CHARACTERIZING THE HEALTH AND WELFARE OF PERIPARTURIENT DAIRY COWS IN A PASTURE-BASED AUTOMATIC MILKING SYSTEM

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# ABSTRACT

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The periparturient period, defined as three weeks prior to and three weeks after parturition, is a time of great physiological, environmental, and social stress for dairy cows. This transition into lactation is often marked by negative energy balance, oxidative stress, and impaired immune function. These factors combine to create the perfect storm to challenge a cow's homeorhesis. The periparturient period has been well characterized in cows in traditional dairies that are milked at a fixed frequency in a parlor and fed a total mixed ration, however, little research on alternative milking and feeding systems exists. One such system is a combined pasture-based automatic milking system (AMS) dairy. This combination of milking system and diet is still relatively uncommon, however, there are many potential benefits for both cows and producers. Despite the growing interest in pastured-AMS dairies, there is little information available regarding how the periparturient cow is impacted by this milking system and diet. Therefore, the aim of this research was to use physiological, production, and behavioral data to evaluate the health and welfare of pastured multiparous and primiparous cows milked in an AMS during the periparturient period. Results indicate that multiparous and primiparous cows experience metabolic and oxidative stress and changes in pro-inflammatory cytokine expression, but that there is not a dramatic difference between parities or during the periparturient period. Non-invasive behavioral measures and production data support these results. Indeed, a well-managed pasture-based system combined with the correct implementation of AMS seems to offer benefits and does not negatively impact cows during the critical periparturient period.

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## KEY TO SYMOBOLS OR ABBREVIATIONS

AMS – Automatic Milking System

DIM – Days in Milk

BCS – Body Condition Score

DMI – Dry Matter Intake

MC – Multiparous Cow

PC – Primiparous Cow

NEFA – Non-esterified Fatty Acid

BHBA – β-hydroxybutyrate

IL1-β – Interleukin 1- β

IL-8 – Interleukin 8

TNF-α – Tumor Necrosis Factor-α

DRTC – Day Relative to Calving

TLR – Toll-like Receptor

CRE – Copper Reducing Units

TMR – Total Mixed Ration

## INTRODUCTION

The periparturient period is a time of great stress and change for a dairy cow. Physiologically, she is preparing for parturition and lactation, while at the same time, she may experience dietary and social changes that may be additional stressors. There is a large body of knowledge describing these changes, especially related to the alterations in metabolic and immune states and the impairment of the immune function, during this critical time, but very little research has examined these parameters in a pastured dairy using an automatic milking system (AMS). The first chapter provides a comprehensive review of the current literature concerning the metabolic and oxidative status of dairy cows during the periparturient period, and addresses some of the possible benefits and drawbacks that AMS technology and a pasture-based diet might offer to cows and producers during this sensitive time. The second and third chapters address the health and behavioral parameters of periparturient cows in such a system.

Much research has been dedicated to understanding and evaluating the metabolic and oxidant status and immune function of dairy cows during the periparturient period (Aikten et al., 2009; Bernabucci et al., 2005; Sordillo et al., 2007); however none of these studies address the impact of milking with an AMS or feeding a pasture-based diet. As this type of dairy becomes more common, in order to optimize health, welfare, and production it is imperative to understand how cows managed in this way are impacted during the transition from gestation to lactation and if there are differences in the metabolic and oxidant profiles and pro-inflammatory cytokine expression of multiparous and primiparous cows. Thus, the second chapter characterizes the metabolic and oxidant stress profiles, as well as examines pro-inflammatory cytokine expression, of primiparous and multiparous pastured-Friesian dairy cows milked by an AMS.

Preparing comprehensive metabolic and oxidant profiles of periparturient cows to evaluate their health status might not always be practical or feasible, especially to meet the everyday needs of a producer trying to understand whether cows are at risk. However, there are behavioral and health parameters that may be collected quickly by the producer or automatically by the AMS to help evaluate the status of a cow. These measures, such as body weight, body condition score, gait score, milk production information, and lying time are non-invasive and could be performed at any time with minimal disturbances to an individual or the herd. Chapter three provides details on the welfare and health status of periparturent Friesian and Holstein dairy cows at pasture-AMS dairies using these measures.

Understanding the impacts of feeding and management systems on dairy cows is imperative to optimizing their health and welfare, especially during the sensitive transition from gestation to lactation. In order to do this, information on how cows respond to difference diets and milking systems must be available, particularly as producers increasingly explore low labor and low input systems. This research is intended to address a gap in the literature on the impact that a pasture-based AMS dairy would have on cows during the transition period. The critical nature of this period for cow health and welfare makes it very important to understand both the possible benefits and drawbacks to placing dairy cows in a pasture-AMS system. Findings here may help to develop recommendations for managing cows, both domestically and internationally, in this highly unique combination of feeding and milking methods.

# CHAPTER 1

# COMPREHENSIVE LITERATURE REVIEW

## INTRODUCTION

The transition period is a physiologically stressful time for dairy cattle. For three weeks before calving to three weeks after calving, cows experience physiological transformation as they prepare for parturition and subsequent production of colostrum and milk. Poor management during this transition phase could have important immunological, metabolic, and behavioral impacts on cows welfare and productivity. Maintaining the balance of optimal health for dairy cows in the transition period could be a challenge in certain production systems. The changes observed around the transition period have been relatively well characterized in traditional confinement operations, but there has been little research to date on what effect pasture nutrition or automatic milking systems (AMS could have on the cow during this sensitive time. This gap in understanding can lead to difficulties in deciding how to best manage cattle in alternative feeding, housing, and milking systems.

One must first look to what is known about these animals in traditional systems in order to best understand what information regarding the immune, metabolic, and behavioral changes is most important to consider when studying transition cattle in alternate production systems. The immune system of dairy cows and the mammary gland are very complex. Maintaining a healthy udder is of vital importance to obtaining high quality milk for production purposes and for the overall health of the cow. Mastitis is one of the biggest health concerns for dairy cattle, as well as being the most costly diseases for producers (Petrovski et al., 2006; Halasa et al., 2007). The most widely used method of detecting mastitis, as well as being part of the infection's formal definition, is the somatic cell count (SCC) of milk in each quarter (Pyörälä, 2003). In addition to being used as a measure of udder health, SCC also acts as an indicator of milk quality (Pyörälä, 2003). The cost is not only incurred from treating ill cows, but also in milk that must be discarded, increased labor to treat the ill animals, veterinary bills, culled cows, and production losses from chronic cows or losses that may last into the next lactation cycle (Petrovski et al., 2006; Halasa et al., 2007).

The amount of milk produced, as well as innumerable environmental factors, influence the metabolic status of the animal. If a cow is expending more energy than she is consuming, especially in the early phase of lactation, a negative energy balance is created that can predispose the animal to several different diseases (Mulligan and Doherty, 2008). Because transition cows cannot ingest enough feed to meet the rapidly increasing requirements of lactation, the resulting negative energy balance could impair immune function (Goff and Horst, 1997). The physiological stressors around calving – parturition itself, beginning a new lactation cycle, increased energy demands and decreased feed intake – strongly challenge the homeorhetic balance of the individual cow (Goff and Horst, 1997). The increased social stress both pre- and post-calving due to frequent regroupings is an additional behavioral challenge that affects the cow during this period of physiological, nutritional, and environmental change (Cook and Norlund, 2004). It is our responsibility as managers and caretakers of dairy cattle to find methods to assure optimal health and welfare of dairy cows during the transition period.

## OXIDATIVE AND METABOLIC STATUS

### *Oxidative Status*

The immunological and oxidative status of dairy cattle is important to consider, especially during the transition period, because of the animals' increased likelihood of illness and infection. A cow's immune system is most impaired the week immediately before calving and the first week after parturition (Goff and Horst, 1997). The incidence of metritis, mastitis, retained fetal membranes, and mammary edema are all common issues that might be seen postpartum, a time when a cow is known to experience oxidative stress (Sordillo and Aitken, 2009). Less frequent milkings at the start of lactation can reduce the period of time a cow is in a negative energy balance (Patton et al., 2006) and can help to lower her risk for metabolic disorders while improving her reproductive performance (Clark et al., 2006). It has been shown that once a day milkings can lower the cow's energy output and lead to improvements in nutritional status, but milk yield will be reduced for the first half of her lactation cycle (Patton et al., 2006). This is not ideal for some producers, depending on individual farm goals, who rely on high milk yields, thus other management and nutritional decisions must be considered. These decisions should take into account the relationships between the physical changes that occur during parturition and the loss of overall antioxidant potentials, which affect the immune and oxidative status of the animal (Sordillo and Aitken, 2009). Studies that have used both *in vivo* and *in vitro* techniques support the notion that oxidative stress during the transition phase could be a major, underlying cause of inflammatory and immune dysfunction for dairy cows (Sordillo and Aitken, 2009).

Oxidative stress is a condition that occurs when the body produces excessive reactive oxygen species (ROS) that antioxidant defenses are unable to neutralize. ROS are a type of free radical (a molecule with at least one unpaired set of electron in the outer orbital that can promote electron transfer with oxidation reduction reactions) that has formed from molecular oxygen.

The free radicals are a normal end product of the mitochondrial electron transport chain or the creation of NADPH (Valko et al., 2007). Low levels of ROS can actually help facilitate normal cell function, but when there are too many present in the body, the excessive protein oxidation can lead to cellular dysfunction or premature protein synthesis (Eaton, 2006). ROS have a large effect on the thiol groups of proteins that regulate many metabolic, signaling, and transcriptional process of a cell (Sordillo and Aitken, 2009). Many of these thiol groups, especially those in blood plasma and red blood cells, are synthesized in the liver, an organ which generally decreases in function after parturition (Berbanbucci et al., 2005). Oxidative stress may help to exacerbate this state, as is evident in the concurrent increase in thiobarbituric acid-reactive substances (TBARS) and Se-glutathione peroxidase (GSH-Px) (Berbanbucci et al., 2005). This shift, decreased thiol groups and increased TBARS and GSH-Px, demonstrates the imbalance of more oxidants present in the body than antioxidants after calving (Berbanbucci et al., 2005). TBARS is a measure related to lipid peroxidation (Berbanbucci et al., 2005), but it is more widely accepted as a good overall marker of total oxidative stress than related solely to lipid peroxidation (Armstrong and Browne, 1994). Selenium-glutathione peroxidase is part of a group of enzymatic antioxidants that serves as the primary defense against intracellular oxidation (Berbanbucci et al., 2005).

An imbalance of ROS relative to antioxidants results from high levels of ROS present in the body, a decline of important antioxidants, or a combination of these two factors (Sordillo and Aitken, 2009). There are three major categories of endogenous antioxidants that work in the body: enzymatic antioxidants, nonenzymatic protein antioxidants, and nonenzymatic low-molecular weight antioxidants (Bernabucci et al., 2005). Enzymatic antioxidants include copper-zinc-superoxide dismutase and selenium-glutathione peroxidase, which are the main form and intracellular defense (Bernabucci et al., 2005). Copper-zinc-superoxide dismutase is the first defense against pro-oxidants that convert free radical superoxide ($^{\bullet}O_2^-$) to hydroxyl ($OH^{\bullet}$), while selenium-glutathione peroxidase converts hydrogen peroxide ($H_2O_2$) to less dangerous reduced forms of the molecule (Bernabucci et al., 2005). Nonenzymatic protein antioxidants are found mainly in plasma and their protein sulfhydryl groups are considered to be important elements of extracellular antioxidant defense (Bernabucci et al., 2005). Nonenzymatic low-molecular weight antioxidants are found in plasma and other extracellular fluids, as well as lipoproteins and membranes (Bernabucci et al., 2005). These antioxidants can be subdivided into water soluble and lipid soluble (Bernabucci et al., 2005). The water soluble antioxidants include absorbic acid and glutathione, which is of special importance because it reacts directly with free radicals and lipid peroxidases and can work to protect cells (Bernabucci et al., 2005).

The excessive ROS then go on to cause oxidative damage to proteins, lipids, DNA, and other macromolecules by acting on the cellular membrane and other components (Bernabucci et al., 2005) This damage occurs throughout the body and can lead to a compromised immune system and inflammatory reactions (Sordillo and Aitken, 2009). Maddox and colleagues (1999) reported that oxidative stress can increase the adhesion of active neutrophils to the endothelial cells of the mammary gland. Additionally, ROS, thiobarbituric acid-reactive substances, and plasma selenium-glutathione peroxidase increase while copper-zinc-superoxide dismutase, plasma thiol groups, and erythrocyte thiol groups decease immediately after calving (Bernabucci et al., 2005). These large changes in the cellular environment likely lead to the imbalance of ROS production being greater than the antioxidants' ability to neutralize them (Bernabucci et al., 2005). This then creates a chain reaction and lipid peroxidation, disrupting the overall homeostasis of the cow (Bernabucci et al., 2005). These could be important contributing factors that lead to the increased incidence of mastitis postpartum.

***Nutritional Influence on Immune Status***

Obesity and high body condition score (BCS) can also be a contributing factor to oxidative stress and metabolic disorders during the transition period. Bernabucci and colleagues (2005) found that cows with a higher BCS pre- and post-calving were more sensitive to oxidative stress, while those animals with lower BCS had lower levels of nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) and showed no signs of metabolic disease. Cows that were scored as fat (BCS = 4) prior to calving compared to those scored as thin (BCS = 2.5) had lower dry matter intake (DMI) and daily milk yields with less lactose and protein; lost more weight (48 kg vs. 27 kg) and more condition (1.2 units vs. 0.52 units); and had a greater incidence of disease (e.g. mastitis, retained placenta, endometritis, ketosis, milk fever, cystic ovaries, ovarian underactivity, hypomagnesaemia, lameness) over the first 10 wk of lactation (Treacher et al., 1986). A greater loss in body weight in overfed versus feed-restricted cows immediately after parturition was also observed by Rukkwamsuk et al. (1998), indicating these animals had a more severe negative energy balance. This observation was supported by blood work results revealing a higher NEFA plasma concentration as well as a higher hepatic triglyceride levels for the overfed cattle compared to the feed restricted group (Rukkwamsuk et al., 1998). Overconditioned cows' livers are not as effective at oxidizing fatty acids as that of thin cows (Goff and Horst, 1997). Extra macronutrients consumed can lead to a weight increase, where the adipose tissue then increases the production of pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) (Dandona et al., 2004).

It has been suggested that oxidative status might be related to metabolic disorders in dairy cows (Bernabucci et al., 2005), which is logical when considering the vast physiological and often dietary changes that occur during this six week period. The final weeks of gestation require a great deal of protein, energy, and minerals to support the fetal-placental mass, but this is still relatively small compared to colostrum (Goff and Horst, 1997). In order for a cow's mammary gland to synthesize 10 kg of colostrum for the newborn calf, 11 Mcal of energy, 23 g of calcium, 9 g of phosphorus, 140 g of protein, and 1 g of magnesium must be supplied in the diet or be pulled from body reserves (Goff and Horst, 1997). Calcium is one mineral of special importance because it is thought to assist with smooth muscle contractions, which are necessary for the teat sphincter to fully close and protect the mammary gland from invading pathogens (Goff and Horst, 1997). When a cow experiences hypocalcaemia, which is commonly observed in most dairy cows at parturition with some animals experiencing such a low calcium level they develop milk fever, this first line of defense is rendered less effective (Goff and Horst, 1997). Energy imbalances that occur around calving as physiological demand increase due to higher requirements for milk production and the inability to consume enough feed to meet these needs are thought to play a role in immunosuppression by acting on different cell types important for defending the body against infection and illness (Kehril et al., 1989; Scalia et al., 2006).

Increased levels of NEFA and BHBA at the individual cow level have been used as markers of negative energy balance during the transition period (Ospina et al., 2010b). The body mobilizes the stored energy from fat through NEFAs, which are then taken up by the liver where they are either oxidized or reesterifired into triglycerides (Ospina et al., 2010a). These triglycerides are then either stored in the liver for later use or they can be exported from the organ as very low density lipoproteins for use in the body (Ospina et al., 2010a). If NEFA levels are excessive, as is common during the periparturient period, the liver cannot store or re-esterify the NEFAs efficiently, leading to releasing the energy by generating heat or accumulating the triglycerides in the body (Drackley et al., 2001). When liver cells are saturated by triglycerides, their function is impaired and they are unable to incorporate acetyl-coenzyme A into the tricarboxylic acid cycle and instead convert it to acetoacetate or BHBA (Goff and Horst, 1997). High levels of both NEFA and BHBA surrounding calving have been linked to the increased likelihood of a cow being diagnosed with a wide range of health concerns during the transition period, including a displaced abomasum (Cameron et al., 1998), clinical ketosis, metritis, or retained placenta (Ospina et al., 2010a). Increased BHBA production is the result of the liver working to remove excessive NEFAs and carnitine palmitoyltransferase-1, both of which regulate ketogensis, the process that produces ketone bodies - BHBA, acetoacetate, and acetone (Hergardt, 1999). High levels of these ketone bodies in bodily fluids (e.g. urine, milk, or blood) found after parturition is characteristic of ketosis, which can then result in hypoglycemia due to impaired gluconeogensis (Goff and Horst, 1997).

Research in both human and animal medicine has demonstrated a relationship between nutritional and immune states (Sordillo et al., 2009). In research completed on human respiratory diseases, an overall inflammatory response of the body has been documented and there is emerging evidence that metabolic status, specifically altered lipid metabolism, directly impacts this systemic inflammatory state (Wood et al., 2009). Fatty acids are the main energy source for the body, but excessive levels, such as superfluous types of NEFA, have been shown to stimulate an innate immune response (Wood et al., 2009). Though NEFA have been recognized to activate the innate immune response, the exact method of this activation has yet to be determined (Shi et al., 2006). Toll-like receptors (TLR) are a type of receptor that initiates the innate immune response by signaling the activation of proinflammatory cytokines, major histocompatibility complex (MHC), co-stimulatory molecules, and chemokines to invading pathogens (Medzhitov, 2001). It has been found that the lipid component of lipopolysaccharide (LPS) on Gram-negative bacteria can trigger the activation of specific TLRs and thus initiate an innate immune response (Shi et al., 2006). These results may suggest that the presences of excessive NEFAs in the body may act in the same way to trigger the TRLs to begin an innate immune response and lead to an overall inflammatory state.

When a cow is under increased metabolic stress, as during the periparturient period, changes in the fatty acid composition of cells important for the inflammatory response could impact how the body reacts to pathogens, though this has yet to be proven in cow endothelia or white blood cells (Sordillo et al., 2009). What has been proven is that cytokines do have catabolic effects on metabolism, specifically; inflammatory cytokines directly alter hepatic metabolic function (Bradford and Eastridge, 2012). One proinflammatory cytokine, TNF-α, can have multiple and varied effects on liver function and nutrient processing (Bradford and Eastridge, 2012). These circulating cytokines, especially if they are linked with an infectious disease near partition, may signal an inflammatory response in the liver (Bradford and Eastridge, 2012).

A connection does exist linking metabolic status and immune function of the periparturient dairy cow, as has been demonstrated in the vast body of research examining the topic. Though some details of the interaction are not fully elucidated, it is apparent that these two systems are closely related and impairment or deficiencies in one will have important impacts on the other. Further research is necessary to continue to parse out the precise mechanisms at work with these two important components of a dairy cow's overall health and transition into lactation.

## MILKING FREQUENCY

### *Differences in Milking Frequency*

Milking frequency can have a large impact on the overall well-being of the cow and her production levels. Benefits to less frequent milkings for the cow include: less milk produced (Davis et al., 1999), leading to the possibility of fewer metabolic diseases due to a lower energy output (Rauw et al., 1998), and reduced handler interactions that may be stressful to the animal (Pajor et al., 2003). Less lameness could also be seen since there would be reduced walking to the parlor and standing waiting to be milked (Tucker et al., 2007), leading to an increase in foot health and locomotion ability (O'Driscoll et al., 2010a). There would be fewer diurnal disruptions for the individual animals, which could be used as one indicator of improved animal welfare (O'Driscoll et al., 2010a).

There are drawback to less frequent milkings for both the cow and the producer. For the animal, she may experience an increase in SCC (Kelly et al., 1998; Smith et al., 2002) and could experience discomfort due to milk accumulation and in the udder (Davis et al., 1998). The inflammatory response occurs 12-24 hours after the last milking occurred and could be partially responsible for the impaired immune function in cows milked less frequently (Davis et al., 1999). The discomfort could lead to less time spent lying down, lying in a position that is somewhat abnormal in order to reduce pressure on the udder, shorter walking strides, a firmer udder, and more milk leakage and kicking in the parlor, as well as increased activity in the hypothathalmic-pituitary-adrenal axis (Tucker et al., 2007). For the producer, the animals could produce less if milked less frequently, especially first lactation cows, who showed a substantial milkfat loss if only milked once a day (Attrill and Holmes, 1993).

Another element that should be taken into consideration when considering milking frequency is the cow's preference for milking frequency and what would naturally occur if the calf was not removed at birth. In a non-production setting where the cow and calf are allowed to reside together, the calf will suckle approximately 4 times in a 24 hour period (Lidfors and Jensen, 1988). When pasture was available for 24 hours a day and cows were milked in an AMS, the animals in one study were voluntarily milked on average 2.3 times per day in a 24 hour period (Ketelaar-de Lauwere et al., 1999). These results may indicate it is part of the natural behavioral repertoire of a cow to be milked multiple times a day and, if given the choice, she will choose to be milked multiple times a day.

A question then arises, how does one determine what is the best milking strategy for the cow and the producer. The context of the benefits of once daily milking compared to multiple milkings per day should be examined more closely. Metabolic diseases may be decreased if there is careful management of the cows' BCS and ration prior to and after calving. This is particularly important in grazing dairy farms where the inherent variability and/or transient decrease in quantity and quality of pasture forage could limit feed intake.

When a cow is "dryed-off" (approximately 60 d prior to calving) to stop lactation and allow time for her body to prepare for the next lactation cycle, the ideal BCS recommended for traditional parlor dairies is 2.75 on a 5 point scale for Holstein or Friesian breeds of cattle, while it should be slightly higher, 3.0, at calving (Mulligan et al., 2006). If cows are over-conditioned (4.0-5.0) during this critical transition period, their DMI drops more dramatically in the three weeks prior to calving than thinner cows (Hayirli et al., 2002), placing them in higher risk for metabolic disorders due to feed intake reduction. Nutrient requirements for the fresh cow depend on many factors (e.g. milk production, parity, etc.), however, the NRC (2001) has published recommendations on meeting the animal's needs during the early state of lactation. A mathematical model predicts that a fresh Holstein cow (DIM = 11; BCS = 3.3; age = 58 mo; BW = 680 kg) producing 25 kg of milk (milk fat = 3.5%; milk true protein = 3.0%; milk lactose = 4.8%) will require: 27.9 Mcal/d energy for lactation;1643 g/d of metabolizable protein for lactation; a minimum of 25-33% NDF; 52.1 g/d of absorbable calcium; 37.3 g/d absorbable phosphorus; 0.27% magnesium (NRC, 2001) to meet requirements for lactation. For grazing dairy cows, an increase in 5 to 10% in requirements above maintenance is caused by grazing activity and must also be considered.

Ensuring proper periparturient body score and nutritional needs postpartum could help to decrease the incidence of metabolic diseases even with frequent milkings in early lactation. With proper training of employees and mindful handling strategies, human-cow interactions can be kept minimally stressful for the herd and herdsman, thus helping to eliminate one of the drawbacks of multiple milkings. Good flooring substrates, short distances to the milking parlor, and a decreased waiting time to be milked could help to improve hoof help and reduce lameness in the herd (Cook and Nordlund, 2009). Selecting for sound bone structure and maintaining a regular hoof trimming protocol would also be positive contributions to increasing leg health of the cows. An additional alternative could be to use an AMS to milk the herd so each individual animal may choose when and how frequently she wants to be milked. Another option could be to use differential AMS routings for access to the AMS and/or areas of the farm based on the individual cow needs postpartum (e.g., managing BCS or foot and leg health) or management needs (e.g., training cows new to the AMS). In fact, differential milking, routing and feeding management of individual cows is one practice that most AMS farmer apply without the need of segregating cows into spatially separated groups.

What must be remembered in any milking system or milking frequency is that these elements do not exist alone. The genetic merit of the cow, feeding system and allotment, social interactions, human-animal interactions, the interactions of all of these elements, and many other factors will contribute to overall milk yield during lactation.

### *Milking Frequency's Influence on Oxidative and Metabolic Stress*

The oxidative and metabolic stress a cow experiences during transition can have major monetary consequences for the producer in terms of quality and quantity of milk. When milk yield is not nutritionally limited (e.g., cows are fed *ad libitum* or there is no limit on energy intake), milking an animal more frequently will yield higher and longer milk production through a lactation cycle and influences the composition of her milk. In one New Zealand grazing study with low producing cows done by Clark et al. (2006), it was shown that the annual values of protein, fat, lactose yields per cow and overall production were higher for animals milked twice daily compared to once daily. Clark and colleagues (2006) tested two breeds of cow: Holstein-Friesian and Jersey on a ryegrass-white clover pasture, and although Jersey production was less impacted by once-a-day milking, both breeds did experience a decrease in production per cow. Work by Dahl and colleagues (2004) agreed with this data, with high producing cows fed TMR *ad libitum* and milked six times per day having more protein, fat, and total solids in their milk throughout the entire study than did the control animals milked three times a day. To produce more milk, the cows must consume more feed to have enough energy to meet their own health requirements and production needs. If a cow is unable or unwilling to do this, her overall health will suffer, as will her milk yield and response to increased milking frequency. However, this general response needs to consider specific differences across dairy systems as both partition of energy (e.g., body weight versus milk production) and milk yield could be greatly affected by differences in feeding systems (e.g., TMR vs. pasture-based) and genetic merit of cows (Dillion et al., 2003).

