating a bimodal milk curve. Cows on S90 had the highest amount of milk harvested during the first 2 min of milk, which downplays the high occurrence of bimodal milk curves for S90. The small amount of milk (0.32 Kg) harvested and >55% of milk being harvested in the first 2 min for S90 counters the idea that a high incidence of bimodal curves (first 60 s in milk phase) occurred for S90 (Figure 10). Our research is in agreement with

previous research indicating 22 to 29% of cows with 0 s lag had bimodal milk curves, whereas 5 to 6% of cows had bimodal milk curves when lag was 90 s (Watters et al., 2011). The occurrence of bimodal milk curves and OT titer for the given treatment were not related, which may be accounted for by differing degrees of udder fill. Sandrucci et al. (2007) recently found a significant decrease in the incidence of bimodal milk curves when the lag time was increased, with which we are in agreement on for manually stimulated cows. In mid-lactation cows bimodal milk curves only occurred with stimulation time 15 s and followed by no latency period (Kaskous and Bruckmaier, 2011), suggesting that extent of udder fill may not be a concern in mid-lactation cows.

Median SCC and PLC for all treatments were $38.5 (x 10^3 \text{ cells/mL})$ and $14.3 (x 10^3 \text{ cfu/mL})$. The SCC did not significantly differ ($P>0.05$) between any of the treatments and medians ranged from 33.5 (M30) to 45 (S90) (Table 12). The PLC was lowest for S90 ($9.8; x 10^3 \text{ cfu/mL}$) and highest for T0 ($15.8; x 10^3 \text{ cfu/mL}$), which were significantly different ($P<0.05$) from each other. The PLC was expected to be higher for T0, S30 and S90 because no cleaning of the teats was performed prior to attachment of the milking. The PLC for T0 and S30 could be explained by unit attachment prior to cleaning of the teats; however, the PLC for S90 was the lowest and is not in agreement with the theory for S30 and S90. The bacterial load for all treatments was at or above the threshold of $10 (x 10^3 \text{ cfu/mL})$, which is recognized by the milk-processing industry to be the upper limit for sanitary milk even though the legal limit is $100 (x 10^3 \text{ cfu/mL})$ in the US. Bacterial counts of $>10 (x 10^3 \text{ cfu/mL})$ with a $\text{SCC} < 45 (x 10^3 \text{ cells/mL})$ for all treatments indicates that bacteria were being removed from the teats and mammary gland during milking of the cow. The high PLC along with the low SCC indicated that the introduction of bacteria into the mammary gland was being minimized within the milking claw.

Oxytocin concentration in blood was < 2.6 pg/mL for all treatments prior to stimulation and this agrees with previous data that indicated no activation of the milk-ejection reflex if OT concentration is < 3.5 pg/mL (Figure 11). Cows on T0 had a significantly higher OT concentration than S30 5 min prior to stimulation: however, the concentration of 2.57 ± 0.2 pg/mL for T0 is a lower concentration than what is required to elicit a milk ejection reflex (Figure 11). At time 0, the concentration of OT for M30 was 6.6 pg/mL, which indicated that cows on M30 responded to manual stimulation and that OT was released into the blood stream. At 1 min after milking-unit attachment all treatments had a blood OT concentration of > 4.6 pg/mL, which is within the range of 3-5 pg/mL that is recognized as the concentration at which the milk-ejection reflex is initiated (Schams et al., 1984). Oxytocin concentration for all treatments did not differ from 2 to 8 min after milking-unit attachment. Stimulation did not take place until milking-unit attachment for T0, S30 and S90 and OT concentration did not differ beyond 2 min--indicating that manual stimulation and stimulation elicit similar OT release. The release of OT by manual stimulation will occur prior to the attachment of the milking unit: however, there is no difference in OT concentration between manual and mechanical stimulation. At 8 min after the attachment of milking unit an intravenous injection of OT was given to obtain the residual milk. The OT concentration after 8 min did not differ between any of the treatments (Figure 12). Our data are in agreement with previous data that reported manual stimulation and stimulation by the milking machine will elicit similar OT profiles (Sagi et al., 1980a, Gorewit and Gassman, 1985).