Related to the animal's overall health and production is how milking frequency impacts udder health, BCS, and weight change postpartum. Animals milked once a day have lower energy outputs, lose less body condition, and lose less weight in early lactation (Davis et al., 1999; O'Brien et al., 2005). These factors have shown to be important in helping to reduce the amount of time a cow will be in a negative energy balance in early lactation and lower her subsequent risk for reproductive failure and metabolic disorders (Patton et al., 2006). With less frequent milkings, an increase in SCC may be seen. Cows milked once a day have higher SCC throughout a year than twice a day cows in two different breeds, though no difference was observed in clinical or subclinical infections despite this difference (Clark et al., 2006). Cows milked six times a day had lower SCC on the first day of testing and maintained lower levels during the first three months of lactation compared to cattle milked three times a day (Dahl et al., 2004). It would seem that for the general health and well-being of the dairy cow, less frequent milking may be beneficial, but when specifically considering udder health and production, there are benefits to multiple milkings per day.

Culling rates must also be considered with general cow health and her productive life. Milking frequency seems to impact reproductive success and the number of cows that remain in-calf within in a given herd. In work done by Clark and colleagues (2006) on seasonally calving grazing cows, it was found that cows milked once a day had five fewer days between calving and subsequent conception compared to cattle milked twice a day. This led to a reduced number of cows culled because of late calving and reduced the need for induced calving to maintain the seasonal management cycle of the herd (Clark et al., 2006). The calving interval was reduced, leading to positive effects on profitability for the producer and welfare and longevity for individual cows (Clark et al., 2006).

### *AMS Technology and Impact*

AMS were introduced commercially in the Netherlands in 1992 with the primary goal to decrease human labor on family-scale dairy farms (Bijl et al., 2007). Since first introduced, the number of dairies employing AMS has grown to over 8000 as reported at the end of 2009, with the majority (>90%) located in north-western Europe (de Koning, 2010). There are two principal motivations in reducing human inputs: social and economic. By investing in an AMS, the farmer and farm employees have more freedom and do not have to be in the parlor multiple times a day with the herd. This has the potential to help improve human quality of life by creating a more flexible schedule and leaving more free time available to those associated with the farm (Mathijs, 2004). Reduced labor translates to lower human expenses (Wade et al., 2004) and more funds available for alternate expenditures on the farm. In one study looking at 31 AMS and 31 parlor farms, the AMS dairies averaged 29% less labor usage than their traditional counterparts (Bijl et al., 2007). It has been shown that it is possible for family dairies to grow in terms of milk production, number of cows, or both measures per year without having to hire additional labor if an AMS is employed (Bjil et al., 2007). There is the possibility of increased production per cow as well because she could choose to be milked more frequently than with scheduled milking, increasing her total milk output and economic value (Wade et al., 2004).

Easing production for humans was one of the main motivators for creating the AMS, but there are many benefits for the cows in this type of milking system. These are perhaps more important than the advantages available to humans because the cows must be comfortable in their milking environment to achieve maximum milk let down. An AMS provides the cow a consistence milking routine, performs proper teat stimulation, and often delivers her a ration concentrate while being milked (Svennersten-Sjaunja and Petterson, 2008). The introduction of AMS could reduce undesirable human-animal interactions that have a large impact on the behavior and production of dairy cows by eliciting a greater fear response (Waiblinger et al., 2002). These consistent, positive effects of AMS milking combine to help optimize oxytocin release and milk let down in each milking session (Svennersten-Sjaunja and Petterson, 2008). Rasmussen and colleagues (1990) demonstrated the importance of a consistent, predictable milking routine in their experiment where the results indicated an increase in milk production when variation was kept to a minimum in the parlor. The feeding of concentrates or roughage during milking has been shown to help increase milk flow and ejection from the udder and consequently decrease total time in the milking unit (Samuelsson et al., 1993). Concentrates are also used as a method to motivate the cattle to voluntarily enter the AMS (Prescott et al., 1998), a behavior that is necessary for the system to be successful in the absence of human labor to move and milk cows. If the cows do not enter by their own choice and must instead be fetched by human labor, the social and economic benefits of the AMS begin to decrease.

An AMS offers unique opportunities for cattle in that producers would have the ability to create a custom milking schedule for each cow so each animal could be milked at a different frequency or have a different interval between milkings (Svennersten-Sjaunja and Petterson, 2008). The AMS also allows for milking frequency to be easily changed based on the animal's stage of lactation while still maintaining a custom schedule if desired (Svennersten-Sjaunja and Petterson, 2008). For transition or early lactation cattle, this could mean more frequent milkings early in lactation to significantly increase total milk production during the lactation cycle (Svennersten-Sjaunja and Petterson, 2005). This flexibility in the milking schedule is an important benefit to the AMS, but it must be managed properly to ensure the robot is operating as efficiently as possible to meet individual cow variations in milking intervals, yield, and duration, as well as the economic goals of the producer, such as the economic revenue of the AMS (André et al., 2010). It is suggested that the optimal operation of the AMS should consider a dynamic milking approach that is frequently updated by real-time outputs to make parameter estimates on optimal milking intervals based on ratios of milk yield to milking duration and overall production response to concentrate feeding (André et al., 2010).

In a study conducted by Dahl et al. (2004), it was found that cows milked six times a day in a traditional confinement dairy produced more milk than animals milked three times a day on the first test day and in the following five test days. If this increase in milking frequency is timed correctly in lactation, persistent effects of increase yield will last throughout the current production period (Dahl et al., 2004). Even when the six times a day milkings cows were reduced to being milked only three times a day, their yield remained higher than animals that only been milked three times per day during the duration of the experiment (Dahl et al., 2004). The window to utilize this increase is very short, as small as twenty-one days after parturition (Dahl et al., 2004). Once this window in early lactation closes, more frequent milkings later in lactation cannot increase yield that compares with the overall increase associated with higher milking frequency in the early postpartum period (Svennersten-Sjaunja and Pettersson, 2008). Only a short duration of time is needed between milkings to see the increase, as little as two hours between milkings will produce the higher yield results when cows are milked on a fixed time schedule (Dahl et al., 2004). However, it is important to note that overmilking can have deleterious effects, such as hardness or discoloration of the udder (Hillerton et al., 2002).

Wagner-Storch and Palmer (2003) showed that an AMS can increase milk yield through increased milking frequency compared to a parlor. In this study, a small subset of AMS cows showed significantly more milk production compared to those animals milked in a parlor . The estimated increase in yields that were observed for the robot were: up to 22 lb for animals milked once or twice a day; up to 14 lb for cattle milked two or three times a day, and up to 7 lb for those milked three or four times a day (Wagner-Storch and Palmer, 2003). The days in milk of the animals milked with the AMS and the parlor were similar, so the significantly higher yield was attributed to the AMS's contribution to increased milking frequency and thus increased production (Wagner-Storch and Palmer, 2003). de Koning et al. (2002) also report an increase in daily milk production (11.4%) in farms that converted from twice a day milking in a conventional parlor to AMS in farms located in The Netherlands. Data from French AMS farms agree with reporting increased milk production, though to a lesser degree (Veysset, et al., 2001). Farms using an AMS for two years or less saw a 3% increase in production while those with the unit for greater than two years reported a 9% milk yield increase (Veysset, et al., 2001). While most of this production response is often the result of flexible milking schedules due to cows having the ability to make choices on milking frequency and milking intervals, other human management aspects, such as improved cow management, controlled feeding, and monitoring, also to explain the progress in AMS dairy farms.

At AMS farms, cows have the greater opportunity to set their own milking schedule, and quite often, increases in milking frequency and production are observed in the absence of detectable metabolic or behavioral distress in dairy cows. Results reported by Abeni and colleagues (2005) showed no significant differences between TMR fed cows milked in an AMS (3.0 ± 0.5 milkings/d) compared to counterparts milking in parlor (2 mikings/d) when looking at BCS and metabolic parameters, such as NEFA and BHBA concentrations. Similar results by Abeni and colleagues (2008) were reported in a twin-cow study where twice a day parlor milking was compared to a flexible milking schedule with an AMS (2.69 ± 0.6 milking/d). In this study, no significant difference in NEFA concentrations or oxidative status were observed despite the clear difference in milking frequency and schedule imposed on the cows (Abeni et al., 2008).

One important aspect related to flexible milking schedules on AMS farms is length of time between milkings and regularity of milking intervals. Long intervals between successful milkings have been shown to negatively impact daily milk yield, with a greater impact on multiparous than primiparous cows (Bach and Busto, 2005). Delamaire and Guinard-Flament (2006) showed that increasing the time between milkings negatively impacts milk production by decreasing the mammary gland's efficiency in nutrient uptake as well as decreasing blood flow to this organ.

Another import benefit of the AMS is the robot's ability to milk each quarter separately. Each individual teat cup monitors the milk flow from the quarter it is attached too. When the flow drops below a specified level, the cup is removed. This is important for udder health to keep teat ends in optimal condition and prevent overmilking any quarter (Hopster et al., 2002). Maintaining a healthy udder will help the cow to be healthier overall.

Although there are many benefits to using an AMS, udder health has been shown to decrease during the transition from a conventional parlor to a robotic system (Hovinen and Pyörälä, 2011). Rasmussen and colleagues (2001) found an increased incidence of new mammary infections during the first year of AMS use compared to previous years in traditional parlor systems, but that elevated SCC levels slowly dropped after three months in the system. Consistently higher SCC were found in AMS milked cows compared to the same animals milked previously at either 2x or 3x a day in a conventional parlor (Kruip et al., 2002). It was suggested that the milking interval, method of teat cleaning, or increased likelihood of the milk canal remaining open with AMS milking might explanations for the higher SCC reported (Kruip et al., 2002). In studying genotype x environment interactions, SCC were again found to be higher in AMS dairies compared to pre-AMS data on the same farms (Mulder et al., 2004). The results of these studies suggest it is especially important to monitor mammary gland health as a new milking method is introduced. As more and more aspects of dairy husbandry become automated, it is still very important that managers and herdsmen observe cows daily to ensure they are in good health and their well-being is not compromised.

## PASTURING COWS IN LOOSE HOUSING DAIRY SYSTEMS

Most dairy cattle in North America are housed indoors (Goldberg et al., 1992), either in free-stalls, tie-stalls, or loose packed beds. Each of these housing systems offers different benefits and challenges to the animals, the producers managing the system, and the consumers looking for more information about their food's origin. From the cow's perspective, in free-stalls and loose packed bed systems cattle have more opportunity to make choices regarding their environment and interaction with conspecifics. They may get up and walk around, choose where to lie, pick a location along the feed bunk, and engage with or avoid conspecifics in the herd. Although these choices allow the cows to exercise some degree of freedom over their environment, the cows also face challenges in these systems. When all cows in a herd have the same free access to focal or spatially limited resources, such as feed or stalls, competitive interactions may exacerbate issues of aggressive behavior, (Phillips and Rind, 2002). Conversely, tie-stalls eliminate this competition because the animals are bedded and fed individually, but this greatly limits the cow's capacity to move around and make choices in her environment.

One alternative housing and/or feeding system, which is most common in New Zealand and Australia, is the pasturing of dairy cows (Hemsworth et al., 1995). Pasturing cattle is not a common practice in North America, but offers many benefits to cows and humans. Producers may have lower labor and cost inputs because they would not need to purchase or deliver feed multiple times per day (Washburn et al., 2002). There may also be a lower environmental impact because manure will be naturally deposited by the cows rather than through artificially spraying fields. Experimentation has demonstrated that dairy cattle welfare in most confinement systems can be improved with increased access to pasture (e.g. Washburn et al., 2002; Hernandez-Mendo et al., 2007) though the research in this area is still emerging and currently somewhat limited.

Pasture may be used in multiple ways and incorporated into many different dairy management systems. Cows fed a TMR may have access to pasture as an alternate lying substrate or pasture may serve as a supplement to a partial TMR diet. Some dairies may choose to use pasture as the primary feed source for cows, supplementing only as necessary based on forage growth rates, climate and precipitation, production goals, or to help motive cows to return to the barn for milking or husbandry practices. When considering the combination of pasture with AMS, the exact purpose of the pasture (e.g., lying area, primary diet, etc.) and amount of time cows have access to it (e.g., 24 h/d or limited time intervals) will impact AMS use. The studies presented below were based on a variety of many possible combinations of pasture usage with different milking systems, including AMS. Thus, the findings should not be generally applied to all systems using pasture but viewed as possible outcomes when pasture is incorporated into systems.

### *Preference and Time Budget*

Several studies have looked at the activity budget of dairy cows in loose housing systems that provide access to grazed pasture. In general, cattle spend at least part of their day outside, but this is largely dependent on weather conditions (Legrand et al., 2009). In temperate weather, cattle will spent the majority of their day on pasture (Ketelaar-de Lauwere et al., 1999), ranging from 13 ± 0.6h/d (Legrand et al., 2009) to 17.2 ± 1.9h/d (Krohn et al., 1992). During this time on pasture, the animals were most often observed lying compared to other postures (Krohn et al., 1992; Ketelaar-de Lauwere et al., 1999; Legrand et al., 2009). There was a greater synchronicity of behavior of pastured cattle compared to those in confinement in regard to lying (Miller and Wood-Gush, 1991; Krohn et al., 1992; Ketelaar-de Lauwere et al., 1999) and eating (Miller and Wood-Gush, 1991; Ketelaar-de Lauwere et al., 1999) behaviors, as well as location in the experimental areas and patterns of movement (Ketelaar-de Lauwere et al., 1999). Synchronicity of behavior is thought to be important for dairy cattle because when there is sufficient space for all the animals to engage in the same behavior at the same time resource competition is reduced. Cattle are a gregarious species (Phillips and Rind, 2002) that have evolved to decide, move, act, and react in the company of conspecifics (Giraldeau and Caraco, 2000). Gregariousness enables both individual animals and groups to synchronize behavior in the absence of competition when space, time budgets and resources are not limited (Giraldeau and Caraco, 2000). Behavioral synchrony is one benchmark that could be used to assess welfare status of dairy cattle in production settings, as high synchrony could indicate low social competition (Miller and Wood-Gush, 1991).

Weather extremes (e.g. very hot, cold, relative humidity, or rainy weather) will impact the activity and behavior of dairy cows in loose housing systems that provide access to pasture. Very warm and or humid weather (i.e., high heat stress index) during the day influenced the location and activity of cows as they sought out shaded areas (Legrand et al., 2009). During rainfall, cattle spent more time indoors, especially at night (Legrand et al., 2009). Rain makes pastures muddy and slippery, subsequently providing a sub-optimal surface for walking and resting. The cattle might dislike the rain itself and this could also influence their choice to remain indoors. During the winter, it was not the weather that directly influenced the cows to remain indoors, but the impact of the weather on the outdoor pasture environment and subsequent management changes to the indoor housing. Frozen, hard, and uneven ground was not often walked upon by cows (Krohn et al., 1992). Walking on a substrate such as frozen ground may make the cattle move very cautiously and facilitate instability in general. Additionally, when pasture is not available as forage during the winter, food must be provided inside, also influencing the cows' preference for location.

Season has also been shown to play a role in the amount of time cows spent eating different feed resources. During summer months, when fresh pasture was available, cows were noted to graze for 4.0 ± 1.3 h/d and consume a mixed ration for 1.3 ± 0.8 h/d (Krohn et al., 1992). The winter saw a much greater amount of time spent consuming the mixed ration (2.2 ± 1.1 h/d) compared to time eating pasture (0.7 h/d, range of 0-3 h/d) (Krohn et al., 1992). Despite the limited amount of fresh pasture available and unfavorable weather conditions of winter, the cattle still chose to spend some portion of their daily time budget in this environment and not remain solely in the barn indicating the importance of assess to pasture.

Time of day also influences the cows' preference for pasture access. In dry, warm weather, cows spent more time on pasture at night (Legrand et al., 2009) because pasture has been demonstrated to be a preferred lying substrate. Cows rest at night and would logically choose to do so in the most comfortable substrate. Cooler night temperatures could also influence an animal's choice to be outside when compared to warmer temperatures experienced during the day.

***Indices of Welfare: Lying Behavior***

Lying is important to good cow welfare and to milk production. In a study by Krohn and Munksgaard (1993), cattle that had access to pasture compared to those in tethered housing spent less time examining the lying surface and kneeling, as well as showed a shorter duration of time from examination to actually kneeling down. These results suggest that cows were more comfortable on pasture because they showed less reluctance to lie down compared to tethered cows. This comfort could be attributed to two different elements in the environment: the lying substrate itself and ease of movement as the cow transitions from standing to lying or lying to standing. If the cattle found the substrate they were lying on more comfortable and supportive to their bodies, it is thought that they would spend less time in transition postures, such as kneeling. Tethering may also have inhibited some of the cow's movement and comfort, making them reluctant to lie down.

Contrary to what was expected, Krohn and Munksgaard (1993) and Hernandez-Mendo and colleagues (2007) found that cattle on pasture actually stood more often and more frequently than confined cows. It is thought that this result occurred because the cattle found the surface more comfortable overall. The cows' increased comfort encouraged them to be in all postures on their preferred surface. This result could also be confounded by the animals grazing on the pastures, standing to better disperse body heat when it was hot outside, or engaging in other behaviors that require standing.

Lying postures performed by pastured cattle are different when compared to those in confinement. Cattle on pasture were more likely to be observed laterally or with their heads on their back or the ground (Krohn and Munksgaard, 1993). These postures are believed to be those in which cattle obtain their deepest rest, thus seeing them on pasture would seem to indicate that this environment provides cows a better resting location compared to being tethered.

### *Indices of Welfare: Group, Agonistic, and Abnormal Behaviors*

Group behaviors showed significant differences when dominant and subordinate animals were in a competitive (pasture plus supplement) or non-competitive (pasture only) situation (Phillips and Rind, 2002). When placed in a competitive feeding environment where cattle were grouped either with a similar ranked conspecific (dominant – dominant or subordinate – subordinate) or opposite ranked conspecific (dominant – subordinate), opposite pairs performed poorly (Phillips and Rind, 2002). All pairings performed equally well when the competition was removed. The competition seems to have reduced the welfare of animals when they were in mixed social groups. In this study, reduced production can be used as one method to assess the situation, but is by no means the sole indicator or welfare. Each animal in the mixed pair may have spent more time participating in behaviors that would either help them maintain their higher social status or not challenge the status quo. All the cattle responded favorably once the need to partake in these activities was removed.

Similar to observed changes in group behavior, pasture access appears to reduce agonistic interactions in general (O'Connell et al., 1989; Miller and Wood-Gush, 1991). Proposed reasons as to why this occurs are related to space and reduced competition. Cattle would be able to distribute themselves and eliminate the need for competition if provided with adequate pasture space. The cows would also be able to avoid or interact with specific individuals without the space restraints of confinement. The environment is dynamic and enriching, thus time devoted to aggressive interactions maybe redirected to other activities, such as exploring or investigating.

Abnormal behaviors showed a strong reaction to pasture access. Redbo (1991) examined the behavior of heifers with established oral stereotypies (tongue rolling or play; bar biting; or excessive equipment licking) prior to, during, and after exposure to pasture. Heifers were tethered indoors before and after having access to pasture. It was reported that heifers spent approximately 11% of their day engaged in oral stereotypies prior to pasture access, displayed no stereotypies while on pasture, and not only did these heifers resume stereotypic behavior, they increased the amount of time to 25% once removed from pasture (Redbo, 1991).

### ***Indices of Welfare: Leg and Udder Health***

In a study conducted by Somers and colleagues (2003), different flooring types in Dutch dairy farms were evaluated for their effect on cow claw health. Cows in zero-grazing dairies had a statistically higher risk of having interdigitial dermatitis and heel erosion, digital dermatitis, sole hemorrhages, sole ulcers, and interdigital hyperplasia compared to cattle with pasture access (Somers et al., 2003).

Pasture access has also been shown to decrease gait score in clinically lame cattle (Hernandez-Mendo et al., 2007). All cows in the study were housed in a free-stall barn and separated into groups of 4 where the average lameness score of the group was a 3 on a 5 point scale. Once separated, groups were randomly assigned to remain in the free-stall barn or be housed on pasture for 4 consecutive weeks; gaits were scored weekly. During the sampling period, cattle housed on pasture decreased their lameness score each week and ended the experiment with a score of approximately 2, while the cows in the free-stalls either remained at the average score of 3 or became more lame (Hernandez-Mendo et al., 2007). This experiment demonstrated that pasture access can help cows recover from leg and hoof injuries that impair their gait and will improve their welfare by decreasing the pain associated with these injuries.

Pasture access was shown to have significantly positive impacts on udder health. Cows on pasture were 1.8 times less likely to develop clinical mastitis and eight times less likely to be culled (Washburn et al., 2002). Teat trampling and teat injuries were less frequently observed in pastured cows compared to those in tie-stalls (Krohn and Munksgaard, 1993.) Another study found that number of udder health issues reported per month were lower in pasture herds compared to confinement (Goldberg et al., 1992). The work of Goldberg and colleagues (1992) would need to be examined further or repeated because the authors stated there were confounding factors in how the data were reported from each individual farm.

### *Indices of Welfare: Nutrition and Overall Health*

Pasture-based diets have lower nutrient inputs than total mixed rations, but are the diet and foraging and feeding methods cattle have adapted to over thousands of years. These lower inputs may mean lower outputs in terms of milk production, but could also mean lower levels of metabolic and immunological stress on the individual. If a cow is producing less, and if this lower production implies less physiological stress, her production level may remain more consistent and risk of illness reduced, providing more income for the producer with no losses due to decreased production, medication and treatment costs, and loss from dumping treated milk. The next step in managing pastured cows would be to find ways to further increase resource use and production efficiently, while simultaneously improving cow health and welfare and reducing the environmental footprint.

Cows exhibit seasonal and diurnal grazing patterns (O'Connell et al., 1989) that are affected by the interaction of changes in both the external environment (e.g. pasture quality, length of daylight, etc.) and the animal during these periods (Linnane et al, 2001). As the season progresses from the cool, wet spring into a warmer, drier summer, the duration of morning grazing bouts were seen to increase, as well as bite and mastication rates, likely due to decreasing grass quality (O'Driscoll et al., 2010b). Several factors may influence intake and grazing behavior, including the characteristics and maturity of the feed (Brundage and Sweetman, 1956); the metabolic and physiological regulators of the individual based on stage of lactation, health status, age, and many other factors (Provenza, 1996); environmental influences, such as temperature and day length (Ketelaars and Tolkamp, 1992); genotype and breed differences (Ketelaars and Tolkamp, 1992); and foraging theory concepts , such as the maximization of energy intake (Ketelaars and Tolkamp, 1992). Many factors, such as individual animal preference and motivation; feed type, availability, and quality; and environment may vary, influence grazing behaviors, but ultimately, the cows will strive to meet their nutritional needs to maintain homeostasis, modifying grazing behavior to account for the animal-sward-environment interaction that changes over the grazing season (Linnane et al., 2001).

Management of pastures and cows will also impact grazing behaviors, duration, and timing of grazing bouts (Taweel et al., 2006). Semi-feral cows have peak grazing bouts at dawn and dusk (Linnane et al., 2001), and similar patterns have been reported for grazing cows milked twice daily in traditional parlor systems (Orr et al., 2001). However, allowing cows pasture access during these times may not be possible depending on the seasonality and the milking schedule of the cows, particularly those milking in parlors. When milked twice daily in a conventional parlor system, cows had peak grazing bouts after the morning and evening milkings (O'Connell et al., 1989), which generally are occur before dawn and dusk, consistent with natural grazing patterns.

### ***Cautions and Concerns about Pasture***

Cows stand to benefit greatly from pasture access, however several concerns exist surrounding managing dairy cattle on pasture. One area of concern is that research is still limited, thus there may be management and behavioral challenges yet to be addressed and/or improved. Further, the three primary areas of concern are human attitudes toward and management of grazeways or walkways between the barn and pasture; the use of appropriate cattle breeds in these systems; and proper nutrition.

A primary concern surrounding the pasture system is human management. Like any dairy operation, proper and careful management of the pasture and facilities is the key to creating a successful environment for the cattle. Grazeway management is one important aspect of pasturing cows (Hemsworth et al., 1995). Grazeways are the walkways that connect pastures to other facilities. Grazeways may not be present on every dairy that offers pasture to cows, but when these walkways are present, they need to be properly maintained. If grazeways are rocky, have debris, or are not well maintained, the potential hoof and leg benefits offered by pasture access could be eroded. Stockperson handling and attitudes are also very important. These individuals may need to be more patient in working with a grazing herd since both cows and humans may be walking longer distances to the parlor or are more spread out over a given space (Hemsworth et al., 1995). Welfare improvements are possible with pasture access, but maximizing overall welfare hinges on proper management practices (von Keyserlingk et al., 2009).