Residual milk ranged from 2.1 to 2.3 ± 0.2 Kg/milking and was not different for any of the treatments (Table 12). Residual milk as a percent of total milk (total milk = milk yield + residual milk) was not different between treatments (Table 12). We were in agreement with

Macuhova et al., (2003) who reported 14.8 to 15.9% residual milk when using mechanical methods for stimulation. Recent data suggested that 11% of the total milk was left in the udder as residual milk at the end of milking (Belo et al., 2009). Negaro and Marnet (2006) classified cows into 2 groups based and residual milk yield and they defined cows with >8% residual milk as having impaired milk ejection. Dutch Friesian and Russian Red Steppe cows had 9 and 25% residual milk, respectively (Brandsma, 1978). Residual milk as a percentage may be higher on the current study because the low-flow threshold was 1kg and/or because cows were milked 3X and therefore may have less mammary pressure. The breed of cow, stage of lactation and age of cow all can influence the amount of residual milk.

CONCLUSIONS

The use of pulsation as a form of stimulation elicits a similar OT profile as that of cows which were subjected to manual stimulation. The impact of not cleaning the teats prior to milking unit attachment resulted in the highest bacterial counts in the stimulation-pulsation group. The high bacterial counts did not affect udder health; the cows on treatment had a median SCC of <45 ($\times 10^3$ cells/mL). The milking unit on-time was lower for cows given 90 s of fast pulsation stimulation, not counting the unit on time during stimulation; however, milk harvest may have begun during the stimulation phase. Less than 0.32 Kg of milk was harvested during the vibration-stimulation phase for S30 and S90; the percentage of milk harvested in the first 2 min was highest for S90. Therefore, extending rapid pulsation stimulation to 90 s does not harvest a quantity of milk high enough to increase the amount of milk harvested in the first 2 min or to reduce the unit on-time significantly. The use of pulsation with increased cycles (300/min) and low vacuum is an adequate form of stimulation to initiate the milk-ejection reflex. The occurrence of bimodal milk curves were the highest when no lag time was allowed and lowest

for manual stimulation plus 90 s of lag time. Mechanical stimulation may be a way to improve milking efficiency (although no time is spent on cleaning the teats prior to milking). Proper cleaning of the teats and detection of mastitis during mechanical stimulation needs further investigation. Milk yield, times milked per day, and the breed of cattle may lead to different responses in milking parameters; therefore, it is important to re-state that the responses in this experiment were conducted on high-yielding Holstein cows milked 3X.

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Table 11. Pretrial data for 30 cows assigned to treatments

Variable	Mean	SE
Parity	2.0	0.2
DIM	163.4	13.1
Milk yield/milking (Kg)	15.0	0.4
Milking unit on-time (s)	266.4	6.8
Average milk flow rate (Kg/min)	3.8	0.1
Maximum milk flow rate (Kg/min)	5.7	0.1

Table 12. Within-trial milk yield, milking unit on time, milk flow rates and milk quality by treatments. Manual stimulation involves forestripping and stimulation by pulsation involves 300 cycles/min with a chamber vacuum of 20 kPa

Variable	Immediate attachment (T0)		Dip plus forestrip and drying and 30 s lag time (M30)		Dip plus forestrip and drying and 90 s lag time (M90)		Stimulation pulsation for 30 s (S30) ¹		Stimulation pulsation for 90 s (S90) ¹	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Milk yield/milking (Kg)	14.5 ^a	0.3	13.7 ^b	0.3	13.7 ^b	0.3	14.2 ^c	0.3	13.7 ^b	0.3
Milking unit on-time (s)	262 ^a	2.1	256 ^b	2.1	259 ^c	2.1	261 ^{ac}	2.1	245 ^d	2.0
Average milk flow (Kg/min)	3.2 ^a	0.1	3.7 ^b	0.1	3.7 ^b	0.1	3.6 ^c	0.1	3.6 ^c	0.1
Maximum milk flow (Kg/min)	5.6 ^b	0.2	5.8 ^a	0.2	5.6 ^b	0.2	5.6 ^b	0.2	5.6 ^b	0.2
Percent of total milk harvested during first 2 minutes of unit on-time (%) ²	40.4 ^a	1.1	47.4 ^b	1.0	49.2 ^c	1.0	45.45 ^d	1.0	57.58 ^e	1.0
Percent of total milk harvested during first 2 minutes of unit on-time (%) ³	40.4 ^a	1.1	47.4 ^b	1.1	49.2 ^c	1.1	44.32 ^d	1.1	55.25 ^e	1.1
Residual milk (Kg/milking)	2.34	0.2	2.26	0.2	2.29	0.2	2.06	0.2	2.21	0.2
Percent residual milk	13.6	0.1	14.0	0.1	13.8	0.1	12.4	0.1	13.4	0.1
Percentile	Median	2.5-97.5	Median	2.5-97.5	Median	2.5-97.5	Median	2.5-97.5	Median	2.5-97.5
SCC(x 10 ³ cells/mL) ⁴	39.8	5.5-245	33.5	9.5-395	44	6.5-480	32.75	6-485	45	5-235
PLC(x 10 ³ cfu/mL) ⁴	15.8 ^a	0-1000	12.5 ^{abc}	0-216	27 ^c	1-1000	13.8 ^{bd}	0-900	9.8 ^d	0-470