The use of breeds selected for success on pasture and/or individuals nurtured on a pasture-based environment is also very important to ensure good welfare and health. In a study conducted by Washburn and colleagues (2002), Jersey cows were found to be better suited to pasture than the Holstein breed. Jersey cows had lower rates of mastitis and cull rates compared to Holsteins, as well as higher body condition scores and reproductive rates (Washburn et al., 2002). Placing an animal in an environment it cannot adapt to or one that is ill suited to the animal's needs compromises welfare due to the fact that the animal is not able to cope with the given situation because of management or environmental constraints, or a combination thereof. Fear, pain, difficult or restrained movement, overstimulation, frustration, or lack of important stimuli may also represent coping challenges for a cow that could negatively impact her welfare, and consequently her health and production (Broom, 1991). For pastures to be a viable production option, cattle that can thrive in a pasture environment and are suited to a grass-based diet should be used to ensure good welfare. Dillion and colleagues (2003) demonstrated the importance of matching the breed of cow and her genetic merit to the environment. In a comparison of two French dual purpose breeds with Holstein-Friesian cattle selected only for milk production on a grass-based system for milk production, the Holstein-Friesian cows were found to have lower survivability due to reduced reproductive success with low fertility and longer periods from calving to next conception (Dillion et al., 2003). The Holstein-Friesian breeds were genetically selected for high milk production while consuming a high concentrate diet, thus there was a mismatch of diet to breed that may have played a role in the reduced survivability.

The metabolic fate of high producing cows also differs when pastured and confined cows are compared or when periparturient cows are rapidly transitioned from indoor TMR feeding to a pasture-based diet. For example, periparturient cows transitioned from a TMR-based to a pasture-based diet had lower blood glucose concentrations during this period and until they had fully adapted to the new pasture-based diet (Kolver and Muller, 1997). These same cattle also had higher BHBA and NEFA concentrations in their blood as a result of greater body weight loss and mobilization of adipose reserves during the transition phase (Kolver and Muller, 1997). If high producing dairy cattle are to be maintained on a pasture diet as their primary source of nutrition, it is important that energy supplementation take place when necessary (e.g., during a drought when pasture quantity and quality decrease) to ensure the good health of the cows (Kolver and Muller, 1997; Petherick, 2005).

## WELFARE AND PRODUCTION IMPLICATIONS

With any housing or feeding system employed for dairy cows, careful consideration should be made to the individual’s and herd’s well-being. Providing optimal welfare for dairy cows, especially transition cattle, is a multifaceted and challenging task. Factors such as good herd management, adequate barn environment, pasture location and quality, AMS management, social dynamics of the herd, and human-animal interactions are just a few items that impact the overall welfare of the animal (Wiktorsson and Sørensen, 2004). Production decisions must balance what is feasible for the individuals managing the dairy as well as what is best for the animals. Though it should not be a determining factor in husbandry decisions, public attitude and perception regarding the dairy industry cannot be ignored and has the potential to influence laws and regulations.

***Cow responses to AMS and Pasture***

Cows in an AMS dairy, compared to a conventional parlor system, have more opportunities to make choices and therefore exhibit a greater degree of control over their environment (Jacobs and Siegford, 2012). Other issues may arise with this increased freedom, such as social isolation during milking, that create new welfare challenges to the individual (Jacobs and Siegford, 2012). In comparing the stress response between primiparous cows milked in an AMS to those in a conventional parlor, Hopster and colleagues (2002) found that AMS cows had lower heart rates and spent more time with their heads inside the feeding trough. They also reported the AMS cows had lower maximum concentrations of nonadrenaline and adrenaline during the milking process, but there was no different observed in the number of steps (a sign of discomfort) during milking or mean oxytocin levels after teat stimulation (Hopster et al., 2002). These results indicate that robotic milking was not any more stressful than traditional milking methods and do not seem to negatively impact the cow's welfare (Hopster et al., 2002).

Other studies have found indicators of chronic stress or discomfort with an AMS system, but this may be due to other elements of the housing system (e.g. cow traffic, AMS model) instead of directly related to the milking method. Though no difference was found during the milking process, Hagen and colleagues (2005) did report the presence of cardiovascular indicators of stress while lying in cows in a forced-traffic AMS compared to those milked in a herringbone parlor. This would indicate that it was the design of the AMS environment and traffic system, not the milking process itself, which is negatively influencing the welfare of the cattle (Hagen et al., 2005). Similar results were found by Gygax and colleagues (2008) when comparing two different AMS (DeLaval Voluntary Milking System VMS1 [DeLaval International AB, Tumba, Sweden] and Lely Astronaut1 [Lely Industries N.V., Maassluis, The Netherlands]) and one auto-tandem parlor. Cows in both types of AMS exhibited more restless behaviors and had higher resting heart rates than those in the auto-tandem system, but the herd milked in the DeLaval system exhibited more of these effects, suggesting that there is a difference between AMS models that impact cow welfare (Gygax et al., 2008). If researchers are able to determine which aspects of the milking process in the AMS are aversive to cattle, scientific recommendations can be made to AMS manufacturers to improve robot design. The cow traffic also differed between the two systems (Lely = free cow traffic; DeLaval = guided/forced traffic), which may have contributed to the higher levels of stress expressed in the DeLaval herd (Gygax et al., 2008) and would agree with the results of Hagen and colleagues (2005).

As discussed above, many welfare benefits have been reported when housed cows have access to pasture for recreation and/or resting. Some European countries have recognized the positive effects of time spent outside of a barn and regulations are in place to ensure the animals have outdoor access (Wiktorsson and Spörndly, 2002). As one example, The Animal Welfare Ordinance (Ministry of Agriculture, 2009) of Sweden states that milking cattle older than six months should have pasture access during appropriate times of year. Pasture access allows for the expression of natural feeding behaviors (e.g. searching, grazing) (Wiktorsson and Spörndly, 2002) and provides an opportunity for extra exercise that may help to reduce hesitations when lying down compared to cows that are tethered with highly restricted movements (Gustafson and Lund-Magnussen, 1995). These results are further supported by the findings of Herlin and Drevemo (1997) that compared the locomotion of dairy cows housed either exclusively in tie-stalls or cubicles (for 2.5 yr) or cows from one of these systems that had been kept on pasture for three months. Through the use of high speed cinematography (100 frames/s) and a kinematic analysis to analyze cow movement, it was found that the slatted floors in the confinement systems and lack of exercise may have affected locomotion because there were greater movement restrictions at the hock and elbow joints and less flexion at the fetlock joint compared to cows that had been on grass for even short periods of time (Herlin and Drevemo, 1997).

Individually, both AMS and pasture offer improvements to cow welfare, but combining the systems offer challenges, however, initial research into AMS-pasture dairies indicates this is a viable system (Jacobs and Siegford, 2012). AMS dairies rely on cows voluntarily choosing to be milked throughout the day in order to be successful (Spörndly and Wredle, 2005), which makes understanding what motivates an individual to visit the robot important (Jacobs and Siegford, 2012). Spörndly and Wredle, (2005) tested the location of drinking water, either on pasture and in the barn or just in the barn, to determine if this was an important motivator for grazing cows to return to the barn. In their finding, the cows without pasture water drank more in the first 30 min upon return to the barn, suggesting this may have been a contributing factor to return to the barn, however, there were no significant difference noted in time on pasture or milking frequency with the cows that had water in both locations (Spörndly and Wredle, 2005).

This suggests that water is an important resource for cows, but it is not necessarily a strong enough force to encourage movement out of the pasture when they appear to have a method to compensate for lack of water on pasture.

Decreases in milking frequency have been noted (daily mean = 2.3 milkings with 24 h access to pasture compared to 2.5 – 2.8 milkings with 12 h or zero-grazing) with pasture access (Ketelaar-de Lauwere et al., 1999). Cows with pasture access on this study spent more time lying in the pasture (80.0-99.6% of the time) than in the barn, but lying times did not differ among treatments, suggesting cows are more comfortable and prefer outdoor access, but restricting it may be advantageous to the producer to increase average milkings per day (Ketelaar-de Lauwere et al., 1999). This decrease in milking frequency may be a normal phenomenon for pasture-based AMS systems due to the lower nutrient intake of the cows, leading to lower milk output. Rather than trying to increase milking frequency of individual animals, it has been found to be more advantageous to increase animals per AMS to maximize robot productivity (Jago et al., 2007).

Creating an effective traffic pattern for pastured-AMS herds is of utmost importance to ensure the success of the system since the cows will be traveling greater distances to be milked (Wiktorsson and Spörndly, 2002). Distance to travel between the pasture and AMS did not significantly impact milking frequency, but sward height did (Ketelaar-de Lauwere et al., 2000). When provided 15 h of pasture access, cows increased their AMS visits from 4.4 to 7.3 and the number of milkings from 2.6 to 3.0 ($P < 0.01$) as sward height decreased and made visits more closely spaced than would be expected by chance (Ketelaar-de Lauwere et al., 2000). Though no significant difference was observed in milking frequency with increased distance between the barn and pasture, it was observed that the first time cows were introduced to a “far” (355 – 360

m) pasture, they spent less time inside and in the feeding area and more time grazing and outdoors than in a "near" (146 – 168 m) pasture or when in the "far" pasture a second time (Ketelaar-de Lauwere et al., 2000). This may indicate that a great habituation time is necessary when the cows are traveling greater distances (Ketelaar-de Lauwere et al., 2000). Wredle and Spörndly (2002) reported no difference in total grazing or lying time for cows in a close (minimum distance 50 m, maximum distance 330 m) pasture compared to a distant (260 m cow track and a maximum of an additional 850 m to the farthest part of the paddock) pasture, but did note that more time was spent outside and a greater percentage of time lying outside in the closer pasture. Distant cows spent more time standing idle or lying on the cow track as the season progressed, perhaps indicating they were not as willing to travel the far distance; no difference was observed for time in the track with the close pasture over the duration of the experiment (Wredle and Spörndly 2002). This experiment highlights the important of mangers being aware of where cows are spending their time as the growing season progresses.

Pastured cows moved in non-random patterns (i.e. with at least one conspecific) to both return to and leave the AMS barn, showed a higher synchronization of behavior in an outdoor system compared to an indoor dairy (Ketelaar-de Lauwere et al., 1999). This has important implications for facility design to ensure bottle necks do not occur at barn entrance/exits when groups of cows return and to minimize the amount of time the AMS is inactive (Wiktorsson and Spörndly, 2002). Forced traffic, as previously discussed, appears to be stressful to cattle, but completely free-flow traffic decreases milking frequency. Sorting gates at barn exits and one-way gates at barn entrances have proven to be successful in managing cow traffic (Wiktorsson and Spörndly, 2002). Another alternative is to design the barn so that cows may only exit to the pasture once they have passed through the AMS, which may also decrease the number fetchings required (Jagtenberg and van Lent, 2000).

As has been demonstrated, grazing can be successfully combined with an AMS when careful consideration is given to cow traffic patterns, type of AMS used, distance to and progression of pasture quality. Additional research would be helpful to further refine recommendations to producer wishing to employ this system and AMS manufacturers to ensure optimal cow welfare.

### *Impacts on Human Management and Public Perception*

The previous section illustrated the unique challenges that will arise in an AMS-pasture system, but there are also different advantages to be had with employing this alternate dairy system. AMS do not simply replace human labor on a dairy, but instead redefine the role of humans. Time spent directly with herd and in highly repetitive tasks may decrease, while tasks such as data monitoring and equipment maintenance may increase (Spahr and Maltz, 1997). Bach and colleagues (2007) expect that, in most cases, human labor will decrease, but if many cows need to be fetched daily, this may not be observed. This again highlights the importance of carefully considering barn construction, traffic flow, and AMS chosen. Many AMS offer automated sensors that can monitor aspects of cow health and production at every milking, including changes in milk production, feed intake, reproductive stage, udder health, and body weight, that could not efficiently be collected as often in conventional parlors (Spahr and Maltz, 1997). A great amount of detailed information on each individual in the herd may be recorded and used to better manage the group and single cows as needed (Spahr and Maltz, 1997). Milk quality can be closely monitored through assessment of color and electrical conductivity of the outputs of each quarter, which is a task other milking systems are not yet capable of (Jacobs and Siegford, 2012). Somatic cell counts may also be directly reported from the AMS, though this feature has yet to receive approval in the United States (Jacobs and Siegford, 2012).

Though it does offer many benefits, pasturing cows will not be feasible or suitable in all environments. Ambient temperatures and precipitation must be considered, as well as the types of plants that can be grown, the amount of time cows will be grazing a given paddock, and the distance the cows must travel from pasture to the AMS (Ketelaar-de Lauwere et al., 1999). Pasturing cows may add additional limits on the time humans interact with the herd since feed delivery is no necessary. Because of this, it will be especially important to make interactions with the herd positive when humans do encounter the cows so the events stress the animals as little as possible. Positive human-animal interactions, especially for dairy cattle, are important to maintain since it has been documented that fear of humans does negatively impact milk production (Breuer et al., 2000). Dairy calves have been shown to be able to discriminate between handlers that interacted with them “negatively” or “positively”, especially in the area where the handling took place (de Passillé et al., 1996). After aversive handling treatments, some calves generalized fear to all humans, but this can be overcome with positive handling experiences (de Passillé et al., 1996). Adult cows can choose between handling treatments and find shouting to be aversive, but tail twisting, if done gently, is not (Pajor et al., 2003). Feeding is a highly rewarding event, but feeding by hand does appear to be as rewarding as presentation in a pail (Pajor et al., 2003), suggesting that even when coupled with a highly positive event, the presence of neutral human may be somewhat stressful and further highlighting the need to create positive handling experiences for dairy cattle.

The other human element of AMS-pasture systems is public perception. The AMS does allow cows to being milked when they choose, generally with little interference (Ketelaar-de Lauwere et al., 1999). This freedom for the cows, coupled with seeing cows in the natural, pastoral setting, may be a more be viewed as more acceptable and providing better welfare for the cattle than a confinement parlor dairy system (Ketelaar-de Lauwere et al., 1999). Indeed, as public concern and awareness of dairy production and perceptions about animal welfare increase, the AMS-pasture system offers a technological answer that does not, with thoughtful design and management, stress the cows more than a conventional parlor (Wagner-Storch and Palmer, 2003). Public views cannot be ignored, but they cannot become the final deciding factor in making husbandry decisions and striving to provide the best welfare possible for a herd.

## CONCLUSIONS

Managing transition cattle is not an easy task. There are many health and production concerns that must be addressed to prevent the cows from becoming overly metabolically or oxdatively stressed, while still maintaining a high milk yield. Careful monitoring of the diet, management of milking frequency, observation of behavioral changes, and diagnostic blood tests are all tools that can assist in making sure cows are minimally stressed during this period. The use of an AMS can assist with monitoring the cow once she enters the milking herd and production data that provides insight into her health status can be collected frequently to alter management strategy to meet her present needs. Combing a pasture-based diet with an AMS may help to reduce metabolic and oxidative stress since the lower nutritional inputs can lead to lower outputs in milk production. Even with this possible advantage, careful management and observation of periparturient dairy cows is vital to ensure there is a proper match of nutrient intake to milk production, especially for genetically high producing cows that may struggle to meet their caloric needs on a primarily pasture-based diet. Reducing health concerns around the critical periparturient period, providing the cows the ability to exercise more control over their environment once they enter the milking herd, and allowing access to pasture for nutritional, behavioral, and physiological needs would all be positive ways to help increase overall dairy cow welfare in areas where such a system would be viable. Research considering a specific system like this and looking into metabolic, immunological, and behavioral factors is necessary to confirm that the individual benefits of pasture access and use of an AMS are retained when these two management elements are combined.

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# CHAPTER 2

# METABOLIC AND OXIDANT STATUS AND CYTOKINE EXPRESSION OF PASTURED PERIPARTURIENT DAIRY COWS IN AN AUTOMATIC MILKING SYSTEM

**Interpretive summary: AMS and pasture impact periparturient health. Elischer**

The periparturient period is a physiologically stressful time for cows. The metabolic and oxidative stress profiles and pro-inflammatory cytokine expression of traditional milking and feeding systems have been well characterized, but the impact of milking frequency in a pasture-based automatic milking system dairy had not been explored. This case study is the first to describe the metabolic and oxidative status and the pro-inflammatory cytokine expression of periparturient cows in a pasture-based AMS dairy.

AMS AND PASTURE IMPACT PERIPARTURIENT HEALTH

**Metabolic and oxidant status and cytokine expression of pastured periparturient dairy cows in an automatic milking system**

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**ABSTRACT**

The periparturient period represents a stressful time for dairy cows as physiological and environmental changes challenge their homeorhesis. Undesirable fluctuations in metabolites and impaired immune defense mechanisms near parturition can severely affect cow health and can have residual effect on performance and longevity. Metabolic and oxidative stress profiles of multiparous and primiparous dairy cows in traditional parlor and feeding systems have been well characterized, but little research has examined these profiles in alternative management systems, such as pastured cows managed with automatic milking systems (AMS). The metabolic and oxidant status of multiparous and primiparous periparturent dairy cows in a pastured-AMS management system has yet to be reported in the literature. Therefore, this case study is aimed at characterizing metabolic and oxidant status and pro-inflammatory cytokine expression of pastured-cows milked with an AMS. Blood was taken from 14 multiparous Friesian-cross dairy cows at -21, -14, -7, 1, 7, 14, and 21 days relative to calving, with 8 primiparous cows sampled only postpartum, for concentrations of insulin, glucose, non-esterified fatty acids, β-hydroxybutyrate, reduced glutathione, oxidized glutathione, antioxidant potential, and four pro-inflammatory cytokines. Milk production and milking frequency data were collected postpartum. Milk production differed on 7 and 14 d between primiparous and multiparous cows. Milking frequency was not significantly impacted by parity. Primiparous cows had significantly higher levels of glucose than multiparous cows, suggesting fatty acids from body reserves were not mobilized as readily, a common characteristic of postpartum metabolic stress. There were no differences in insulin, NEFA, or BHBA concentrations between multiparous and primiparous cows postpartum, though day relative to calving significantly impacted insulin and NEFA. Primiparous cows also had significantly higher antioxidant potential, reveling better overall

protection from damage occurring during cellular oxidation. Expression of pro-inflammatory cytokines fluctuated postpartum, indicating a differential immune response over time between the multiparous and primiparous cows. Results from this case show that pastured-periparturient cows milked with an AMS were not highly metabolically or oxidatively stressed, however, differences in response exist between primiparous and multiparous cows.

## INTRODUCTION

The periparturient period defined as three weeks prior to and three weeks following parturition, is a physiologically stressful time for dairy cattle. The physical, dietary, environmental, and social changes observed during this period have been well characterized in traditional confinement operations that milk in parlors, but little research has examine the effect of voluntary milking in an automatic milking system (AMS) on metabolic and immunological parameters of pastured periparturient dairy cows. This gap in understanding could lead to difficulties in deciding how to best manage cattle in these particular production systems.

The quality and quantity of milk produced, as well as numerous environmental factors, influence the metabolic status of the cow. If a cow is expending more energy than is consumed, especially in the early phase of lactation, a negative energy balance (NEB) can predispose the cow to different metabolic diseases (Mulligan and Doherty, 2008) and impair immune function (Goff and Horst, 1997). One method the body uses to adapt to a NEB is to mobilize adipose tissue and release non-esterified fatty acids (NEFA) as an alternate fuel source (Sordillo, et al., 2009), but excessive circulating levels of NEFA have been linked in humans with the initiation of a systemic inflammatory response (Wood et al., 2009). The precise mechanism by which NEFA activates the immune system remains unknown, however, research shows it may be similar to the way in which the lipid portion of lipopolysaccharide (LPS) stimulates toll-like receptor 4 (TLR4; Shi et al., 2006) to activate acute phase cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1, and IL-8, and the inflammatory response (Sordillo et al., 2009).

On the other hand, it has been well established that nutrient deficiencies are commonly associated with decreased immune function, specifically with antibody binding and the complement system, the response of secreted antibodies, phagocyte activity, cytokine levels, and cell-mediated immunity (Chandra, 1999). Energy balance postpartum is not solely related to milk yield, but is also dependent on the amount, quality, and types of feeds (Ingvarsten et al., 2003). The physiological stressors around calving – parturition itself, beginning a new lactation cycle, increased energy demands and limited feed intake capacity – strongly challenge the homoeostatic balance of a cow (Goff and Horst, 1997).

A cow's immune system is most impaired in the week immediately before and the week immediately after parturition (Goff and Horst, 1997). The incidence of metritis, mastitis, retained fetal membranes, and mammary edema are all common issues postpartum, a time when a cow is known to naturally experience oxidative stress (Sordillo and Aitken, 2009). Oxidative stress is a condition that occurs when the body produces excessive reactive oxygen species (ROS) that antioxidant defenses are unable to neutralize. These ROS are a type of free radical (a molecule with at least one unpaired set of electron in the outer orbital that can promote electron transfer via oxidation reduction reactions) that has formed from molecular oxygen. Excessive ROS cause oxidative damage to proteins, lipids, DNA, and other macromolecules by acting on the cellular membrane and other components (Bernabucci et al., 2005). This damage occurs throughout the body and can lead to a compromised immune system and inflammatory reactions (Sordillo and Aitken, 2009). For example, neutrophils exhibit impaired microbicidal mechanisms, as well as decreased chemokinesis and ingestion of invading pathogens (Kehril et al., 1998); lymphocytes are less responsive (Wells et al., 1977); and complement and immunoglobulin concentrations decrease (Stavel et al., 1991). Studies that have used both *in vivo* and *in vitro* techniques support the notion that oxidative stress during the periparturient phase could be a major, underlying cause of inflammatory and immune dysfunction for dairy cows (Sordillo and Aitken, 2009).

It has been suggested that oxidative status is related to metabolic disorders in dairy cows (Bernabucci et al., 2005). Negative energy balance around calving is thought to play a role in immunosuppression by acting on different cell types (e.g., neutrophils, lymphocytes) and proteins (e.g., immunoglobulins, complement) crucial to the body's defense against infection and illness (Kehril et al., 1989; Kehrli et al., 1990; Scalia et al., 2006). The precise reason cows experience such a high level of immunosuppression around parturition has yet to be fully elucidated, however, there is strong evidence that metabolic changes associated with calving and lactogenesis affect immune function (Goff, 2006). Glucocorticords are important in the immune response because they enhance bone marrow production of neutrophils, an important step at parturition assist in tissue remodeling for the dam after expulsion of the offspring (Burton et al., 2005). However, increased neutrophil attraction to the reproductive tract leaves peripheral tissues more susceptible to infection and illness, and may be a major contributing factor to immunosuppression at parturition (Burton et al., 2005). Direct immunosuppression is influenced by insufficient consumption of vitamins and trace minerals along with the reduced presence of antioxidants (Goff, 2006). In addition, indirect impairment of immune function near parturition occurs through decreased dry matter intake, leading to negative energy and protein balances, as well as the possible development of hypocalcemia from diets with a high cation-anion difference or low levels of magnesium (Goff, 2006). Less frequent milkings at the start of lactation may reduce the length of NEB (O'Driscoll et al., 2010) and may lower the risk of metabolic disorders while improving reproductive performance (Clark et al., 2006). However, more frequent milkings mean higher milk production, which is a desirable production response (Dahl et al., 2004). Management decisions need to move beyond simply assessing production to take into account the relationships between the physical changes that occur during parturition and overall immune and oxidative status of the animal (Sordillo and Aitken, 2009).

An AMS allows producers the ability to create a custom milking schedule for each cow at a frequency ideal for her nutritional inputs and stage of lactation (Svennersten-Sjaunja and Petterson, 2008). This flexibility of individual milking schedules is an important benefit of the AMS, but it must be managed properly to ensure the robot is operating as efficiently as possible to meet individual cow variations in milking intervals, yield, and duration, as well as the economic goals of the producer (André et al., 2010).

The objective of this study was to characterize the metabolic and oxidative status and cytokine gene expression of multiparous and primiparous pastured periparturient cows milked by an AMS. It was hypothesized that pastured cows milking with an AMS would experience changes in oxidative and metabolic status and pro-inflammatory cytokine expression after parturition, but that there would not be extreme changes in any parameters of interest. Further, age was expected to be influence a cow's response to parturition, with multiparous cows exhibiting greater changes that primiparous cows. Cows that milked at a higher frequency were expected to show a stronger response than cows that milked less often.

## MATERIALS AND METHODS

### *Animals and Husbandry*

All procedures were approved by the Michigan State University Institutional Animal Care and Use Committee prior to the start of the experiment.

Fourteen multiparous (MC) and eight primiparous (PC) Friesian-cross bred dairy cows were enrolled based on calving date (Table 1). The study was conducted from 12 April 2012 to 26 July 2012 at a commercial dairy farm in central Michigan with 111 cows in the milking herd at the time of the study. The diet of the cows was pasture-based, with occasional supplementation of hay (harvested previously from the pasture) depending on growing conditions. The temperature during the experimental period ranged from 8.3 to 34.4°C, with a mean of 22.7 ±1.2°C. A total of 84.6 mm of precipitation was reported during the study period. Multiparous cows were enrolled 21 d prior to expected parturition date and PC within 1 d after parturition.

Dry cows were housed on pasture as one group and were rotated between pastures as necessary to meet the grazing needs of the cows throughout the spring and summer. Plant species in the pasture were the same for dry cows as the lactating herd. Water was available *ad libitum*. Indoor access was limited, but there was a pack-bedded barn available for calving if necessary. Cows were allowed to calve on pasture without human interference. After parturition, both the dam and offspring were moved into a transition barn. Within 12 h of parturition, the calf was moved into an individual hutch and the dam joined the milking herd.

The milking herd was managed as a single group under the same breeding, feeding, grazing, and AMS milking protocols. The cows were housed in a loose housing system with 24 h/d access to outdoor pasture and to an indoor AMS barn providing 69 sawdust-bedded free stalls. Water was available indoors only, *ad libitum* from two automatic water troughs at opposite ends of the barn. Two Lely A3 Astronaut AMS (Lely, Maassluis, the Netherlands) were available for milking 24 h/d except for a total of 40 min/AMS/d when the units closed for cleaning and any repairs or servicing that was necessary. All cows had equal access to both

AMS units; no management, traffic, or barn design restrictions were placed on the animals for AMS use. All cows wore a transponder around their neck for individual identification by the AMS and routing to pasture via a sort gate.

The lactating herd was rotationally grazed on 101.2 ha of pasture subdivided into 26 paddocks. The pasture contained a mixture of red clover (*Trifolium pretense*) and white clover (*Trifolium repens*) and orchard grass (*Dactylis glomerata)*. Pasture access from the barn was controlled via an automated sort gate (Lely, Maassluis, the Netherlands). The distance from the barn exit to the farthest corner of the closest paddock was 0.19 km, and the distance to the farthest corner of the farthest paddock was 1.21 km. Cows were allowed on pasture based on restrictions set by the producer for number of milkings/d and maximum allowable interval between milkings. Cows pushed through a one-way manual gate for reentry to the barn. Cows were fetched to the AMS as necessary based on standard farm protocols. Fresh cows were allowed to milk a maximum of 5 times in a 24 h period, with a minimum of 3 milkings per 24 h, as long as their expected yield was at least 8.18 kg/milking. During milking, all cows were fed grain based on their level of production (multiparous and primiparous minimum = 2.27 kg concentrate/day; multiparous maximum = 7.26 kg concentrate/day, primiparous maximum = 5.85 kg concentrate/day).

***Data Collection***

Sampling days for MC were -21, -14, -7, 1, 7, 14, and 21 days relative to calving (DRTC). Primiparous cows were only sampled 1, 7, 14, and 21 d after calving. Cow weights were automatically recorded by the AMS at every successful milking visit, thus only postpartum data for weights are available. The AMS also reported frequency of successful and failed milkings, as well as number of refusals from the AMS; duration of time between milkings; and milk yield (Table 2.1).

Individual BCS were scored on a 1 – 5 point scale (0.25 increments), adapted from the methods described by Wildman et al. (1982) and Ferguson et al. (1994). These scores were completed through visual assessment of the cow without palpation. Scores were taken while the cows were restrained for blood sampling. At least two trained individuals scored the focal animal and these scores were averaged to reach the one score recorded.

Blood was collected from the coccygeal tail vein via venipuncture with Becton Dickinson Vacutainer needles and collection tubes (Franklin Lakes, NJ, USA). Twenty gauge single-use needles were used to collect blood in EDTA(K2) tubes (for analysis of insulin, glucose, NEFA, and BHBA) and heparin tubes (for the analysis of antioxidant potential [AOP], reduced glutathione [GSH] and oxidized glutathione [GSSG]). The tubes were inverted several times after blood was added to mix the blood with the anticoagulant and immediately placed on ice until processing

Pasture samples were collected weekly during the experimental period from the paddock the milking cows accessed the day of sampling. A 0.5 m x 0.5 m square was constructed out of 0.64 cm plastic PCV pipe created a fixed 0.25 $m^2$ area. The square was placed in three random locations spread throughout the paddock to account for spatial variation in botanical composition, forage availability and quality. Using clippers, all live plants (dead plant material and roots excluded) were cut within 2.5 – 5.2 cm above ground as a representative sample of the forage available for the herd. All cuttings were placed in a brown paper bag and then dried at 60°C for at least 48 h or until all plant matter was dry as determined by visual and tactile inspection. Dried samples were stored in a dry, sealed plastic tub until the end of the experiment.

Fifteen weekly samples were collected. Upon completion of the experimental period, all forage samples were ground with a 5 mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). Grounded samples were composited by month (consisting of three to five weekly composite samples) and analyzed for DM, OM, NDF, ADF, and CP. (Cumberland Valley Analytical Services, Inc. Maugansville, MD, USA; Table 2.2).

***Laboratory Procedures***

Blood for metabolic assays was collected in blood collection tubes were kept on ice until further processing in the laboratory. Tubes were centrifuged for 30 min at 706 x *g* at 4°C. Plasma was then removed, aliquoted, and stored at -20°C until metabolic assays were completed.

Insulin and glucose plasma samples were sent for analysis to the Diagnostic Center for Population and Animal Health at Michigan State University (East Lansing, MI, USA). Insulin was assessed via a radioimmunoassay (Human Insulin RIA Kit; Millipore Corporation, Billerica, MA). Glucose was quantified by the hexokinase G-6-PDH method using an Olympus AU640e analyzer. The reagent necessary for the assay was obtained from Beckman Coulter, Inc. (Brea, CA).

NEFA and BHBA assays were performed on plasma samples using commercial enzymatic colorimetric kits (NEFA: NEFA HR kit, Wako Chemicals USA, Richmond, VA; BHBA: procedure no. 2440, Stanbio Laboratory, Boerne, TX).

Blood for oxidative stress assays was collected in blood collection tubes were kept on ice until further processing in the laboratory. Reduced glutathione (GSH) is an important antioxidant that becomes oxidized to form oxidized glutathione (GSSG). The ratio of GSH/GSSG indicates the level of oxidative stress. This ratio decreases as the body is exposed to increasing levels of oxidative stress, increasing the GSSG concentration present. The GSH/GSSG ratio of each cow on all sampling days was evaluated using Bioxytech GSH/GSSG-412 Kit (Oxis Research, Burlingame, CA). Whole blood was aliquoted from the heparin collection tubes and a thiol scavenger (M2VP) was added to the samples analyzed for GSSG; untreated whole blood samples were analyzed for GSH. All samples were frozen at -80°C until further processing according to manufacturer instructions. The ratio of GSH:GSSG was determined by the presence of 5-5'dithiobis(2-nitrobenzoic acid) and NADPH. In brief, a spectrophotometer was used to measure the change in absorbance at 412 nm over a 3 min incubation period of both standards (0 to 1.5 m*M* GSSG) and samples. The ratio of GSH:GSSG was subsequently calculated through a standard curve and the reaction rates of the samples.

Total antioxidant potential (AOP) in plasma samples was evaluated with a commercial kit (Bioxytech AOP-450; Oxis Research, Foster City, CA), to determine the copper reducing power of antioxidants present. Briefly, plasma samples and standards were diluted 1:40 with the provided dilution buffer. Each diluted sample or standard was plated in duplicate (200 μL/well) and read at 450 nm in the Wallac Victor$^3$ 1420 Multilabel Counter (Perkin Elmer, Wellesley, MA) for a reference value. Copper solution (50 μL) was added to each well and incubated at RT for 3 min. A stop solution (50 μL) was then added to cease the reaction and the plate was again read at 450 nm. The differences between the two readings was compared against a six-point standard curve to determine the copper reducing power of the sample.

Whole blood was stimulated with LPS (10 ng/mL; Lipopolysaccharide E. Coli 0111:B4; Sigma-Aldrich, St. Louis, MO) or media alone as a control (RPMI media, Sigma-Aldrich, St. Louis, MO) as previously described by Røntved and colleagues (2005) with slight modifications. Briefly, 3 mL of whole blood was aliquoted into two sterile 50 mL tubes and either 3 μL of LPS or RPMI was added. These samples were incubated at 37°C for 3.5 h. Total RNA was isolated (Qiagen QIAamp RNA Blood Mini Kit; Qiagen, Valencia, CA) and quantified with a NanoDrop (NanoDrop ND-1000; Thermo Scientific, Wilmington, DE) to determine the RNA concentration. A random subset of samples (44 total; 17.5%) were assessed on a Bioanalyzer chip for RNA quality (RNA 6000 Pico kit, Agilent Technologies, Santa Clara, CA). Next, cDNA was synthesized High Capacity cDNA reverse transcriptase kit with RNA inhibitor (Applied Biosystems by Life Technologies, Grand Island, NY) and samples were plated and diluted with sterile RNase free water to a standardized 35.0 ng/μL RNA.

All primers used in this study were were TaqMan® Gene Expression Assays acquired from Applied BioSystems (Grand Island, NY), derived from the *Bos taurus* genome Specific assay identification numbers are stated below. Real-time quantitative PCR (qPCR) was carried in an Applied Biosystems 7900ht Sequence Detection System (Applied Biosystems by Life Technologies, Grand Island, NY) using TagMan Gene Expression Assays (Applied Biosystems by Life Technologies, Grand Island, NY). The PCR was performed in triplicate using a 10-μL reaction mixture per well, containing 2.0 μL of cDNA template, 5.0 μL of TagMan Fast Universal PCR Master Mix (2x, Applied Biosystems by Life Technologies, Grand Island, NY), 0.5 μL of TagMan Gene Expression Assay 20x Mix (Applied Biosystems by Life Technologies, Grand Island, NY), and 2.5 μL of sterile water. The thermal cycling conditions for the 3-stage PCR were: stage 1 at 50°C for 2 min; stage 2 for 10 min at 95°C; and stage 3 holding at 95°C for 15 s, followed by 60°C for 1 min with stage 3 repeated 40 times.

Targeted genes were amplified with the reaction mix described above. Three genes were included as endogenous controls: eukaryotic translation elongation factor 1 alpha 1 (EEF1A1, Bt03223795_g1), ribosomal protein S9 (RPS9, Bt03272016_m1), and phosphoglycerate kinase 1

(PGK1, Bt03225857_m1). The reference gene for ΔCt calculations was the average log of all housekeeping genes listed above. The mean of 1 d postpartum control MC was used as the reference expression point for ΔΔCt calculations for each gene of interest. Experimental genes of interest were TNF-α (Bt03259154_m1), IL-8 (Bt03211906_m1), IL-1β (Bt3212745), and osteopontin (Bt03212107_m1). These cytokines were selected because they are crucial in mediating the acute inflammatory response.

### *Statistical Analysis*

Only postpartum data were available for both PC and MC, thus only the statistical results from postpartum data are reported. All statistics were performed using SAS 9.2 (Statistical Analysis Software, Cary, NC, USA) with significance declared at $P < 0.05$. A mixed model (PROC MIXED) was used to analyze all metabolic and oxidative stress parameters, as well as milk production and milking frequency data. The model was as follows:

$$Y_{ijk} = \mu + DRTC_j + P_k + (DRTC)(P)_{jk} + e_{ijk}$$

Where $Y_{ijk}$ is the dependent variable for the metabolic or oxidative stress or milking parameter of interest from $cow_i$ on the day relative to $calving_j$ (DRTC, 1, 7, 14, or 21d) of $parity_k$ (P, MC or PC). Day relative to calving was a repeated measure. For each response variable, the covariate structure for parity was chosen based on lowest BIC and AIC values. Least square means ± standard error of the least square means are presented. Data were adjusted using the Tukey-Kramer method in SAS for multiple pair-wise comparisons.

A mixed model (PROC MIXED) was also used to evaluate qPCR data using the $2^{-\Delta\Delta CT}$ method to access relative expression as described by Hill and colleagues (2011). The model for cytokine expression was:

$$Y_{ijk} = \mu + DRTC_j + LPS_k + P_l + (DRTC)(LPS)_{jk} + (LPS)(P)_{kl} + (DRTC)(P)_{jl} + (DRTC)(LPS)(P)_{ljk} + e_{ijkl}$$

Where $Y_{ijk}$ was the $-\Delta\Delta CT$ of the cytokine of interest for the $i^{th}$ cow on the $j^{th}$ DRTC with the $k^{th}$ LPS treatment (control or LPS stimulated) of $parity_l$ (MC or PC); cow was treated as a random effect. Least square means from the model were reported as $-\Delta\Delta CT$ values, thus the base (2) was raised to the reported LSM to determine relative expression of each cytokine. Stand errors were calculated and reported based on LS means, SE of LS means, and the $2^{-\Delta\Delta CT}$ value. Only MC data were used for prepartum relative expression values.

## RESULTS

### *Milk Production and Milking Frequency*

Body condition score and BW across the postpartum period significantly differed between MC (2.9 ± 0.1; 470.0 ± 14.8 kg) and PC (2.7 ± 0.1; 357.2 ± 13.0; $P < 0.01$; Table 2.1). Milk production was significantly impacted by DRTC, parity, and the interaction of DRTC and parity ($P < 0.01$; Fig 2.1A). There was a significant increase in milk production from 1 d (7.5 ± 1.3 kg) to 7 d (20.5 ± 0.9 kg; $P < 0.05$) for MC and consistent production of about 22 kg/d for 14 and 21 d postpartum. Primiparous cows' yield increased less dramatically between 1 d (7.0 ± 1.8

kg) and 7 d postpartum (12.3 ± 1.3 kg), However for PC, milk yield on 14 d (14.6 ± 1.3 kg) and 21 d (17.1 ± 1.4 kg) significantly increased from milk yield on 1 d ($P < 0.05$). Multiparous cows produced significantly more milk ($P < 0.05$) on 7 d (20.5 ± 0.9 kg) and 14 d (22.3 ± 1.0 kg) than PC, but yields on 21 d (MC: 22.4 ± 1.1 kg) did not differ significantly.

Day relative to calving impacted milking frequency ($P < 0.05$), but parity or the interaction of parity and DRTC did not play a significant role (Fig. 2.1B). There were more milkings/d on 21 d (2.55 ± 0.2) compared to 1 d (1.7 ± 0.1; $P < 0.05$). However, milking frequency on d 7 d (2.3 ± 0.2) and d 14 d (2.2 ± 0.2) were not significantly different from each other, 1 d, or 21 d.

***Metabolic Assays***

Day relative to calving significantly impacted plasma concentrations of insulin, glucose, and NEFA ($P < 0.01$) (Fig. 2.2 A, B, and C). Insulin concentrations declined at 14 d for both PC (33.1 ± 9.0 nmol/L) and MC (43.7 ± 6.8 nmol/L) compared to 7 d (MC: 65.9 ± 6.8 nmol/L; PC: 72.0 ± 9.7 nmol/L), but at 21 d (MC: 80.5 ± 6.8 nmol/L; PC: 64.9 ± 9.0 nmol/L; NS). Primiparous cows had greater average glucose concentration (3.8 ± 0.08 mmol/L) than MC (3.6 ± 0.06 mmol/L; $P < 0.05$) (Fig. 2.2B). Glucose concentrations were significantly higher in MC at 1 d (3.8 ± 0.07 mmol/L) compared to 7 d (3.4 ± 0.06 mmol/L) and 21 d (3.4 ± 0.10 mmol/L; $P < 0.05$). Glucose concentrations of PC did not differ significantly from each other during the postpartum period. Average plasma NEFA concentrations were significantly higher on 1 d (512.92 ± 44.6 μEq/L; $P < 0.05$) compared with NEFA concentrations on all other days (7 d: 385.5 ± 45.2 μEq/L; 14 d: 328.58 ± 44.1 μEq/L; 21 d: 290.0 ± 43.6 μEq/L); however, parity did not a significantly effect NEFA concentrations (Fig. 2.2C). Parity or DRTC did not have a significant effect on plasma concentrations of BHBA (Fig. 2.2D).

### *Oxidative Stress*

Plasma AOP was significantly affected by both parity ($P < 0.05$) and the interaction of DRTC and parity ($P < 0.01$; Fig. 2.3A). Primiparous cows had increased values of copper reducing units, the units of AOP measurement, on 14 d (619.2 ± 43.3 CRE) and 21 d (678.7 ± 38.5 CRE) compared to MC on 14 d (447.0 ± 32.7 CRE) and 21 d (419.5 ± 29.1 CRE), but PC and MC did not differ significantly on 1 or 7 d postpartum. Reduced glutathione was effected by DRTC ($P < 0.01$), but not parity (Fig. 2.3B). The average concentration of GSH declined after parturition with a significantly lower concentration on 7 d (226.2 ± 13.1 μM) compared to 1 d (287.8 ± 13.6 μM). There was a significant interaction of parity and DRTC ($P < 0.05$) for GSSG. Multiparous cows and PC had similar 1 d concentrations (MC = 4.0 ± 0.7 μM vs. PC = 4.1 ± 1.2 μM). Primiparous cows' values declined on 7 and 21 d, but were elevated on 14 d (Fig. 2.3C). The ratio of GSH:GSSG was significantly impacted by DRTC ($P < 0.05$), but parity and the interaction of DRTC and parity were not significant (Fig. 2.3D). The ratio declined from 1 d (199.0 ± 40.2) to 7 d (133.5 ± 23.1), then increased and remained stable on 14 d (238.71 ± 125.1) and 21 d (236.3 ± 36.9).

### *qPCR and Cytokine Gene Expression*

Blood stimulated with LPS differed significantly from control blood on all days for both multiparous and primiparous cows, displaying higher levels of expression for IL-1β, IL-8, osteopontin, and TNF-α ($P < 0.001$; Fig. 2.4). Interleukin-8 (Fig 2.4B) was significantly affected by DRTC ($P < 0.05$), with gene expression on 21 d (0.67 ± 0.4) significantly lower than on 1 d (1.6 ± 0.4), 7 d (1.4 ± 0.4), and 14 d (1.3 ± 0.4) postpartum. There was no impact of parity or any interactions on IL-8 expression (Fig 2.4B). Day relative to calving significantly affected TNF-α ($P < 0.01$) with significantly lower relative expression on 1 d (2.7 ± 0.4) than on all other days (7 d: 3.4 ± 0.4; 14d: 3.6 ± 0.4; and 21 d: 3.2 ± 0.4). Interactions of parity and LPS treatment significantly affected expression of TNF-α ($P < 0.05$). There was no difference between the relative expression of MC control (0.6 ± 0.5) and PC control (0.6 ± 0.6), however, expression in both controls differed from expression in MC LPS stimulated (4.5 ± 0.5) and PC LPS stimulated blood (6.2 ± .6). Multiparous cow LPS stimulated samples (4.5 ± 0.5) were significantly lower from PC LPS samples (PC LPS: 6.2 ± 0.6; $P < 0.05$; Fig. 2.4D). There was no significant effect of parity, DRTC, or any significant interactions on the expression of IL1-β or osteopontin (Fig 2.4A, C).

## DISCUSSION

The periparturient period is a physiologically stressful time for dairy cows, generally marked by increased metabolic stress, a depressed immune system, and changes in cytokine gene expression. These measures have been well studied and characterized for cows milked in traditional parlors and fed a TMR diet, but limited research exists on how a pasture-based diet or an AMS impacts metabolic and immune parameters of cows in the transition period. The objectives of this study were to characterize the metabolic, immune, and cytokine expression profiles of multiparous and primiparous pastured periparturient cows milked by an AMS.

Milk production by MC cows remained stable at approximately 22 kg/d through the final two weeks of the experimental period, while the yield of PC increased from 14.6 to 17.1 kg/d between 14 d and 21 d. There are few reports in the literature describing milk production for Friesian dairy cows in the first three weeks postpartum in any feeding or milking system that can be used for comparison with the results reported here. Two such studies described milk production of Friesian cows from parturition to 21 d postpartum, but milk yields from these two studies were presented graphically, without reporting the precise value or standard error of milk production between 1 d and 21 d postpartum. However, the yield from PC and MC on 21 d observed in the current study appears to be in line with graphed data reported by Roche and colleagues (2006) for New Zealand Friesians consuming a pasture-based diet (milking system and frequency unknown), but is less than TMR-fed Italian Friesians milked in an AMS (Abeni et al., 2005).

Several studies have reported milking frequencies for Friesian cows in an AMS, but at later periods of lactation: 3.0 ± 0.5 for cows in the first 154 DIM on a TMR diet (Abeni et al., 2005); 2.6 to 2.8 ± 0.07 for cows averaging 191 ± 2.13 DIM fed a TMR on the first day of the 7 mo experimental period (Bach et al., 2007); 2.6 to 3.0 ± 0.1 for pastured-cows ranging from 39 to 274 DIM at the onset of the experiment (Ketelaar-de Lauwere et al., 2000); and 2.5 to 3.1 ± 0.1 for cows 109 ± 8.9 DIM when the study began and fed a grass-maize silage mix (Hermans et al., 2003). At 21 d postpartum, MC and PC cows in the current study milked 2.9 ± 0.3 and 2.25 ± 0.4 times/d, respectively. Although the average milking frequency of PC in the current study was lower, the MC frequencies were similar to those reported elsewhere. This would suggest that milk yield is not necessarily dependent on milking frequency in AMS dairies, but may be more influenced by diet and housing changes related to pasture.

Decreases in milking frequency have been noted when cows have unlimited pasture access. For example, in one study an average of 2.3 milkings/d were observed when cows had

24 h access to pasture compared to an average of 2.5 or 2.8 milkings/d when cows had 12 h of pasture or zero-grazing, respectively (Ketelaar-de Lauwere et al., 1999). Cows with unlimited pasture access in that study spent more time lying in the pasture (80.0 – 99.6% of the time) than in the barn, suggesting the cows were more comfortable lying outdoors; however, restricting pasture access may help increase the average number of milkings per day (Ketelaar-de Lauwere et al., 1999). Jago and colleagues (2004) reported even lower milking frequencies for Friesian cattle with 24 h unrestricted pasture access (1.42 – 1.91 ± 0.15 milkings/d). The milking frequencies in the current study were higher than results reported by Jago and colleagues (2004), but did not reach the frequency reported for zero-grazing systems (Ketelaar-de Lauwere, et al., 1999; Abeni et al., 2005); however, milking frequency did increase from 1d to 21 d for both MC and PC.

Milking frequency and milk production are important components of metabolic health postpartum. More frequent milkings increase the annual values of protein, fat, total solids, and overall yield (Dahl et al., 2004; Clark et al., 2006). However, increased production leads to greater metabolic pressure that may result in NEB. With the onset of lactation, glucose and insulin concentrations in the blood generally decrease because these metabolites are necessary for milk synthesis (Ingvarsten, 2006). While glucose and insulin decline, levels of NEFA and BHBA are elevated, indicative of a NEB in the periparturient period (Pedernera et al., 2010). The flexibility of an AMS allows for cows to be milked more frequently and could thus lead to increased metabolic stress.

Abeni and colleagues (2004) compared the glucose, NEFA, and BHBA profiles for primiparous and multiparous Friesian cows milked twice a day in a parlor to cows in an AMS with 3.0 ± 0.5 milkings/d and found no significant differences in metabolite concentrations despite increased milking frequency in the AMS. Milk yields for cows in the AMS and parlor systems also did not differ (Abeni et al., 2005). In a study of twin cows with one twin milked in a parlor twice-a-day and the other milking in an AMS (2.69 ± 0.6 milking/d), AMS cows were found to have slightly higher NEFA concentrations and lower blood glucose values, indicating these cows had more difficultly adapting to lactation, however, the feeding and traffic system for the AMS in this study were thought to be the main factors contributing to this difference (Abeni et al., 2008). Interestingly, the oxidative stress of AMS milked cows was lower than that of parlor-milked cows in early lactation, as demonstrated by lower reactive oxygen metabolites in the blood (Abeni et al., 2008), in agreement with the present study.

Insulin levels generally rise in late pregnancy and are followed by a declining concentration during early lactation in the postpartum period (Ingvarsten and Andersen, 2000). Glucose concentrations are recommended to be at or above 3.0 mmol/L (Mulligan et al., 2006), with a decline in glucose concentration noted after calving as glucose is shifted to the mammary gland for milk synthesis. Non-esterified fatty acid concentrations are recommend to stay less than 0.4 mmol/L (LeBlanc, 2010); and BHBA levels should not exceed 1200 – 1400 µmol/L (LeBlanc, 2010). If NEFA and BHBA levels exceed these recommended concentrations, an individual cow may be more prone to developing metabolic diseases, thus these values serve as guidelines to help evaluate overall health status and likelihood of developing an illness. In the current study, PC had generally higher glucose and insulin and lower NEFA and BHBA concentrations compared to MC, suggesting PC were in better metabolic state than MC. The differences in metabolite concentrations between the two parities were likely due to the differences in milk yield. Multiparous cows had significantly higher average daily yield (18.2 ±

0.9 kg/d) compared to PC (12.8 ± 1.2 kg/d), and thus were experiencing a higher level of metabolic stress, corroborated by the results of the blood assays.

Although the exact connection between metabolic and oxidant statuses remains to be fully elucidated, there are several important known interactions. Glucose is vital for proper metabolic and immune function; it is the main metabolic fuel for all cells in the body (LeBlanc, 2010). Low levels of glucose have been linked to a less effective pathogen-killing oxidative burst from polymorphonuclear neutrophils and are often seen at the same time as decreases in GSH concentrations (Ingvartsen et al., 2003), both of which impair host defenses. Reduced glutathione is a major non-enzymatic regulator of intracellular redox homeostasis, and in periods of oxidative stress, GSH concentrations will decrease as GSSG increase (Sordillo et al., 2007).