¹Stimulation pulsation involves 300 cycles/min with a chamber vacuum of 20 kPa

²Percent of total milk harvested during the first 2 minutes of unit on-time including mechanical stimulation time (S30 = 150 s and S90 = 210 s)

³Percent of total milk harvested during the first 2 minutes of unit on-time including mechanical stimulation time, minus the milk harvested during the mechanical stimulation time for S30 and S90

⁴Values presented are actual values; however, statistical analysis performed on log₁₀ transformed values
Values within row and with different superscripts are significantly different at ($P < 0.05$)

Table 13. Incidence of bimodal milk curves by treatment. Manual stimulation involves forestripping and stimulation by pulsation involves 300 cycles/min with a chamber vacuum of 20 kPa

Treatment	Total	Bimodal (%)
Immediate attachment (T0)	333	21 ^a
Dip plus forestrip and drying and 30 s lag time (M30)	347	14 ^b
Dip plus forestrip and drying and 90 s lag time (M90)	350	7 ^c
Stimulation pulsation for 30 s (S30) ¹	337	14 ^{bd}
Stimulation pulsation for 90 s (S90) ¹	352	17 ^{ab}

¹Stimulation pulsation involves 300 cycles/min with a chamber vacuum of 20 kPa

Figure 10. Bimodal milk curve

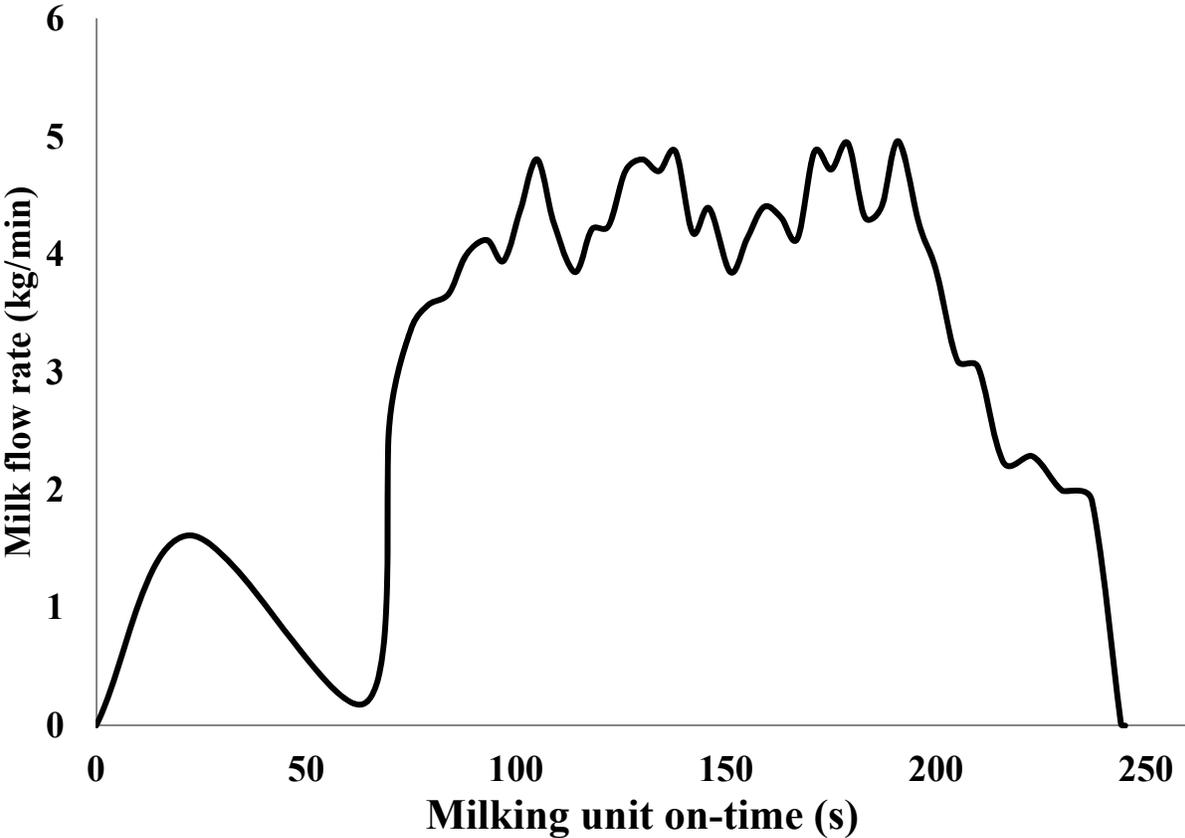


Figure 11. Oxytocin concentration (pg/mL) prior to, during and after milking for 5 treatments. Treatments were immediate attachment of the milking machine T0 (●); dip plus forestrip and drying with 30 s lag time M30(○); dip plus forestrip and drying with 90 s lag time M90 (▼); stimulation pulsation for 30 s S30 (Δ); and stimulation pulsation for 90 s S90(■). Lines with arrow depict time point when stimulation began with treatment listed above.