In the present study, concentrations of GSH postpartum in pastured Friesian cattle were lower at 1 d (287.8 ± 13.6 μM) and 21 d (261.1 ± 12.0 μM) compared with data published from Holstein cows fed a TMR (0 d: 488.4 ± 48.3 μM; 21 d: 341.5 ± 19.5 μM; Sordillo et al., 2007). Multiparous Friesian cows in the present study had higher GSH concentrations than PC on all days postpartum, but these concentrations did not significantly differ. Multiparous cow GSH concentration remained stable from 14 d (268.7 ± 14.4 μM) to 21 d (268.2 ± 14.4 μM) while PC concentrations rose over the same period (14 d: 231.4 ± 19.1 μM; 21 d: 254.1 ± 19.1 μM). These results are interesting because PC had higher AOP concentrations than MC on 7, 14, and 21 d postpartum, which could indicate PC were better protected from cellular oxidation than MC. The lower GSH concentration for PC show that even with one strong set of immune defenses, the body may not be completely protected and could still be subjected to immune assaults from a different quarter, here the effects of oxidative stress. Primiparous and multiparous cows may be experiencing different types of metabolic and immune stress in the periparturient period, and their bodies responding as necessary to maintain homeostasis and homeorhesis.

Exactly how glucose contributes to GSH function is unknown, however, both of these substances exhibited a similar pattern for PC and MC from 1 d through 14 d postpartum: concentrations declined from 1 to 7 d and increased at 14 d. Glucose concentrations for both MC and PC declined on 21 d (PC: 3.7 ± 0.2 mmol/L; MC: 3.4 ± 0.1 mmol/L) compared to 14 d (PC: 3.9 ± 0.09 mmol/L; MC: 3.6 ± 0.07 mmol/L) and were very near the values obtained on 7 d (PC: 3.7 ± 0.08 mmol/L; MC: 3.7 ± 0.08 mmol/L). Primiparous cows had significantly higher glucose concentrations than MC from 7 d to 21 d, which agrees with the findings of Wathes and colleagues (2006). Primiparous cows produced less milk, thus less glucose must be partitioned to the mammary gland for milk synthesis, leaving more glucose available to circulate throughout the body to fuel other functions, such as supporting PC growth. Holstein-Friesian cows fed a TMR and milked twice daily had lower glucose concentrations on 11 d (3.0 ± 0.1) and 21 d (3.2 ± 0.1) postpartum (Renyolds et al., 2003) compared to glucose concentrations of MC and PC in the present study, however, cows in the study by Renyolds and colleagues (2003) were producing a much larger volume of milk on their sampling days (11 d: 36.4 ± 1.2 kg/d; 21 d: 41.3 ± 1.2 kg/d) than either MC or PC in the current study.

As metabolic demands increase, so does the demand for oxygen, increasing the presence of ROS that must be removed to prevent cellular damage from oxidative stress (Sordillo and Aitken, 2009). Antioxidants, the first line of defense in removing ROS, are dependent on sufficient vitamin and mineral availability to ensure proper functioning (Goff, 2006). At times of decreased feed intake, as is common during the periparturient period, vitamins and minerals supply is more limited and antioxidant function may be impaired (Goff, 2006). Bernabucci and colleagues (2005) found a positive relationship of ROS to NEFA ($r = 0.32$, $P < 0.05$) and BHBA ($r = 0.40$, $P < 0.05$), further demonstrating a link between increased metabolic stress and oxidative stress.

Oxidative stress is associated with many different disease states, however, it has not yet been established whether excessive ROS are the result or cause of impaired health (Sordillo and Aitken, 2009). Quantifying the antioxidant potential is means of estimating overall antioxidant protection, with higher values indicating more protection from oxidant stress (O'Boyle et al., 2006). Cows in the current study maintained high AOP concentrations after parturition through 21 d, with PC values higher than MC on all days except immediately postpartum. In fact, the antioxidant defenses of PC increased over time, contrasting with published data reporting either a decline in antioxidants postpartum (Sordillo et al., 2007) or an increase in ROS (Bernabucci et al., 2005). However, the high AOP concentration contrasting with the low NEFA and BHBA levels found in cows on this study supports the relationship of oxidant and metabolic stress suggested by Bernanbucci et al. (2005).

Pro-inflammatory cytokines are also linked with metabolic and immune states. Evidence is emerging that metabolic status, specifically altered lipid metabolism, directly impacts systemic inflammation (Wood et al., 2009). For example, superfluous NEFA have been reported to trigger an immune response, possibly by acting similarly to the lipid portion of LPS (Shi et al., 2006). Increased NEFA concentrations and alterations of lipid metabolism are common during the periparturient period, thus a concomitant increase in pro-inflammatory cytokine expression is considered typical. Additionally, oxidative stress acts directly on TLR, with the exact mechanism of this relationship under intense investigation (Cuschieri and Maier, 2007). Toll-like receptors initiate the innate immune response by specifically activating pro-inflammatory cytokines, major histocompatibility complex, co-stimulatory molecules, and chemokines (Medzhitov, 2001). The combination of increased metabolic and oxidative stress near parturition contributes to increase pro-inflammatory cytokine production, further impairing cow health. If, however, cows are not under increased stress, cytokine expression should remain low, as was demonstrated by the metabolic, oxidant, and cytokine expression results of this study.

In agreement with reported results, samples stimulated with LPS had much higher expression of acute phase cytokines (TNF-α, IL-8, and IL1-β; Sordillo et al., 2009). Acute phase cytokines are protein molecules released from numerous cells of the innate immune system that are important signaling molecules to initiate an immune response. There was a significant effect of day ($P < 0.05$) on the expression of IL-8; 21 d ($0.7 \pm 0.4$) had the lowest level of expression, with no difference between 1 d ($1.6 \pm 0.4$), 7 d ($1.4 \pm 0.4$) and 14 d ($1.3 \pm 0.4$). Although not statistically different, PC showed a greater relative expression in both control and LPS stimulated samples of IL-8 to MC on all days except 7 postpartum. The expression of IL-8 fluctuated over time for PC, with 1 and 14 d exhibiting a higher relative expression than 7 and 21 d postpartum for both control and stimulated samples. Control samples of MC rose from 1 d to 7 d where the expression peaked, and then declined thereafter, while LPS stimulated samples slowly declined from 1 to 21 d postpartum. The greater fluctuation in PC IL-8 expression may reflect the new physiological changes that are occurring as cows enter their first lactation cycle. The initiation of a new lactation combined with the transition from a pasture-only environment to sand-bedded freestalls might impact PC more strongly than MC due to differences previous experiences; however, further research into this specific question would be necessary. Younger cows have been shown to have an increased immune response to *E. coli* infusion, exhibiting only a moderate inflammatory response compared to older cows under the same *E. coli* dose that have a varied response of moderate to severe inflammation (Vangroenweghe et al., 2004a; Vangroenweghe et al., 2004b). Indeed, parity plays a role in the immune response, as demonstrated by decreased neutrophil function with forth parity or greater cows (Gilbert, et al., 1993); decreased number of circulating peripheral leukocytes and the expression of integrins (van Werven et al., 1997); and compromised bactericidal behavior of polymorphonuclear leukocytes immediately following parturition in multiparous cows compared to primiparous cows (Mehrzad et al., 2002). Interleukin 8 is a pro-inflammatory cytokine that draws neutrophils to sites of infection and may have an important role in modulating mastitis induced by Gram-negative bacterium (Alluwaimi, 2004). Increased expression of IL-8, along with IL-1β and TNF-α, by activated macrophages at the site of infection heightens the production of adhesion molecules (Oviedo-Boyso et al., 2007). The decline in IL-8 expression at 21 d compared to 1, 7 and 14 d shows a decrease in immune response as cows moved further into lactation. Exactly where or how IL-8 acted to attract neutrophils cannot be determined by the current study, however, the declining relative expression of IL-8 over time indicates a decreased need for neutrophils.

Interleukin 1-β expression for PC and MC displayed a similar pattern to IL-8: PC had a higher level of expression compared to MC in both control and stimulated samples. Primiparous cows displayed the same decrease at 7 d in IL-1β expression as seen in IL-8 expression, however, Il-1β remained elevated through 21 d postpartum. Multiparous cows had their highest expression in unstimulated IL-1β samples on 7 d, with relative expression decreasing on 14 and 21 d postpartum. Upon recognition of invading pathogens in the mammary gland, macrophages release several pro-inflammatory cytokines, including IL-1β, to attract neutrophils to the site of infection (Oviedo-Boyso et al., 2006). In addition to be being important for attracting neutrophils to the mammary gland, IL-1β also up-regulates inducible oxygen radical formation, but does not affect phagocyte efficiency (Alluwaimi, 2004). The increased expression of IL-1β in the stimulated cells is expected since this cytokine is in known to be highly reactive to Gram-negative bacteria, such as *E. coli* (Oviedo-Boyso et al., 2006). Toll-like receptor 4 is highly sensitive to LPS stimulation and can induce nuclear factor-κB (NF- κB) activation, which is necessary for the up-regulation of pro-inflammatory cytokines from endothelia cells (Bannerman and Goldblum, 2003). If the cells did not strongly react to the LPS stimulation, it would suggest the innate immune system was somehow impaired and that host defenses were deficient. Primiparous cows seemed to be especially sensitive to LPS stimulation compared to MC, indicating that these cows would likely mount a more severe inflammatory response to an infection than MC. Although PC were producing less milk than MC, the new adaptations to lactation may make the PC more sensitive to stresses and thus initiate a greater immune response.

Tumor necrosis factor -α is a toxic molecule secreted by macrophages, neutrophils, and epithelial cells (Oviedo-Boyao et al., 2006), as well as cytotoxic lymphocytes, such as natural killer (NK) cells, that may induce apoptosis in altered cells (Sordillo et al., 1997). Periparturient dairy cows have significantly higher levels of TNF-α compared to cows later in lactation, which may contribute to the mammary gland's acute response to coliform mastitis (Sordillo et al., 1995). In this study, TNF- α was more highly expressed by PC than MC and stimulated cells had higher relative expression compared to control samples. As with IL-1β, higher expression in stimulated cells would be expected because TNF-α is one of the first cytokines to respond during the early stages of infection (Oviedo-Boyao et al., 2006). Both PC and MC samples stimulated with LPS, as well as control PC mRNA, had peak relative expression at 14 d postpartum; while control MC peaked at 7 d. Fourteen days postpartum seems to be a time of increased challenge for the PC, as demonstrated by increased cytokine expression and a sharp rise in GSSG concentration. Metabolically, by 14 d PC seemed to recover from the stress caused by beginning lactogenesis as seen in declines in insulin and NEFA and rise in glucose concentrations, but the immune system appeared to take longer to fully adapt and recover from the stresses surrounding parturition. Multiparous cows do no exhibit this same point of challenge at 14 d, rather, they have fluctuations in all metabolites, indicators of oxidative stress, and gene expression through the 21 d postpartum, suggesting MC are experiencing stress, but not as strongly as PC and that MC recover more quickly.

In total, increased expression of IL-8, IL-1β, and TNF-α described above has been related to the severity of certain strains of bacterium causing intramammary gland infections, specifically coliform strains (i.e., *E. coli*; Oviedo-Boyso et al., 2007). These pro-inflammatory cytokines are some of the first expressed and are important mediators to help recruit other cells to the site of infection and to initiate the adaptive immune response if the innate response is unable to successfully destroy the invading pathogens.

Osteopontin is a cytokine important for up-regulating and promoting the cell-mediated immune response and enhancing immune defense against mycobacterial infections (Karcher et al., 2008). This cytokine helps to mediate cell migration and adhesion, as well as playing a role in the cell-mediate immune response (Lund et al., 2009). The relative expression of osteopontin was the lowest of the four examined in this study, even after stimulation with LPS. Other research has demonstrated that the relative expression of osteopontin in control and concanavaline A stimulated peripheral blood mononuclear cells remained low for non-Johne's disease infected cows during the periparturient period (Karcher et al., 2008). Neither parity nor

DRTC significantly affected the expression of osteopontin, but MC and PC displayed opposing patterns in their osteopontin gene expression. In both control and LPS stimulated samples, MC had the highest relative concentration at 7 d compared to all other days, though none of these values significantly differed. Primiparous cows expressed osteopontin lowest on 7 d in both control and stimulated samples compared to 1, 14 and 21 d, but again this difference was not significant. Osteopontin expression in PC followed the same pattern of IL-8: increased expression on 1 and 14 d, with lower expression on 7 and 21 d postpartum, which also coincided with glucose and GSSG concentration patterns in the current study. The observed decreases in expression on 7 d could be related to increased milk yield observed from 1 to 7 d postpartum when the metabolic and immune systems of PC may still be adapting to the to the new stress and increased demands of lactation. Multiparous cows may be more adapted to the increased demands placed on their body at the onset of lactation and express stress in less acute ways, though the overall response might be higher because of increased milk production.

The interactions of metabolic status, oxidant status, and cytokine expression demonstrate that as one system is altered, the others' function are also changed, which may further compromise a cow's health if either the metabolic or immune system is unduly stressed. Maintaining optimal metabolic health and immune function is crucial for an overall healthy cow, contributing to a positive state of welfare, especially during the difficult periparturient period. Multiparous and primiparous cows appear to react to the stress of lactation in different ways. Other factors may confound these results, such as the impact of DRTC, changes in feed intake, the changes in forage quality over the experimental period, or climate. Further investigations into these specific areas would be necessary to further understand the magnitude of parity's influence on these results. Multiparous cows may experience a different degree of physiological stress, likely due to increased milk production compared to PC. Although both MC and PC did exhibit signs of metabolic and oxidative stress and changes in immune function during the periparturient period, the use of an AMS combined with a pasture-based diet did not appear to stress cows beyond what they are considered capable of coping with in this case study. A large degree of variation from farm to farm may exist, thus further research at additional locations should be completed to assess how MC and PC at pastured-AMS dairy farms respond during the periparturient period. Metabolites, indicators of oxidative stress, and pro-inflammatory cytokines responded in the patterns that would be expected, however, values reported here did not show a great deal of change over the experimental period. Even though metabolic and oxidative stress appeared to be low in the MC and PC in this study, the differing results of MC and PC would suggest that different management strategies may need to be considered to optimize the health, welfare, and production of pastured PC and MC milking by an AMS.

## CONCLUSIONS

Combining a pasture-based diet with an AMS did not negatively impact the metabolic or oxidative status of multiparous or primiparous periparturient dairy cows beyond what is considered normal as they transition between pregnancy and lactation. Although the cows in this study did experience metabolic and oxidative stress and had an altered immune state, as expected during the periparturient period, these responses did not appear to be severe enough to impair cow health and production. Both parities experienced physiological changes in the patterns that would be expected during this critical period. Thus despite providing cows with a lower energy diet and more frequent and variable milking, combining pasture with AMS does not seem to add additional distress to the metabolism or immune state of periparturient dairy cows.

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APPENDIX

**Table 2.1** Physical and milking characteristics of multiparous and primiparous pastured-periparturient Friesian-cross dairy cows milked in an AMS over a 21 d postpartum period[1].

| | Multiparous Cows | Primiparous Cows | *P* values |
|---|---|---|---|
| Number of Cows | 14 | 8 | |
| Parity | 2.9 ± 0.5 | 1.0 ± 0.0 | 0.01 |
| BCS | 2.9 ± 0.1 | 2.7 ± 0.1 | <0.0001 |
| BW (kg) | 470.0 ± 14.8 | 357.2 ± 13.0 | <0.0001 |
| Milk Yield/Milking (kg) | 19.9 ± 1.0 | 13.2 ± 0.7 | <0.0001 |
| Total Milkings in 21 d | 56.9 ± 3.5 | 42.6 ± 2.7 | 0.03 |
| # Milkings/Cow/d | 2.6 ± 0.2 | 1.9 ± 0.2 | <0.0001 |
| Refusals | 228.5 ± 77.3 | 26.4 ± 7.7 | NS[2] |
| Failures | 3.3 ± 1.0 | 5.0 ± 1.8 | NS |
| Milking Interval | 9 h 2 min ± 1 h | 12 h 15 min ± 1 h 29 min | <0.0001 |

[1]Values reported as least square means ± SE

[2]NS = not significant

**Table 2.2**. Analyzed nutrient composition of monthly pasture (orchard grass, red and white clover) samples collected over the experimental period (April to July 2012).

| Nutrient | Collection Month | | | |
|---|---|---|---|---|
| | April | May | June | July |
| Dry matter (%) | 93.9 | 93.9 | 93.5 | 94.0 |
| Organic matter[1] | 90.9 | 89.6 | 91.3 | 91.1 |
| Neutral detergent fiber[1] | 39.6 | 52.9 | 53.8 | 62.1 |
| Acid detergent fiber[1] | 24.4 | 33.2 | 30.4 | 40.2 |
| Crude protein[1] | 16.2 | 15.1 | 12.4 | 11.8 |

[1] % of dietary DM

**Figure 2.1.** Milk production (A) and milking frequency (B) for 14 multiparous (◆) and 8 primiparous (■) pastured periparturient dairy cows milked in an automatic milking system. A) Milk production (DRTC, $P$ <0.0001; parity, $P$ <0.01; interaction of DRTC and parity, $P$ < 0.01). B) Milking frequency (DRTC, $P$ < 0.05). Asterisks indicate significant between primiparous and multiparous cows on a given day ($P$ <0.05).

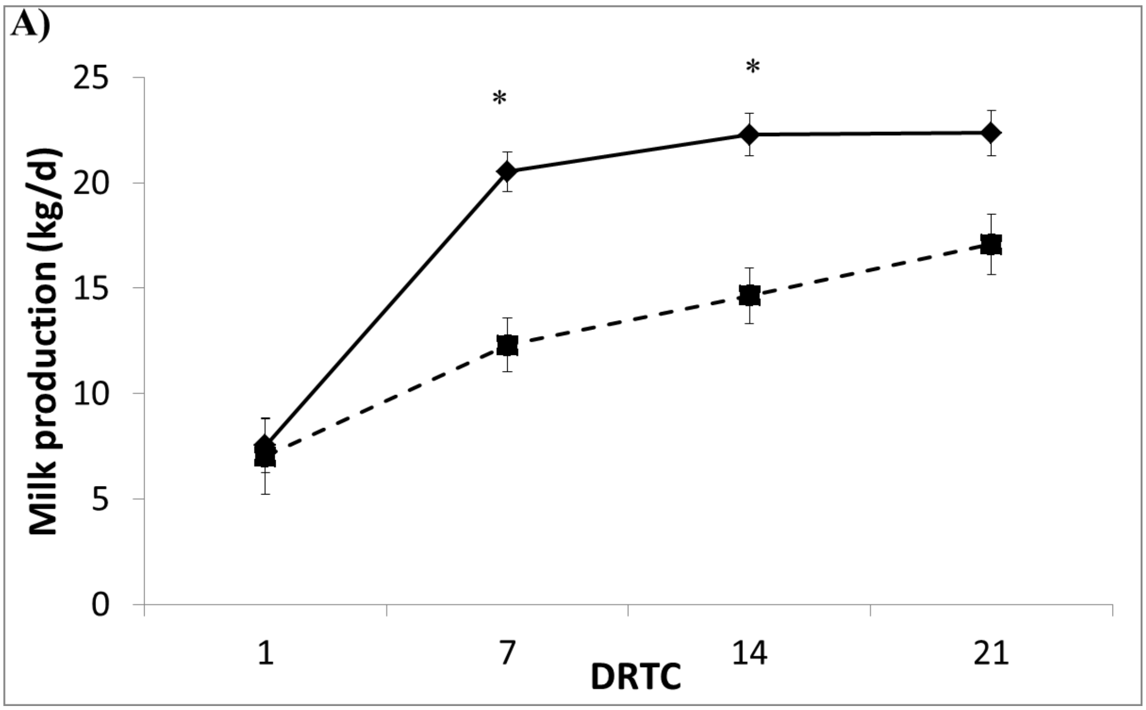


**Figure 2.1 (cont'd)**

B)

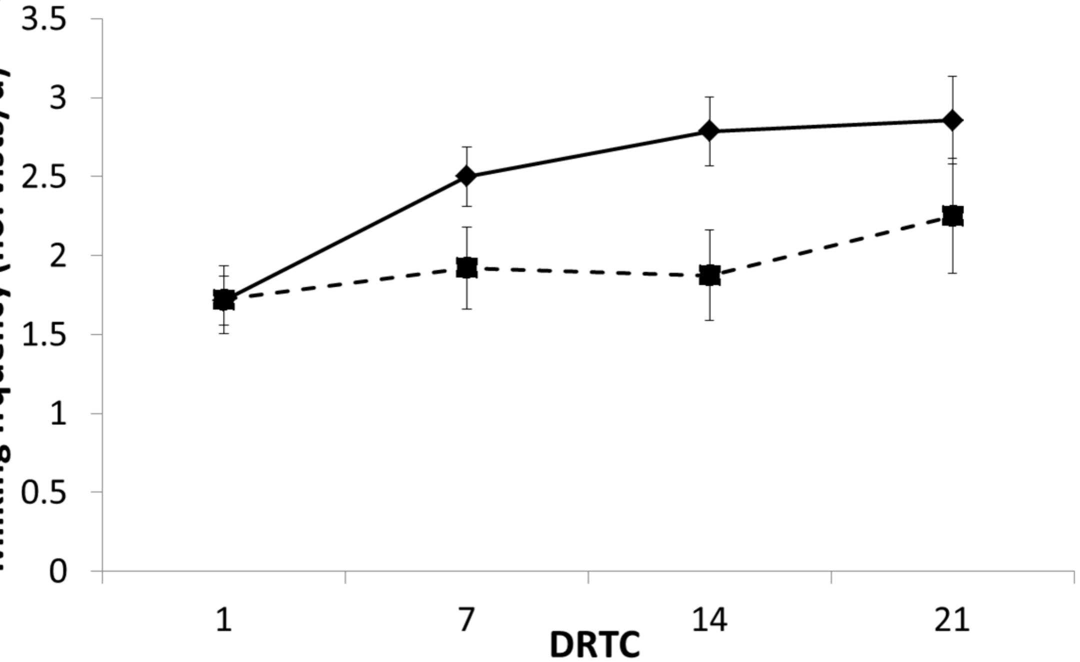
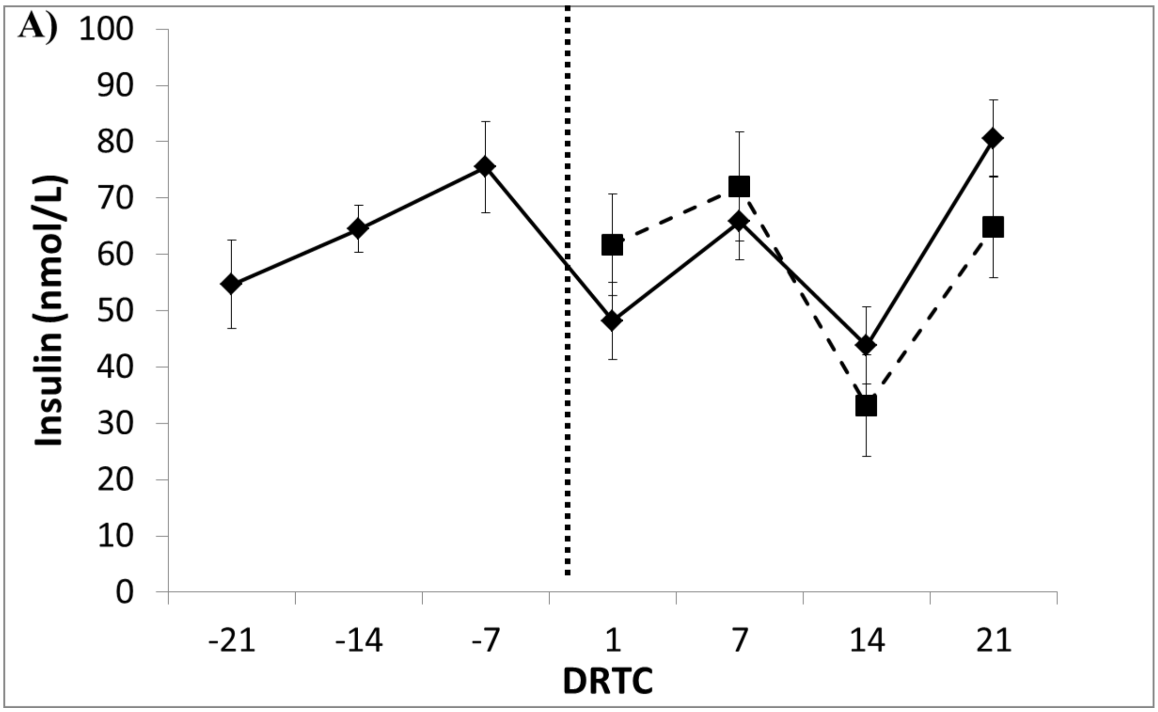


**Figure 2.2.** Metabolic parameters for 14 multiparous (◆) and 8 primiparous (■) pastured periparturient dairy cows milked in an automatic milking system. A) Insulin (DRTC, $P < 0.01$) (B) Glucose (DRTC, $P < 0.01$; parity, $P < 0.05$; interaction of DRTC and parity, $P < 0.05$) C) NEFA (DRTC, $P < 0.01$) D) BHBA.



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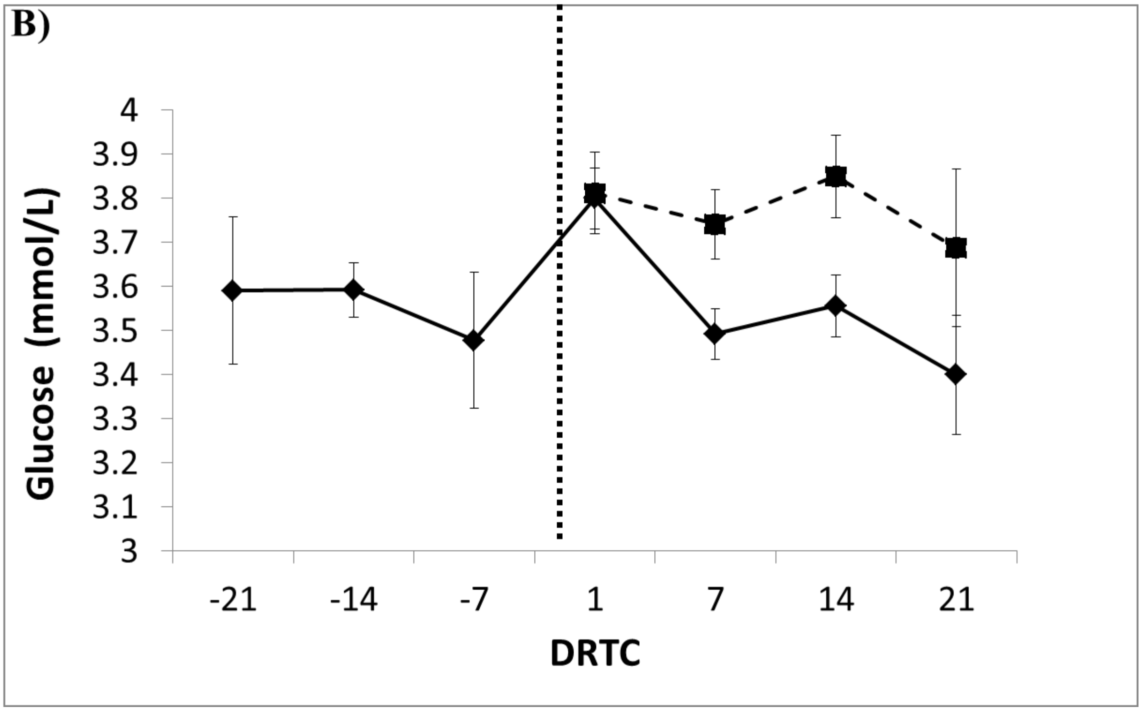


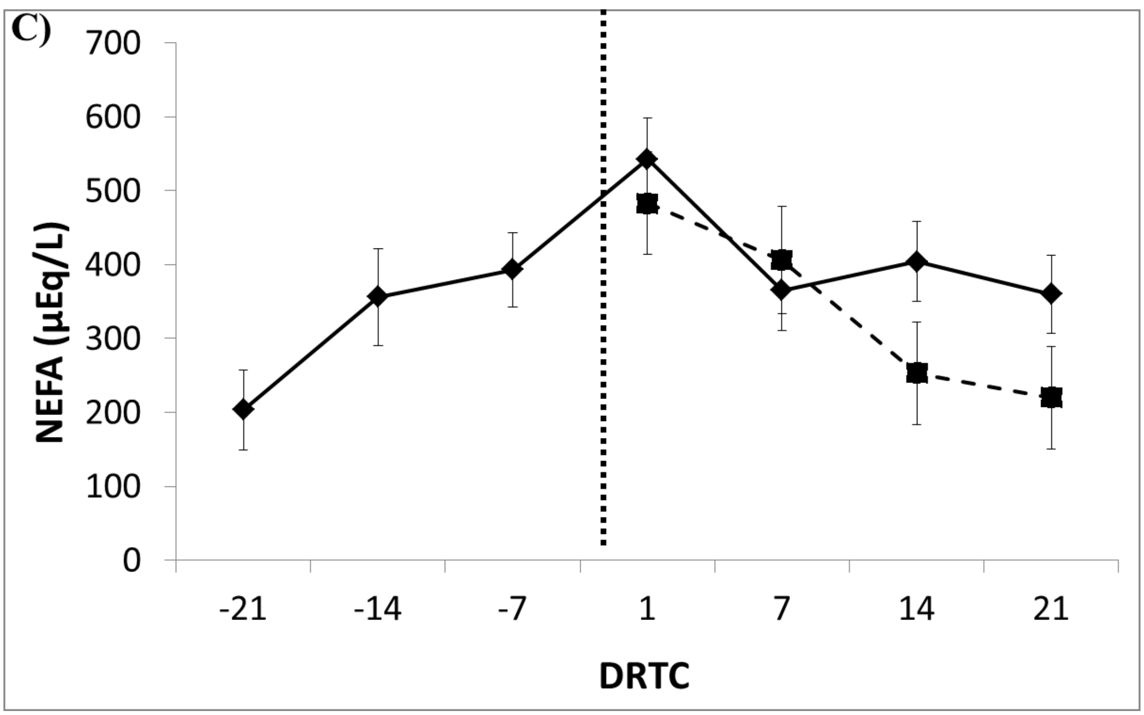

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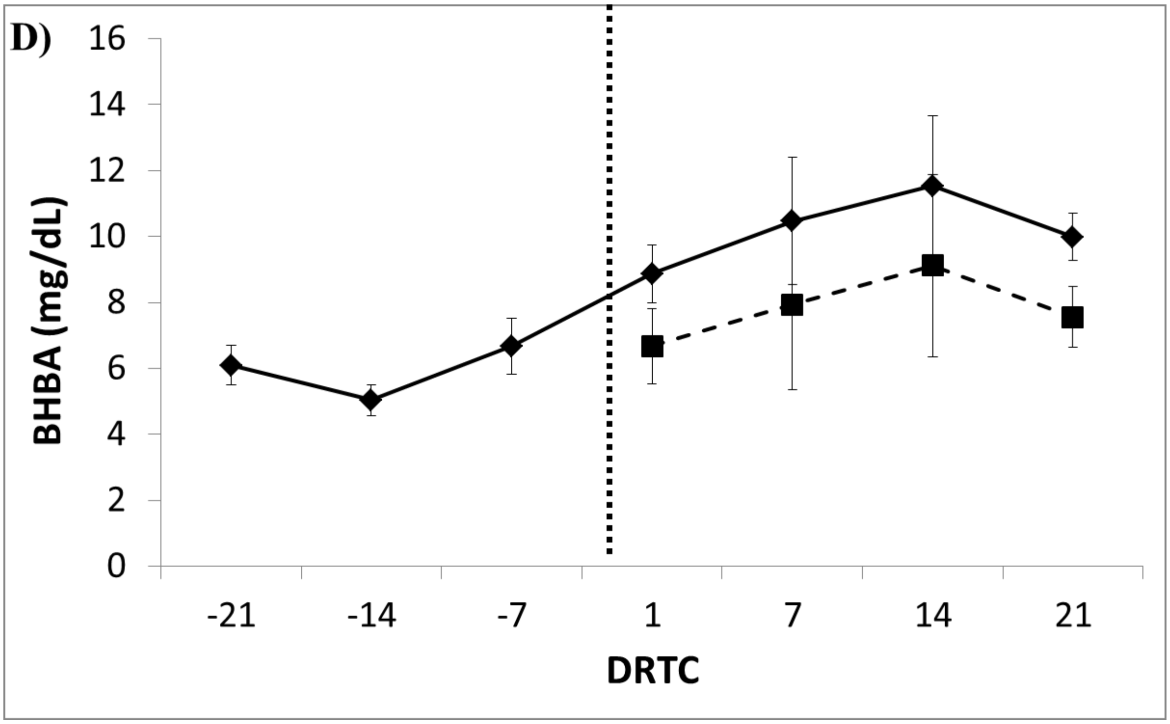
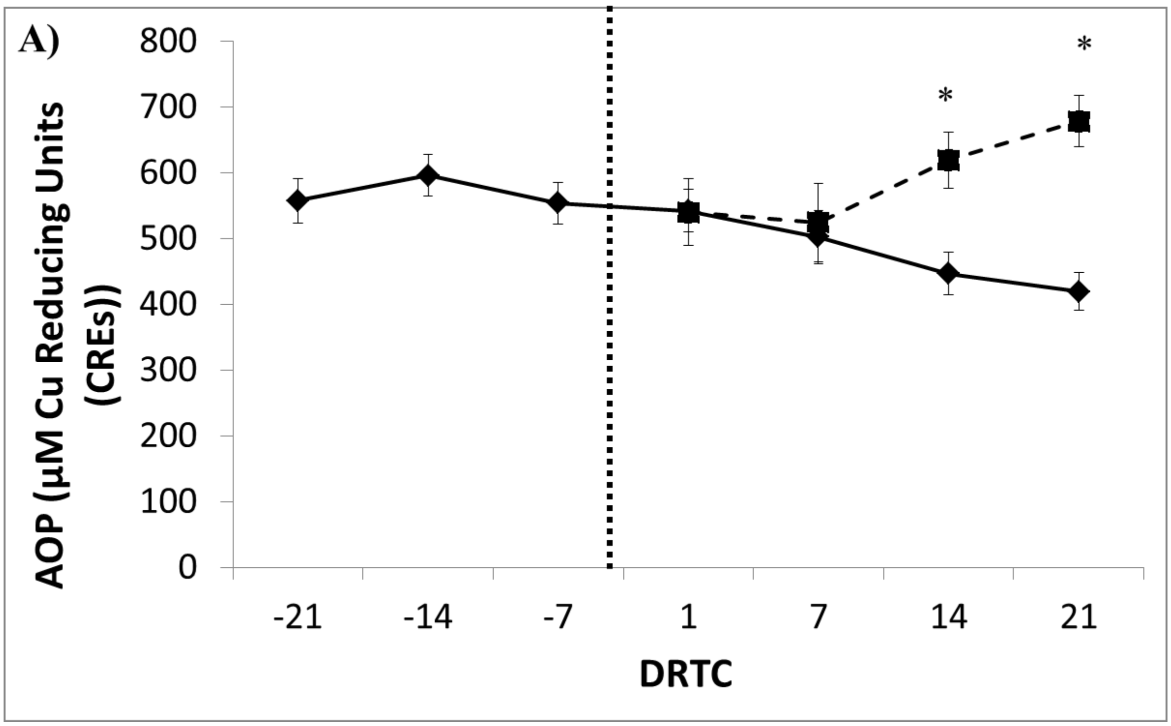


**Figure 2.3.** Oxidative parameters for 14 multiparous (◆) and 8 primiparous (■) pastured periparturient dairy cows milked in an automatic milking system. A) AOP (parity, $P < 0.05$; interaction of DRTC and parity, $P < 0.01$) B) GSH (DRTC, $P < 0.01$) C) GSSG (interaction of DRTC and parity, $P < 0.05$) D) GSH:GSSG (DRTC, $P < 0.05$). Asterisks indicate significant between primiparous and multiparous cows on a given day ($P < 0.05$).



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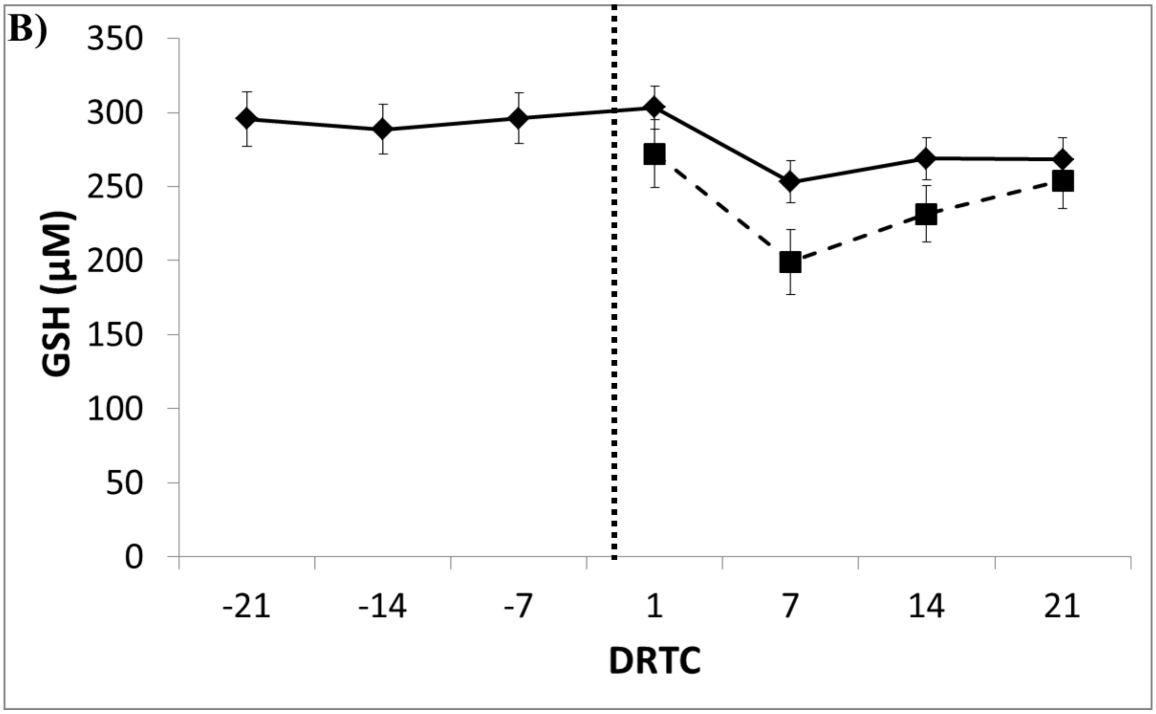


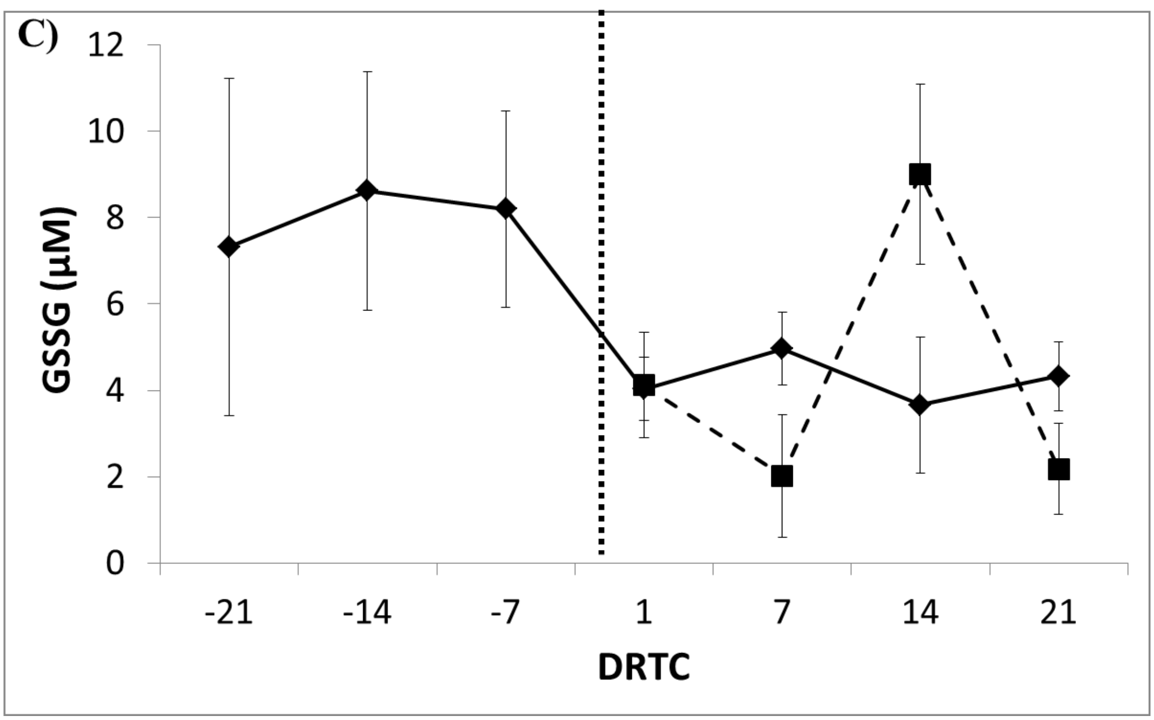

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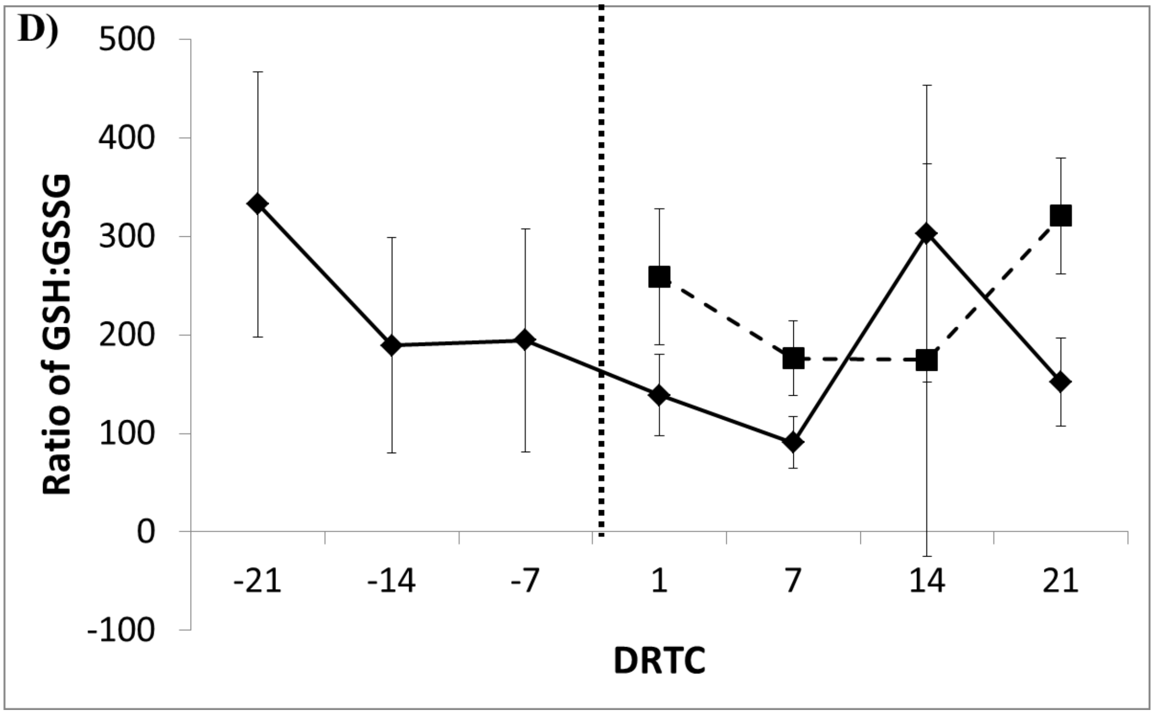
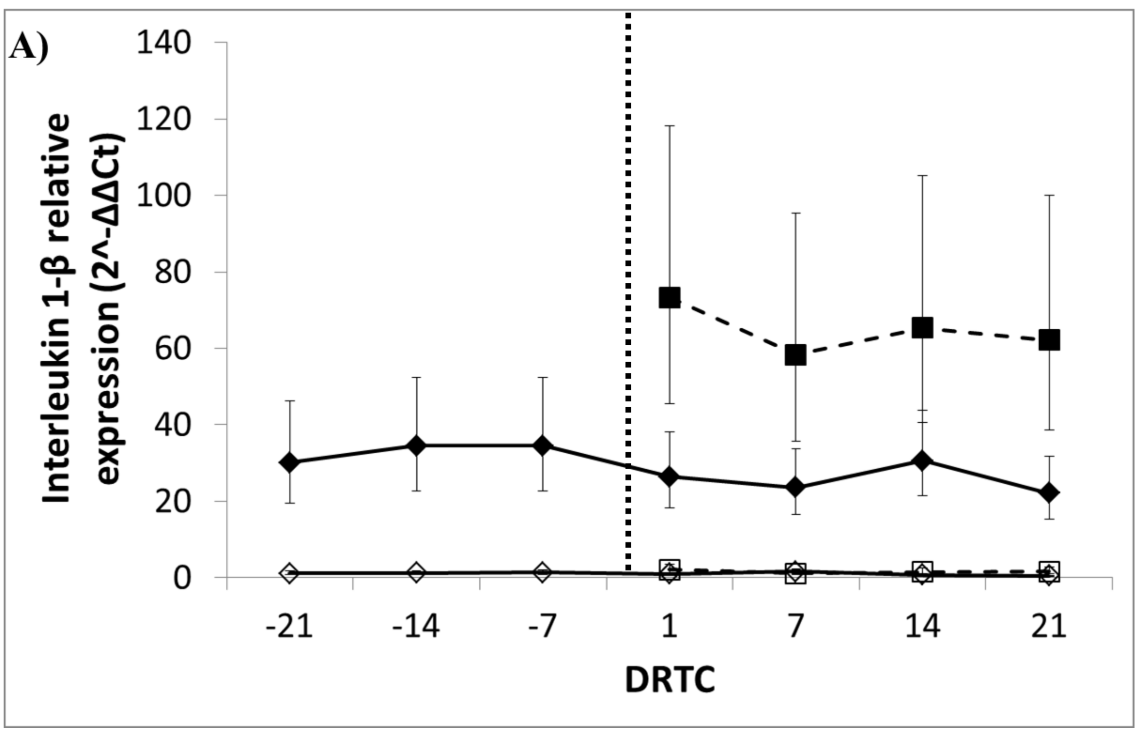


**Figure 2.4.** Cytokine gene expression data for 14 multiparous (◆) and 8 primiparous (■) pastured periparturient dairy cows milked in an automatic milking system. Alterations in mRNA (from whole blood) expression of A) interleukin 1-β, B) interleukin 8 (DRTC, $P < 0.01$), C) osteopontin and D) tumor-necrosis factor-α (DRTC, $P < 0.01$; the interaction of parity by LPS stimulation, $P < 0.05$) of control (solid line) and lipopolysaccharide (LPS) treated (dashed line) obtained on 1, 7, 14, and 21 days relative to calving. Data were analyzed by the $2^{-\Delta\Delta Ct}$ method with 1 d control MC as the reference expression point. Data are reported as least square means ± SEM. Asterisks indicate significant between primiparous and multiparous cows on a given day ($P$ <0.05). Expression levels in control samples compared to those receiving LPS stimulation were significantly different ($P < 0.001$) for all days and all cytokines (not marked on graphs).