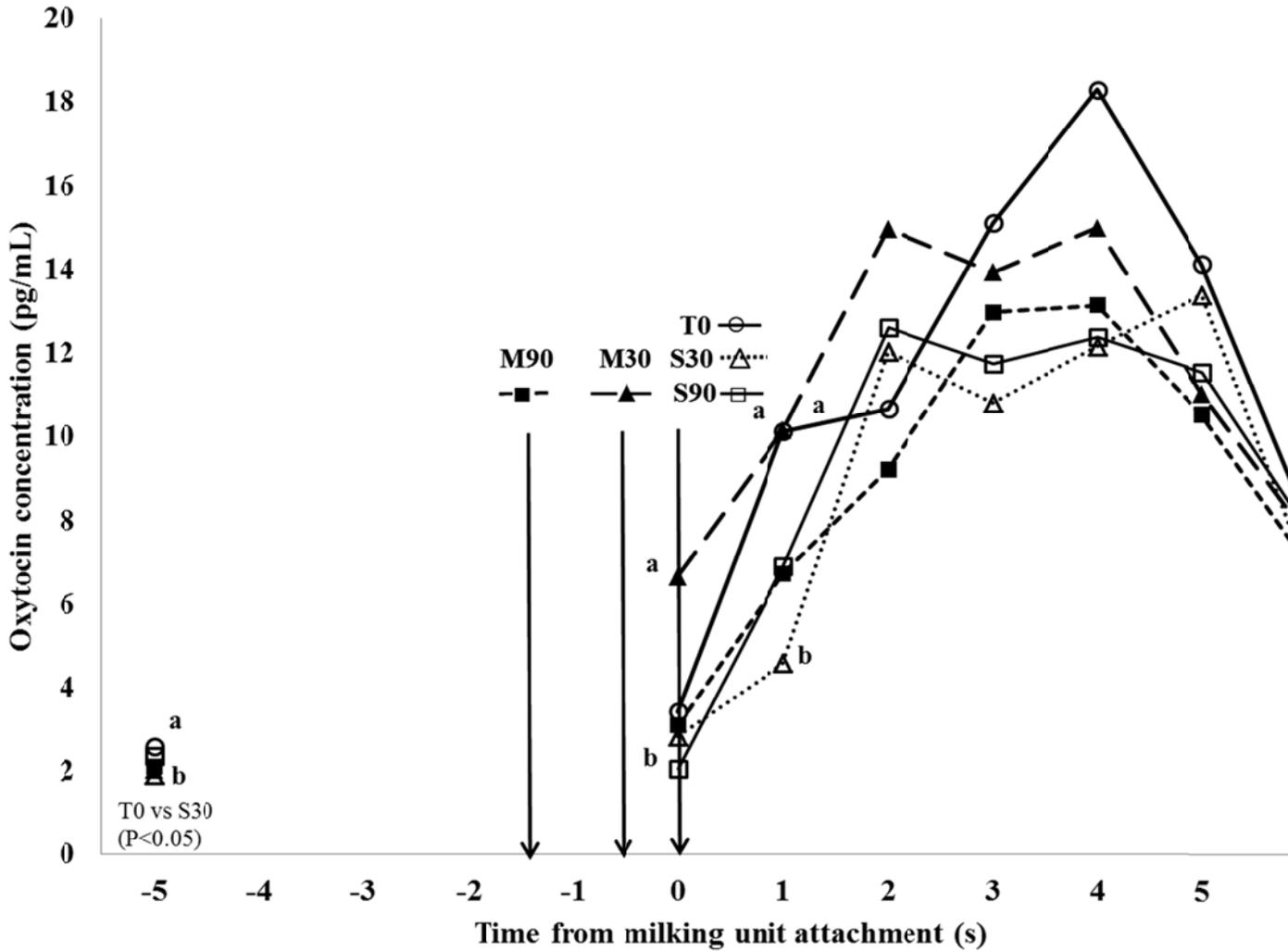
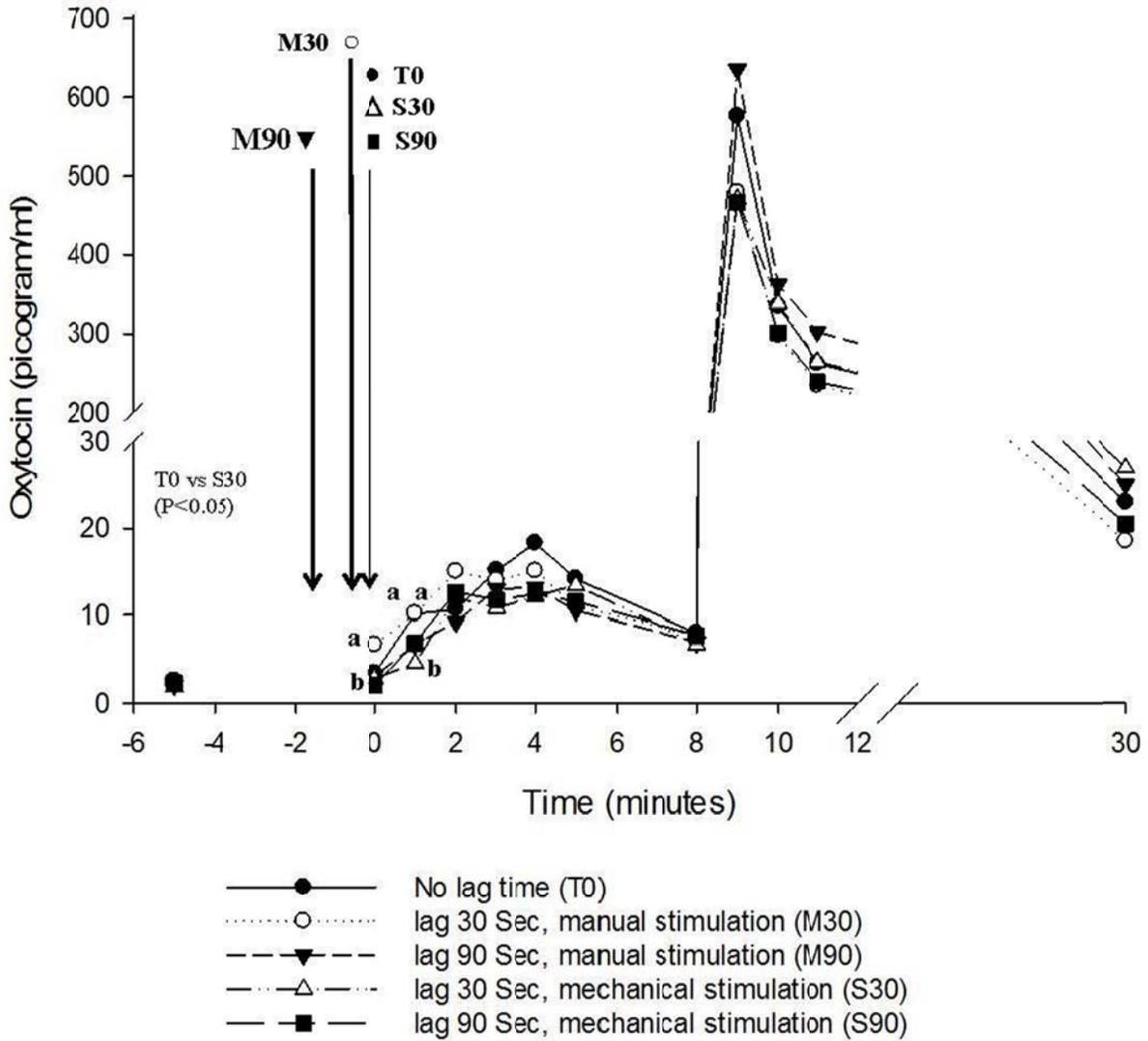


Figure 12. Oxytocin concentration (pg/mL) prior to, during and after milking for 5 treatments. At 8 minutes after milking unit attachment an injection of oxytocin was administered. Treatments were immediate attachment of the milking machine T0 (●); dip plus forestrip and drying with 30 s lag time M30(○); dip plus forestrip and drying with 90 s lag time M90 (▼); stimulation pulsation for 30 s S30 (Δ); and stimulation pulsation for 90 s S90(■). Lines with arrow depict time point when stimulation began with treatment listed above.