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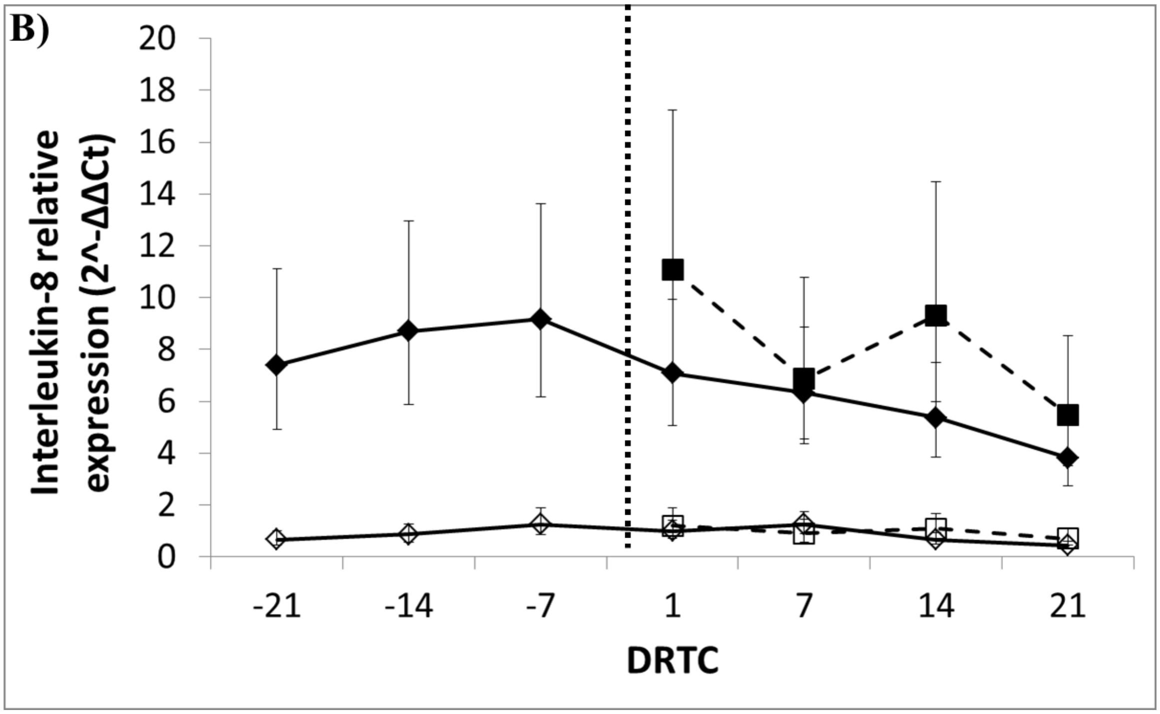


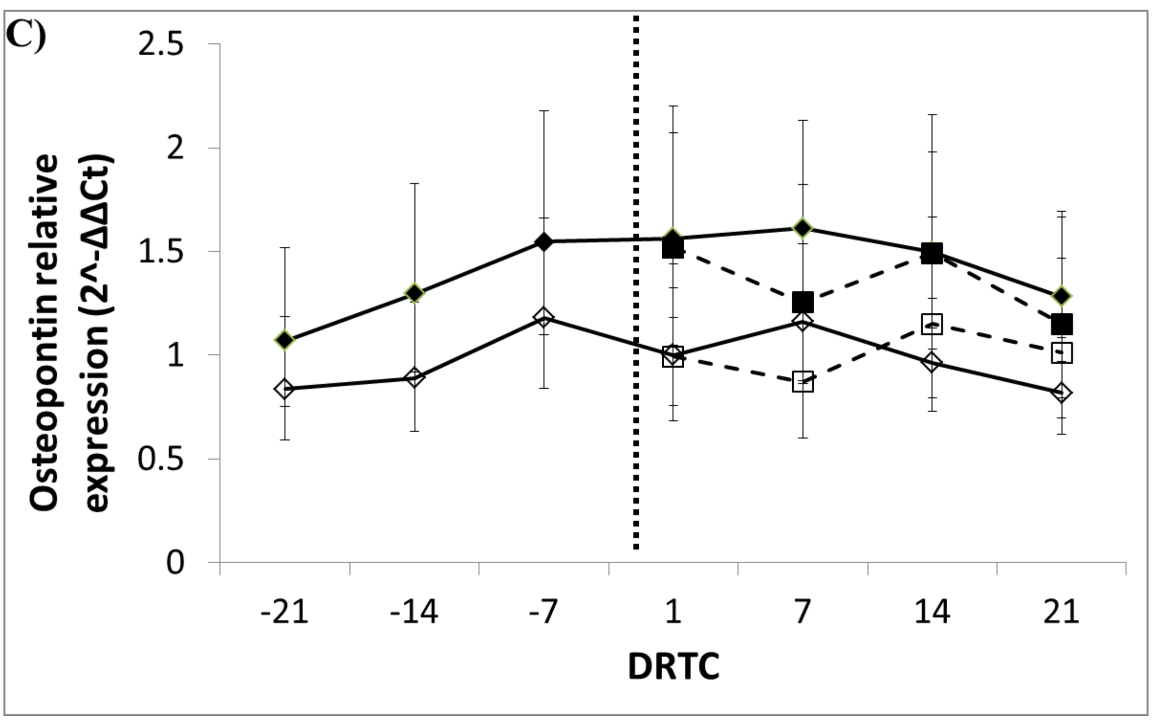

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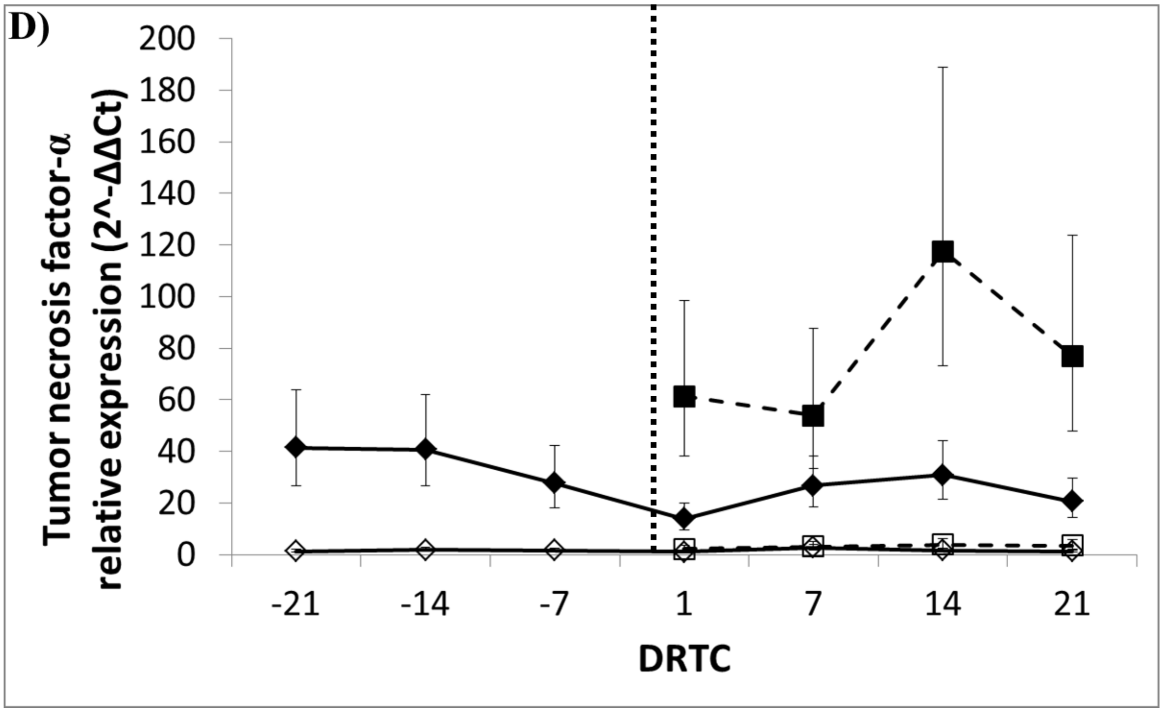

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# CHAPTER 3

# BEHAVIORAL, PHYSICAL, AND PRODUCTION PARAMETERS OF PERIPARTURIENT DAIRY COWS ON PASTURE AND MILKING WITH AN AUTOMATIC MILKING SYSTEM

**Interpretive summary: Welfare of periparturient pastured AMS cows. Elischer**

Near calving, dairy cows experience physical and behavioral stress. How periparturient cows respond in confinement dairies has been well documented, but research examining their responses in other systems is limited. This research examined behavior, physical measures and production parameters of periparturient dairy cows in a pasture-based automatic milking system. Cows experienced minimal fluctuations in weight or body condition score, increased milk production over time, and did not experience lameness issues during the postpartum period despite milking 2.3-2.6 times per day at variable intervals, suggesting minimal physiological stress, contributing to positive welfare status.

Welfare of periparturient pastured AMS cows

**Behavioral, physical, and production parameters of periparturient dairy cows on pasture and milked with automatic milking systems**

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**ABSTRACT**

The periparturient period is a time of great physical, environmental, and behavioral change for dairy cows. This complex combination of factors can negatively impact the welfare of a cow when not managed well. The impact of transition on a dairy cow's welfare in traditional confinement dairies has been relatively well characterized, however, little research has explored how alternative systems, such as automatic milking systems (AMS) and pasture-based diets, affect a cow during this sensitive time. Pasture-based dairies are being combined with AMS in several areas of the world, most notably New Zealand, Australia, and Europe. The ability of cows to milk voluntarily and have constant access to food without the need for human assistance may increase animal welfare during the transition period. However, cows must also walk more and are consuming feed with less energy, which could conversely have a negative impact on welfare. Thus, the aim of this study was to characterize the behavior and welfare of pastured cows milking in an AMS during the periparturient period to elucidate the impact of these systems on dairy cattle welfare. Results indicate that cows were under minimal physiological stress, as demonstrated by small fluctuations in weight and body condition score, increasing milk yield, and low gait scores relating to excellent foot and leg health. Contrary to many AMS studies, milking interval variability and milking frequency were not correlated with significant, production or behavioral responses. This study provides the first evidence that combining pasture and AMS does not negatively impact the welfare of cows during the transition from late gestation to lactation.

# INTRODUCTION

The periparturient, or transition, period is a physiologically, environmentally, and behaviorally stressful time for dairy cattle that can affect a cow on many levels, which could negatively impact her overall welfare. Poor welfare is not only a major concern for the well-being of the cow, but also for the producer who, for example, may experience financial losses from decreased milk production or increased veterinary costs if cows become ill or injured. The changes observed around the transition period have been relatively well characterized in traditional confinement operations using milking parlors, but there has been little research on what effect pasture nutrition or automatic milking systems (AMS) have on the cow during this sensitive time. This gap in understanding can lead to difficulties in deciding how to best manage cattle in alternate feeding, housing, and milking systems.

Automatic milking systems were commercially introduced in the Netherlands in 1992 with the primary goal of decreasing human labor on dairy farms (Bijl et al., 2007), but there are many possible advantages for the cow. A properly functioning AMS provides the cow with the option to milk voluntarily, performs proper teat stimulation, and often delivers a concentrate supplement while the cow is being milked (Svennersten-Sjaunja and Petterson, 2008). Many of these AMS attributes combine to help optimize oxytocin release and milk let down during each milking session (Svennersten-Sjaunja and Petterson, 2008). For example, feeding concentrates or roughage during milking has been shown to help increase milk flow and ejection from the udder and consequently decrease total time in the milking unit (Samuelsson et al., 1993).

An AMS can allow producers to customize milking schedules for their herd so that each cow could potentially be milked at a different milking frequency or have a different interval between milkings without using additional labor (Svennersten-Sjaunja and Petterson, 2008).

Desired milking frequency can be adjusted depending on the stage of lactation, expected yield, or parity of the cow (Svennersten-Sjaunja and Petterson, 2008). This flexibility in setting individual milking schedules is an important benefit of the AMS, but it must be managed properly to ensure the robot is operating efficiently to balance and meet individual cow variation in milking intervals, yield, and duration, as well as the economic goals of the producer (André et al., 2010).

Given the flexibility of milking frequency and intervals that can occur when cows control their milking routine in an AMS, managers must be aware of the length of time that passes between milkings and whether these intervals are consistent or variable. Long intervals between successful milkings have been shown to negatively impact daily milk yield, particularly for multiparous cows in confinement operations (Bach and Busto, 2005). Delamaire and Guinard-Flament (2006) showed that increasing the time between milkings negatively impacted milk production by decreasing the blood flow to and efficiency of nutrient uptake by the mammary gland. However, in pasture-based AMS systems, where energy inputs are lower, increasing the interval between milkings from ~13 h to ~17 h was not related to reduce milk yield in low producing cows (Jago et al., 2007).

Perhaps even more important than the actual duration of time between milkings is the variability in the amount of time that elapses between milkings. Bach and Busto (2005) found that cows with a high coefficient of variance (>27%) in their weekly milking intervals produced significantly less milk, and multiparous cows were more sensitive to these effects than primiparous cows. This decrease in milk yield was not due to limits in udder storage capacity, nor due to difference in milk flow rates (Bach and Busto, 2005). Rather, the irregular milking intervals appeared to impair milk synthesis at the mammary level as a result of a decline in metabolic activity of secretory cells or decreased cell proliferation (Bach and Busto, 2005). However, many factors (e.g., feeding system, machine function, incomplete or failed milkings, social competition etc.) besides variability of milking interval may also influence milk production alone or in combination. Therefore, variability of milking interval cannot be considered in a vacuum apart from other environmental or management influences.

Pasturing cows is also a way of adding management flexibility to dairy systems. By relying on a pasture-based diet, producers may have lower labor and cost inputs because they do not need to purchase feed from external sources or deliver feed to cows multiple times per day (Washburn et al., 2002). Beyond having fresh feed available at all times, there are other health and behavioral benefits to pasturing dairy cows. In a study conducted by Somers and colleagues (2003), different flooring types on Dutch dairy farms were evaluated for their effect on cow claw health. Cows in zero-grazing dairies had a statistically higher risk of having interdigitial dermatitis and heel erosion, digital dermatitis, sole hemorrhages, sole ulcers, and interdigital hyperplasia compared to cattle with pasture access (Somers et al., 2003). Even limited pasture access has also been shown to decrease gait score in clinically lame cattle (Hernandez-Mendo et al., 2007). Beneficial changes in lying behavior also occur as a result of providing pasture to cows, including quicker transitions from standing to lying suggesting it is easier to lie down on pasture than in a stall (Krohn and Munksgaard, 1993). Cattle on pasture were also more likely to be observed lying laterally or with their heads on their back or the ground (Krohn and Munksgaard, 1993), postures that are believed to provide the deepest rest for cows.

Individually, both AMS and pasture offer potential improvements to cow welfare, but combining the systems creates challenges. However, initial research into AMS-pasture dairies indicates this is a viable system when managed with attention to the unique attributes of the system (see Jacobs and Siegford, 2012 for a review). For example, it can be a challenge to obtain the desired number of voluntary milkings per day when cattle are pastured. However, Jago and colleagues (2004) demonstrated that having a sorting unit near the pastures rather than near the AMS to direct the cows to be milked or back to pasture was effective at getting cows to milk at the desired frequency and reduced the need for fetching. Additionally, Jago and Kerrisk (2011) found that both multiparous and primiparous pastured cows can quickly adapt to a pastured AMS dairy with or without training prior to entering the milking herd, though training eased the process.

The objective of this study was to characterize the lying and miking behavior, production, and physiological responses of multiparous cows of two different breeds at two different pasture-based AMS farms during the sensitive periparturient period. It was hypothesized that pastured cows milking with an AMS, regardless of farm or breed, would maintain a healthy body condition score over the periparturient period, exhibit low weight loss after parturition, maintain a low gait score during the periparturient period and have uniform lying times postpartum. Further, differences in dairy management would affect milking behavior, and breed would influence all parameters of interest.

## MATERIALS AND METHODS

All procedures were approved by the Michigan State University Institutional Animal Care and Use Committee prior to the start of the experiment.

### *Animals and Husbandry*

The study was conducted from 12 April 2012 to 26 July 2012 at a commercial dairy farm (Farm 1) in central Michigan with 111 cows in the milking herd at the time of the study. Fourteen multiparous Friesian-cross bred dairy cows (Table 3.1) were enrolled based on calving date, approximately 21 d prior to expected parturition. The diet of the cows was pasture-based, with occasional supplementation of hay (harvested previously from the pasture) depending on growing conditions.

Dry cows at Farm 1were maintained as one group that was moved between pastures as necessary to meet the grazing needs of the cows throughout the summer. Plant species in the pasture were the same for dry cows as the lactating herd. Water was available *ad libitum*. Indoor access was limited, but there was a pack-bedded barn available for calving if necessary. Cows were allowed to calve on pasture without human interference. After parturition, both the dam and offspring were moved into a transition barn. Within 12 h of parturition, the calf was moved into an individual hutch and the dam joined the milking herd.

The milking herd was managed as a single group under the same breeding, feeding, grazing, and AMS milking protocols. The cows were housed in a loose housing system with 24 h/d access to outdoor pasture and to an indoor AMS barn providing 69 sawdust-bedded free stalls. Water was available indoors only, *ad libitum* from two automatic water troughs at opposite ends of the barn. Two Lely A3 Astronaut AMS (Lely, Maassluis, the Netherlands) were available for milking 24 h/d except for a total of 40 min/AMS/d when the units closed for cleaning and any repairs or servicing that was necessary. All cows had equal access to both AMS units; no management, traffic, or barn design restrictions were placed on the animals for AMS use. All cows wore a transponder around their neck for individual identification by the AMS and routing to pasture via a sort gate.

The lactating herd was rotationally grazed on 101.2 ha of pasture subdivided into 26 paddocks. The pasture contained a mixture of red clover (*Trifolium pretense*) and white clover (*Trifolium repens*) and orchard grass (*Dactylis glomerata)*. Pasture access from the barn was controlled via an automated sort gate (Lely, Maassluis, the Netherlands). The distance from the barn exit to the farthest corner of the closest paddock was 0.19 km, and the distance to the farthest corner of the farthest paddock was 1.21 km. Cows were allowed on pasture based on restrictions set by the producer for number of milkings/d and maximum allowable interval between milkings. Cows pushed through a one-way manual gate for reentry to the barn. Cows were fetched to the AMS as necessary based on standard farm protocols. Fresh cows were allowed to milk a maximum of 5 times in a 24 h period, with a minimum of 3 milkings per 24 h, as long as their expected yield was at least 8.18 kg/milking. During milking, all cows were fed grain based on their level of production (multiparous and primiparous minimum = 2.27 kg concentrate/day; multiparous maximum = 7.26 kg concentrate/day, primiparous maximum = 5.85 kg concentrate/day).

The temperature at Farm 1 one ranged from 8.3 to 34.4°C, with a mean of 22.7 ±1.2°C with a total of 84.6 mm of precipitation reported over duration of the experiment from April to July.

The study was conducted from 13 April 2012 to 3 August 2012 at a dairy (Farm 2) in west central Michigan with approximately 120 cows in the milking herd during the time of the study. Five multiparous Friesian and nine multiparous Holstein cows were enrolled based on expected calving date approximately 21 d prior to expected parturition based on artificial insemination records and confirmed pregnancies (Table 3.2).

Dry cows were grazed as a single group on 8 ha of pasture comprised of orchardgrass (*Dactylis glomerata*), endophyte free tall fescue (*Festuca arundinacea*), red clover (*Trifolium pratense*) and alfalfa (*Medicago sativa*). The group was rotated between pastures as necessary to meet the cows' grazing needs throughout the summer. Dry cows did not have access to a barn or other indoor structure. Water was available *ad libitum* from at least one automated waterer per pasture. Dry cows were moved to a close-up grassy paddock near the lactating herd and generally allowed to calve without human interference. Once a cow calved, the dam and offspring were separated within the first 12 h, as per normal farm protocols. The dam joined the milking herd and the calf moved into an individual hutch.

The milking herd was grazed as two groups with 24 h/d access to one of 2 equally sized pens in a freestall AMS barn. Stalls (n = 58/group; 1.22 m x 2.44 m) contained waterbed mattresses that were top-dressed with wood shavings 2x/wk. Automated ally scrappers ran 2x/d, and stalls were manually cleaned daily, with additional shavings added as needed. Each pen had one Lely A3 Astronaut AMS (Lely, Maassluis, the Netherlands) located at the south end of the pen. Cows could only access the AMS associated with their pen. The cows had free access to the AMS 24 h/d, except for 60 min/d when the AMS automatically closed for cleaning (twice a day with 30 min/cleaning) and during times of repair or servicing on the AMS. Milkings occurred on a voluntary basis, but twice a day, any unmilked cows that remained in the barn that had not milked in ≥ 16 h were fetched by farm staff to be milked. Water was available *ad libitum* in the barn only, from two automatic water troughs per pen.

Access to pasture (rye grass and clover or orchard grass, fescue, alfalfa, and red and white clover; 40 ha total, subdivided into 40 total individual paddocks (1 ha each) with hot wire fencing) was controlled via automated sorting gates (Lely, Maassluis, the Netherlands) at the north end of the barn, opposite the AMS. Successful milking events (i.e., milkings occurring within the pre-established intervals) were used as criteria to grant access to pasture or to draft unmilked cows back into the milking barn. The distance from the barn exit to farthest corner of the closest paddock was 0.27 km; the distance to the farthest corner of the farthest pasture was 0.56 km. During the study period, cows were allocated new pasture every 16 to 24 h/d. While being milked in the AMS, fresh cows were fed increasing amounts of a pelleted concentrate until 30 DIM; afterward, pellet feeding was based on their level of milk production (minimum = 2.27 kg concentrate/day; maximum = 5.67 kg concentrate/day). Additionally, all lactating cows were allotted 1.36 kg/d of coarsely ground corn distributed from a CosMix feeder (Lely, Maassluis, the Netherlands) located near the exit of the AMS in each pen.

Fresh cows were allowed to milk a maximum of 5.5 times in a 24 h period during the first 30 d of lactation. The minimum number of milkings for fresh cows was 4x/d, or every 6 h. If a cow was expected to produce more than 9.09 kg of milk at her next milking, she was allowed to milk more frequently.

The range of temperatures at Farm 2 was 4.4 to 35.0°C, with a mean temperature of 22.0 ± 2.1°C and total 25.4 mm of precipitation through the experimental period from April to August.

All cows at both farms wore a transponder around their neck for individual identification by the AMS and sort gates. Length of time since previous milking and time until next milking were automatically calculated by the AMS management program (Lely, Maassluis, the Netherlands) and were the factors that determined whether an individual cow was allowed to milk at the AMS and, at the sort gates, whether the cow was directed to pasture or back into the barn. Every study cow was also fitted with an IceQube pedometer (IceRobotics, Edinburgh, Scotland) on the rear pastern above the fetlock the first day the cow was enrolled in the study, and the pedometer was removed after 21 d. It should be noted that although all 28 cows were fitted with a pedometer upon being enrolled in the study, only data from 11 cows (nine Friesians at Farm 1 and two Friesians and six Holsteins at Farm 2) are reported with regard to time spent lying due to equipment malfunction that was not discovered until pedometers were removed at the end of experiment. All other response parameters (BCS, weight, milk yield, milking frequency, milking interval, and gait score) were collected from the 28 focal cows for the entire length of the study period.

### *Data Collection*

Sampling days for all cows at both farms were -21, -14, -7, 1, 7, 14, and 21 days relative to calving (DRTC). The sampling consisted of making non-invasive observations of gait and body condition score (BCS) and collecting data on milk production, AMS visitation, and body weight from the AMS. At least two trained individuals were on hand to complete data collection on all sampling days.

Cow weights were automatically recorded by the AMS at every successful milking visit, thus only postpartum data are available for body weight. The AMS also reported frequency of successful and failed milkings, as well as number of refusals by the AMS, duration of time between milkings, and milk yield (Table 3.1).

The gait of individual cows was scored on a 1 – 5 point scale in whole number increments, adapted from the methods of Sprecher and colleagues (1997). Cows were assessed walking on a straight, level surface. Individual BCS were scored on a 1 – 5 point scale (in 0.25 increments), adapted from the methods described by Wildman et al. (1982) and Ferguson et al.

(1994). These BCS scores were determined through visual assessment of the cow without palpation. Both gait score and BCS were assessed by at least two trained individuals on each sampling day.

***Statistical Analysis***

Only postpartum data were initially analyzed because the impact of milking interval variability and milking frequency were the main points of interest related to the health and production parameters of the pastured-AMS dairies. The variability of milking interval was determined by taking the standard deviation of all milking intervals occurring within a 7 d period. Thus, the interval variability calculated for a cow on 7 d was the standard deviation of all milking intervals from the first day postpartum through 7 d; variability on 14 d was calculated using interval data from 8 d through 14 d; and 21 d variability was calculated using interval data from 15 d to 21 d. Statistics to evaluate the strength of relationships between interval variability and milk frequency with the response parameters were performed in SAS 9.2 (Statistical Analysis Software, Cary, NC, USA) using a Spearman correlation analysis (PROC CORR). This procedure was carried out to check the strength of the relationships of milking interval variability and milking frequency to the response parameters of interest between Friesian cows at both farms and between Friesian and Holstein cows at Farm 2. The results of these analyses indicated weak relationships between both milking interval variability and milking frequency with BCS, weight, gait score, lying time, and milk yield for Friesians cows at both farms (Table 3.3) and with Friesian and Holstein cows at Farm 2 (Table 3.4). Artificially creating groups of high, medium, and low variability or frequency was decided against because it would decrease the resolution of the data set. Since neither milking interval variability nor milking frequency was found to be a good predictor of the responses of interest, they were not included in the final model statement examining changes in other parameters of interest.

Comprehensive statistical analyses were also conducted in SAS 9.2 with significance declared at $P < 0.05$. A mixed model (PROC MIXED) was used to analyze all behavior, production, and physiological responses of interest for the Friesian cows at Farms 1 and 2. The model was as follows:

$$Y_{ijk} = \mu + DRTC_j + F_k + (DRTC)(F)_{jk} + e_{ijk}$$

Where $Y_{ijk}$ was the dependent variable of interest from $cow_i$ on the $DRTC_j$ (7, 14, 21d) at $farm_k$ (Farm 1 or Farm 2), with mean $\mu$ with residuals $e_{ijk}$. Day relative to calving was a repeated measure and farm was a random effect. For each response variable, the covariate structure for farm was chosen based on lowest BIC and AIC values. Least square means ± standard error of the least square means are presented. Data were adjusted using the Tukey-Kramer method in SAS for post hoc multiple pair-wise comparisons.

The statistical analysis of Friesian and Holstein cows at Farm 2 was also carried out in SAS 9.2 with significance declared at $P < 0.05$ using a mixed model (PROC MIXED) to analyze all behavior, production, and physiological parameters. The model was as follows:

$$Y_{ijk} = \mu + DRTC_j + B_k + (DRTC)(B)_{jk} + e_{ijk}$$

Where $Y_{ijk}$ was the dependent variable of interest from $cow_i$ on the $DRTC_j$ (7, 14, 21d) of $breed_k$ (Friesian or Holstein), with mean $\mu$ with residuals $e_{ijk}$. Day relative to calving was a repeated measure and breed was a random effect. For each response variable, the covariate structure for breed was chosen based on lowest BIC and AIC values. Least square means ±

standard error of the least square means are presented. Data were adjusted using the Tukey-Kramer method in SAS for post hoc multiple pair-wise comparisons.