CHAPTER 5

CONCLUSIONS AND RECCOMENDATIONS

The release of OT, which is required for the milk ejection reflex can be initiated by manual and mechanical methods. The flow of OT from the PP to the mammary gland occurs within approximately 20 s after the initiation of stimulation. Timing of milking unit attachment after the initiation of the milk ejection reflex is more variable because of the number of times a cow is milked daily. Harvesting healthy milk in the quickest and safest manner is the goal of the milking routine and timing of milking unit attachment is one component that dairy managers have control over.

Increasing the lag time beyond 60 s, which has been used for 12 h milking intervals was beneficial because cows milked 3X daily start milk ejection later than cows milked with 12 h intervals. The release of OT was not determined between 2X and 3X cows; however, the neuroendocrine reflex for OT release is not expected to differ. Lag time did not affect cows in early lactation; however, a lag time of 90 s was beneficial for late lactation cows. Extending the lag time to 90 s decreased unit on-time while maintaining milk production; therefore average milk flow rate increased. Increasing the lag time to 90 s also led to the highest level of milk harvested in the first 2 min for late lactation cows. Udder fill is greatest in early lactation and in cows with long milking intervals and decreases with advancing stage of lactation and shorter milking intervals. Increasing lag time to 90 s is thought to allow for the delay in milk ejection in cows milked 3X daily and therefore increases the average milk flow rate.

Foremilk has often been thought of as being the worst portion of milk from a quality standpoint. Data suggests that the highest SCC is in the foremilk when compared to the alveolar fraction of milk. Microorganisms are the main cause of infection and an increase in SCC, but

little information is available on the comparison of foremilk and alveolar bacteria counts. Milk that is of high quality <100,000 cells/mL the foremilk and harvested milk fractions are similar; however when the SCC is >350 the foremilk is not a good predictor of the harvested milk fraction. The bacteria count of foremilk and harvested milk are similar when the PLC is <10,000 cfu/mL. This indicates that when the udder is healthy <100,000 cells/mL that the foremilk is similar. A bacteria count of 10,000 cfu/mL is considered high by dairy processing standards. It is important to distinguish between the amount of bacteria present and the type of bacteria. Cows with a high PLC (>10,000 cfu/mL) and a low SCC <200,000 cells/mL indicate that the cow may have a superior immune system or that the bacteria present may not be considered major pathogens. The use of foremilk as an indicator of the harvested milk portion may be possible if the SCC of the fore milk is 200,000 or less.

Stimulation by high vibration pulsation was compared to manual stimulation to see if OT concentrations in plasma were similar as well as milking time characteristics. Oxytocin profiles were similar between treatments if time of stimulation was taken into consideration. Mechanical stimulation for 90 s had the highest amount of milk harvested in the first 2 min and the shortest milking unit on-time. Increased lag time to 90 s with improved milking characteristics on 3x daily cows supports the theory of delayed milk ejection release in 3X cows. The use of high vibration pulsation for stimulation was researched without sanitizing the teat prior to milking unit attachment. The process of milking the cow will remove organic matter from the teat. The bacteria count of milk harvested by high vibration pulsation or immediate attachment of the milking unit was 10,000 cfu/mL. The removal of milk that has a high SCC and PLC is required with high vibration pulsation.

Holstein cows milked 3X daily have a shorter milking interval and benefit from a lag time of at least 90 s. It is recommended that lag time for all cows milked 3X be at least 90 s. If mechanical stimulation by high vibration pulsation is used then 90 s of stimulation time is recommended. The use of a combination of manual stimulation and high vibration stimulation is an option too. Upon removal of the teat disinfectant the milk unit could be attached in high vibration pulsation mode until 90 s of total lag time is achieved. The combination of both manual and mechanical methods allows for a shorter time until the milking unit is attached and less variation in the time from first tactile stimulation until milking unit attachment. Development of technology that can determine the SCC both in-line and in real time will be required for the use of high vibration pulsation in order for quality milk to be harvested. A process to sanitize the teat and remove the sanitizing solution along with abnormal milk prior to milk harvest should be researched further.