Pre- and postpartum data were available for BCS and gait score, thus both of these parameters were analyzed across the entire experimental period (-21, -14, -7, 1 7, 14, and 21 DRTC) as well as during the postpartum period with the production parameters of interest.

## RESULTS

### *Behavioral, Production, and Physiological Responses: Friesian Cows at Farms 1 and 2*

Cows at Farm 2 (3.2 ± 0.1) had higher average BCS than cows at Farm 1 (2.9 ± 0.1; $P < 0.05$). There was a significant effect of DRTC ($P < 0.01$) on BCS across the entire sampling period, but the interaction between farm and DRTC was not significant (Fig. 3.1A). Body condition score slowly decreased over time from -21 to 21 d, with the score at -21 d (3.34 ± 0.07) differing significantly from 7 d (3.07 ± 0.07), 14 d (2.99 ± 0.07) and 21 d (2.91 ± 0.07; $P < 0.05$) postpartum (Fig. 3.1A). Cows at both farms experienced a decline in BCS at 14 d postpartum, with BCS on 14 d (2.9 ± 0.1) differing significantly from BCS on 21 d (3.1 ± 0.1; $P < 0.05$). However, the cows' BCS on 7 d (3.0 ± 0.1) did not differ significantly from BCS on 14 or 21 d.

The main effect of DRTC was significant ($P < 0.01$) for milk yield, as was the interaction between farms and DRTC ($P < 0.05$; Fig. 3.1B). Cows at both farms showed increased milk yield from 7 d to 21 d, with milk yield on 7 d (8.5 ± 0.6 kg) significantly lower ($P < 0.05$) than yield on 14 d (10.6 ± 0.6 kg) and 21 d (10.1 ± 0.6 kg). Milk yield on 14 d for the cows at Farm 2 (13.0 ± 1.1 kg) was significantly higher ($P < 0.05$) than yield of Farm 1 cows on 7 d (7.3 ± 0.6 kg), 14 d (8.3 ± 0.6 kg) and 21 d (8.42 ± 0.6 kg) and cows at Farm 2 on 7 d (9.6 ± 1.1 kg), but did not differ significantly from milk yield by cows at Farm 2 at 21 d (11.7 ± 1.1 kg).

Day relative to calving and the interaction between farm and DRTC were not significantly related to gait score (Fig. 3.2A), time spent lying (Fig. 3.2B), or body weight (Fig. 3.1C). During the periparturient period, gait score of cows at both farms varied little and did not significantly differ on any day. The range of scores for cows at Farm 1 was 1.24 ± 0.17 to 1.71 ± 0.15, while the range Farm 2 cows was 1.27 ± 0.25 to 1.83 ± 0.23.

The cows at Farm 1 had an increase in lying time at 14 d (7 h 34 min ± 38 min) compared to 7 d (6 h 18 min ± 38 min) and 21 d (5 h 27 min ± 38 min). Farm 2 cows showed a differing pattern, with the lowest amount of lying time on 14 d (6 h 46 min ± 1 h 20 min) compared to lying times on 7 d (7 h 15 min ± 1 h 20 min) and 21 d (7 h 15 min ± 1 h 20 min). Body weight of cows at both farms declined from 7 to 14 d (Farm 1 7 d = 474.8 ± 13.0 kg, 14 d = 468.5 ± 14.0 kg; Farm 2 7 d = 469.7 ± 21.7 kg, 14 d = 488.83 ± 23.4 kg). Farm 2 cows had a slight increase at 21 d (490.5 ± 23.5 kg), while the weight of cows at Farm 1 continued to decline at 21d (465.9 ± 14.0 kg).

***Behavioral, Production, and Physiological Responses: Friesian and Holstein Cows at Farm 2***

Body condition score of cows over the entire experimental period was significantly impacted by DRTC ($P < 0.01$) and the interaction of DRTC and breed ($P < 0.05$; Fig. 3.3A). Friesian cows lost less condition over time and had a higher score as 21 d postpartum (3.35 ± 0.15) compared to -21 d prepartum (3.2 ± 0.19), but these values did not significantly differ. Friesian cows had the lowest BCS on 14 d after calving (3.00 ± 0.18), which differed significantly from 21 d; there were no other significant differences between days for Friesian cows. Holstein cows had a greater change in BCS during the periparturient period, with the highest BCS on -7 d (3.36 ± 0.12) and the lowest on 21 d (2.58 ± 0.12). All days postpartum significantly differed from all prepartum days for the Holstein cows ($P < 0.05$). The only day that significantly differed between the cows of each breed was 21 d postpartum (Friesian: 3.35 ± 0.15; Holstein: 2.58 ± 0.12, $P < 0.05$).

Milk yield was significantly impacted by DRTC ($P < 0.01$) and the interaction of DRTC and breed ($P < 0.05$; Fig. 3.3B). Significantly less milk/milking was produced on 7 d (9.74 ± 0.89 kg/milking) compared to 14 d (11.85 ± 0.89 kg/milking) and 21 d (12.21 ± 0.89 kg/milking; $P < 0.05$); while milk yield on 14 d and 21 d did not differ. Friesian cows produced significantly less milk on 7 d (9.61 ± 1.42 kg/milking) compared to 14 d (12.97 ± 1.42 kg/milking; $P < 0.05$); but production on 7 d did not differ from 21 d (11.74 ± 1.42) and 14 d and 21 d did not differ. Holstein cows increased milk production between 7 d and 21 d, with the yield per milking on 7 d being significantly lower (9.87 ± 1.06 kg/milking) than 21 d (12.68 ± 1.06 kg/milking; $P < 0.05$). Milk production per milking on 14 d (10.73 ± 1.06 kg/milking) did not significantly differ from 7 or 21 d. Friesian cow compared to Holstein cow milk production per milking did not significantly differ on any day.

Day relative to calving had a significant impact on weight ($P < 0.05$) for both Friesian and Holstein cows, but there was no interaction of breed and day relative to calving (Fig. 3.3C). Cows weighed significantly less on 21 d (541.03 ± 11.99 kg) compared to 7 d (561.73 ± 11.99 kg; $P < 0.05$) postpartum; cow body weight on 14 d (549.23 ± 11.99 kg) did not significantly differ from 7 or 21 d. The body weight of Friesian cows did not differ from 7 d through 21 d (7 d: 496.72 ± 19.22 kg; 14 d: 490.46 ± 19.22 kg; 488.84 ± 19.22 kg). Holstein cows weighed significantly more on 7 d (626.74 ± 14.33 kg) compared to 21 d (591.60 ± 14.33 kg; $P < 0.05$); but weight on 14 d (609.62 ± 14.33 kg) did not differ from that on 7 d or 21 d postpartum.

There were no significant differences in gait score or time spent lying on any day or for the interaction of DRTC and breed (Fig. 3.4). The lowest overall gait score was on 14 d (1.82 ±

0.21), but this did not differ significantly from gait score on 7 d (2.08 ± 0.25) or 21 d (2.09 ± 0.18). Both Friesian and Holstein cows had the lowest gait score on 14 d (Friesian: 1.20 ± 0.34; Holstein: 2.44 ± 0.26), but these scores did not differ from those of 7 d (Friesian: 1.6 ± 0.40; Holstein: 2.56 ± 0.30) or 21 d (Friesian: 1.4 ± 0.28; Holstein: 2.78 ± 0.21). Friesian cows had lower gait scores than Holstein cows on all DRTC. The lying time for Friesian and Holstein cows decreased from 7 d (8 hr 39 min ± 52 min) to 14 d (7 h 37 min ± 52 min) and 21 d (7 h 45 min ± 52 min), but none of these durations differed significantly. Friesian cows spent the least amount of time lying on 14 d (6 h 46 min ± 1 h 30 min) compared to 7 d and 21 d (7 h 15 min ± 1 h 30 min), but times were not significantly different. Lying time of Holstein cows decreased over the postpartum period (7 d: 10 h 4 min ± 52 min; 14 d: 8 h 29 min; 21 d: 8 h 15 min ± 52 min) and Holstein cows generally spent more time longer than Friesians, but there were no significant differences between the breeds.

***Milking Interval Variability and Milking Frequency***

Although not good predictors of the production and physical response parameters, milking interval variability and milking frequency were analyzed for differences over the postpartum period for time, breed or farm, and the interaction of time with breed or farm. Day relative to calving significantly impacted the variability of milking interval at Farm 1 and Farm 2 ($P < 0.05$), but there was no interaction of farm and DRTC (Fig. 3.5A). The variability in interval was longest on 7 d (3 h 50 min ± 33 min), which significantly differed from the variability seen on 14 d (2 h 20 min ± 12 min; $P < 0.05$); however, variability on 21 d (2 h 55 min ± 24 min) did not differ from that of 7 d or 14 d. Milking frequency was also affected by DRTC ($P < 0.05$); however, there was no interaction of farm and DRTC. The milking frequency on 1 d (1.93 ± 0.13 milkings/d) was significantly lower than that seen on all other days postpartum (7 d: 2.46 ± 0.14 milkings/d; 14 d: 2.68 ± 0.17 milkings/d; 21 d: 2.57 ± 0.19 milkings/d; $P < 0.05$); frequency of milking on 7, 14, and 21 d did not differ (Fig. 3.5B).

In comparing Friesian and Holstein cows at Farm 2, there were no significant effects of DRTC or the interaction of breed and DRTC on the variability of milking interval or milking frequency during the postpartum period (Fig. 3.5 C, D). The greatest variability in milking interval was seen on 7 d (5 h 10 min ± 1 h 2 min), but this did not significantly differ from variability on 14 d (2 h 48 min ± 16 min) or 21 d (3 h 27 min ± 39 min). The lowest milking frequency was on 1 d (2.16 ± 0.22 milkings/d) and the highest on 14 d (2.53 ± 0.22), but these did not differ. Milking frequency on 7 d (2.33 ± 0.22 milkings/d) and 21 d (2.22 ± 0.22 milkings/d) were intermediate and did not differ from each other or 1 and 14 d.

## DISCUSSION

The periparturient period is a time of great stress for dairy cows as they calve, begin lactation and a milking routine, experience a change in diet, and are moved from the social and physical environment of the dry cow group to that of the milking herd. A cow's homeostasis is severely challenged as she copes with these internal and external challenges. Behavioral, non-invasive physical measures, and production parameters can be collected that provide insight into how well a cow is adapting and coping during this time. These parameters have been well characterized in traditional milking parlor and feeding systems, but no research has examined the impact of a pasture-based diet combined with an AMS during the periparturient period. Therefore, the aim of this study was to characterize some of the behavioral, physical, and production parameters of multiparous periparturient Friesian and Holstein dairy cows during the postpartum period.

The impact of the variability of the milking interval was one of the main topics of interest in this research. A large degree of variation in milking interval has been linked to lower milk production (Bach and Busto 2005) and positively correlated with increased somatic cell count (Mollenhorst et al., 2011). Somatic cell counts were not analyzed in the present study; however, data on milk yield was collected. Contrary to the findings of Bach and Busto (2005), variability in milking interval over the first 21 d of lactation was not a good predictor of milk production. High variability was not associated with lower milk yield, nor were very regular milking intervals linked to higher yields; in fact, no strong relationship in either direction was detected. Further, it was found that variability was not strongly correlated with any of the behavioral or physical response variables of interest.

Grove and colleagues (2004) reported an increase in time between milkings when cows had an increased gait score (i.e., increased lameness) in an AMS dairy. These results are logical because if locomotion is compromised, a cow will be less willing to walk to the AMS to be milked. The cows on this study maintained excellent leg and foot health, as demonstrated by the low gait scores seen across the 42 days of the study. One important factor that may have contributed to healthy gait scores was the pasturing of cows at both farms. Pasture access has been shown to increase leg and hoof health for even mildly lame cows (Hernandez-Mendo et al., 2007), thus constant pasture access pre- and postpartum was likely an important factor in maintaining low gait scores. The benefits associated with pasture access may be even more important for some breeds than others; recent research has found that Holstein cows have a higher incidence of lameness compared to other breeds (e.g., Jersey; Barker et al., 2010), even when housed under the same conditions (Baranski et al., 2008). The New Zealand Friesian cows in this study did not experience a high incidence of lameness, nor would a high incidence be expected, because this breed is anecdotally considered to be more sound in bone structure and frame compared to Holstein cows, though no research has directly evaluated lameness in New Zealand Friesian dairy cows. In the current study, Holstein cows had a higher overall gait score than the Friesians housed under the same conditions at Farm 2. Further investigation examining where each breed of cow spends the most time (i.e., inside the barn on concrete versus outside on pasture) and assessment of conformation could help to determine exactly why this difference in gait score exists at this farm. However, it is important to point out that on average cows of both breeds and at both farms had low gait scores (< 2) and would not be considered clinically lame. These low rates are in contrast with the prevalence of 25% clinical lameness (≥ 3) reported for cows in dairies in Wisconsin and Minnesota (Cook, 2003; Espejo et al., 2006).

One somewhat counterintuitive result of pasturing cows is a reported decrease in lying time (Hernandez-Mendo et al., 2007; Krohn and Munksgaard, 1993). Cows housed in confinement with no pasture access have been found to spend, on average, 11.9 ± 2.4 (SD) h/d lying, with a range of 3.9 to 17.6 h/d (Gomez and Cook, 2010). Pastured cows, however have daily lying times closer to 10 h (Hernandez-Mendo et al., 2007; Krohn and Munksgaard 1993). This decrease in lying time is likely attributable to the time it takes for cows to walk from the barn to the pasture and time spent on their feet grazing. Adequate lying time is very important to cow health and behavior and is often used as an indicator of cow health, with 12 h of lying time considered an ideal target. However, the decrease in lying time noted in studies of cows in pasture systems does not seem to be negatively correlated with gait score, health or production.

Lying times observed at both farms during this experiment were lower (< 8 h/d on average) than those reported for pastured cows in the literature. Holstein cows at Farm 2 lay down for longer total duration (range: 8 – 10 h) than Friesian cows (range: 6 – 7 h), and were closer to the reported average of 10 h (Hernandez-Mendo et al., 2007; Krohn and Munksgaard 1993) on 7 d postpartum, but lying time decreased to approximate 8.5 h on 14 d and 21 d. There are several possible factors that may contribute to the difference between durations here and those reported elsewhere. Cows in this study were in the early postpartum period, which may alter the standing and lying needs of the animal. Huzzey and colleagues (2005) examined standing behavior in free-stall housed Holstein cows during the periparturient period and reported significant differences in lying time pre- and postpartum (prepartum: $12.3 \pm 0.3$ h/d, postpartum: $13.4 \pm 0.3$ h/d; $P = 0.02$), but relatively consistent lying times during early lactation. Lying times in the current study, although low, did not vary greatly in the postpartum period, agreeing with the pattern described by Huzzey and colleagues (2005), suggesting that the events of parturition may not be responsible for low lying durations. However, additional research on pastured cows in early lactation should be conducted to see if there is an interaction of pasture with the postpartum period that differs from the postpartum period in a free-stall environment.

Distance to pasture may impact lying times; if the cows have to spend more time walking to reach the available paddock, it may reduce the amount of time they are able to spend lying (Ketelaar-de Lauwere et al., 2000). Time of the year, temperature, and precipitation will also impact lying times, as cows may move indoors to seek shade on hot or humid days but may not lie down as much once indoors (Legrand et al., 2009; Ketelaar-de Lauwere et al., 1999). As reported earlier, the mean temperature at both farms during the experimental periods was high: $22.7 \pm 1.2$°C at Farm 1 and $22.0 \pm 2.1$°C at Farm 2. The thermoneutral zone of dairy cattle is 5 - 25°C (Roenfeldt, 1998), and at temperatures greater than 26°C, the cow is no longer able to cool herself and enters into a state of heat stress (Kadzere et al., 2002). The mean temperature during the study period at both farms was near the upper limit of the thermoneutral zone, with high temperatures on some days well exceeding it, thus cows likely favored the shaded, indoor facility during these times even though they may not have had access to their preferred lying substrate.

The method of recording lying behavior may also have contributed to discrepancies between the current study and previous findings. IceQube pedometers with a three-dimensional accelerometer were used in this experiment, but other pedometers may record precise lying times differently. However, a study by Elischer and colleagues (submitted) validating the use of these pedometers to report lying times in pastured dairy cows reported a high correlation (r = 0.972, between lying durations recorded by IceQubes and duration recorded using live observations. Although all pedometers appeared to be functioning normally when activated and placed on the cow's leg at the start of this study, malfunctions were discovered in some of the units upon removal, decreasing the number of cows in the data set. Had all devices been working normally and a complete data set available, reported lying times may have differed.

Breed differences may account for the difference in lying times as they do for the differing gait score. Hernandez-Mendo and colleagues (2007) reported that cows without pasture access had higher gait scores and lay longer than those with pasture access. Although both Friesian and Holstein cows at Farm 2 had the same access to pasture and same housing conditions, the Holstein cows did lie for longer durations. Further research into the lying durations of cows at both farms and for both breeds during the periparturient period should to be conducted to help understand the differences in these times compared other reported results.

The frequency of milking early in lactation can have a major impact on milk yield through the current lactation cycle; more frequent milkings may lead to mean higher milk production (Dahl et al., 2004). However, high milk production can be detrimental to the cow if insufficient calories are consumed, leading to failure to meet both maintenance and lactation needs, placing a cow in negative energy balance and under metabolic stress that may predispose the animal to several different diseases (Mulligan and Doherty, 2008). An AMS can allow more milkings to take place per cow compared to even a 3x/day parlor system if the cow voluntarily enters the stall as often is she is typically allowed (for example, 5-5.5x/day at the farms studied here). However, much like the findings for milking interval variability reported above, in the current study milking frequency was not found to significantly impact any of the response variables over the experimental period.

The average number of milkings per day per cow for both farms (Farm 1: 2.60 ± 0.24 milkings/d; Farm 2: 2.27 ± 0.38 milkings/d) and both breeds at Farm 2 (Friesian: 2.27 ± 0.38 milkings/day; Holstein: 2.36 ± 0.27 milkings/d) was higher than the rate previously reported by Jago and colleagues (2004) for pastured Friesian cows (1.42 – 1.91 ± 0.15 milkings/d). The cattle in the previous study were managed using a selection unit located in the pasture, at a distance from the AMS, to direct cows back to the pasture or to the AMS based on minimum milking intervals of 6 – 12 h and were not given supplemental feed in the AMS (Jago et al., 2004). The cows in the present study did not have to pass through a remotely located sorting system, had shorter milking interval restrictions, and were given concentrates in the AMS, which could account for the higher frequency seen here. Friesian cross-bred cows with 24 h unrestricted pasture access and no interval restrictions milked 2.3 ± 0.1 times per day in an AMS where they were provided with concentrate during milking based on parity (Ketelaar-de Lauwere et al., 1999), similar to the results obtained here. Ketelaar-de Lauwere et al., (1999) did not report milk production results, however, Jago and colleagues (2004) reported somewhat higher production (11.96 – 16.40 ± 1.21 kg/milking) compared to Farm 1 (8.03 ± 0.57 kg/milking) but at levels similar to Farm 2 (11.44 ± 0.95 kg/milking) in the present study. What must be accounted for in these reported yields is that the Friesian and Holstein cows in this study were very early in lactation (21 d postpartum), compared to cows studied by Jago and colleagues (2004), that ranged from 7 – 38 DIM (23 cows) and 347 – 376 DIM (4 cows). The milking frequency and yield of cows in the current study did not differ greatly from the two studies mentioned above, however neither Jago and colleagues (2004) or Ketelaar-de Lawuwere and colleagues (1999) reported on the health status of the cow; i.e., whether there were indicators that the combination of milking frequency and production in the AMS in conjunction with a pasture-based diet were meeting the physiological needs of the cow.

Changes in BCS and weight during early lactation are non-invasive methods typically used to assess the metabolic status of cows, particularly those that are not so compromised as to be clinically diagnosed with a problem. Some AMS are equipped to record a cow's weight whenever she is milked, automating this process. The ideal BCS at calving for a Friesian dairy cow is 3.0 on a five point scale (Mulligan et al., 2006), and being over this value can negatively impact health at parturition. Bernabucci and colleagues (2005) reported that high BCS cows were more sensitive to oxidative stress and had higher levels of blood metabolites indicative of metabolic stress.

Cows in the present study did not display extreme changes in BCS, weight, or milk production during the first 21 d postpartum. Body condition scores at both farms and both breeds at Farm 2 were approximately 3.0 at 7 d postpartum and changed very little over the next two weeks. Holstein cows had the greatest change in BCS and weight from 7 d (BCS: $2.8 \pm 0.1$; weight: $626.7 \pm 14.3$ kg) to 21 d postpartum (BCS: $2.6 \pm 0.1$; weight: $591.6 \pm 14.3$). However, the changes recorded in Holstein cow BCS and weight were not different from ranges considered acceptable that have been reported elsewhere (BCS between 3.0 – 3.2 immediately after calving, 2.8 – 3.0 by 14 d postpartum for TMR-fed cows milked with an AMS; Abeni et al., 2005).

Milk production did differ significantly between the two farms, with Farm 2 having higher yield; however, comparing yield within farm, a steady increase in production was seen from 7 to 21 d. The same was true of the milk yield seen in Friesian and Holstein cows at Farm 2: there was a steady increase in yield from 7 to 21 d and no difference in milk production from the two breeds. If there had been a substantial loss in BCS or weight, or a decline in milk production instead of the observed increase, these would be causes of concern that the cows were unable to meet their nutritional needs to maintain health and lactation. However, losses in weight or decreases in BCS were not observed, milk yield continued to increase, and there were no behavioral indications of impaired health based on lying times or gait score, thus the welfare of the pastured-Friesian cows milked with an AMS did not seem to be compromised during the periparturient period.

The differences observed in milking interval variability and milking frequency relative to DRTC when considering both farms could be due to physical differences in the farms and AMS settings since there were no significant differences observed between breeds at Farm 2. Milking variability was greatest 7 d postpartum for Farm 1 and Farm 2 compared to all other days, suggesting the cows were still adjusting to the milking system during the first week of lactation. The cows may have also been adapting to the new social grouping and dominance structure to determine milking order. Cows that have a higher social rank have been found to visit an AMS more frequently and at preferred times of day compared to lower ranking cows (Ketelaar-de Lauwere et al., 1996). It may take time for milking cows to fully establish a social hierarchy, especially since it could change daily as new animals enter or leave the milking herd, thus the initial high variability may be the result of this. Variability decreased over time, suggesting the animals adapted to the routine of milking and their social role in the milking herd. Milking frequency was lowest on 1 d postpartum, again, likely due to the cow becoming acclimated to the system. Also, depending on when the cow was moved in to the milking herd during the day, she may have had more or fewer opportunities to visit the AMS on her first lactation day. Individual farm management strategies could also be involved. If a cow was walked directly the AMS upon being moved into the milking herd versus being moved to the milking herd but not directly walked to the AMS, this would also impact the number of visits she achieved on the first day of lactation. Since there were differences noted between the two farms but no significant differences between Friesians and Holsteins at Farm 2, differences in management protocols between Farm 1 and Farm 2 were likely responsible for the differences in milking interval variability and frequency in the beginning of the postpartum period.

## CONCLUSIONS

In the present study, milking interval variability and milking frequency were not found to significantly correlate with milk yield, BCS, weight, gait score, or amount of time spent lying during the first 21 d postpartum for pastured multiparous Friesian or Holstein dairy cows milked by an AMS. All behavior and production measures indicated that cows were healthy and not under significant physiological stress during the first 21 d postpartum, a time when cows are susceptible to various diseases and infections. Holstein cows seemed to be under greater stress than Friesians based on the greater decease in weight and BCS between the two breeds, though data here were within the range considered normal for a healthy Holstein during transition. Lying times for cows at both farms and of both breeds were low, though average gait scores were $<2$, indicating good hoof and leg health. Low levels of physical and behavioral stress should contribute to a positive state of welfare, which is important for providing optimal care to cows. The combination of a pasture-based diet with an AMS may not be a viable option for every dairy; however, it seems to offer multiple health benefits to cows in terms of behavior, production, and health. Further research is needed to see if and how these benefits extend beyond the periparturient period.

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APPENDIX

**Table 3.1.** Characteristics of the multiparous Friesian cows used in this study during the 21 d postpartum period at Farm 1 and Farm 2 (least square means ± SE).

| | Farm 1 | Farm 2 | *P* values |
|---|---|---|---|
| Number of Cows | 14 | 5 | |
| Parity | 2.86 ± 0.50 | 2.00 ± 0.00 | NS |
| BW (kg) | 469.97 ± 14.84 | 474.60 ± 11.84 | 0.0059 |
| Milk yield (kg)/d | 19.85 ± 0.95 | 25.33 ± 3.78 | <0.0001 |
| Total milkings in 21 d | 56.93 ± 3.45 | 48.20 ± 5.85 | NS |
| No. milkings/Cow/d | 2.60 ± 0.24 | 2.27 ± 0.38 | 0.0005 |
| Refusals | 228.50 ± 77.32 | 14.2 ± 6.40 | <0.0001 |
| Failures | 3.29 ± 0.96 | 1.60 ± 0.60 | <0.0001 |
| Milking Interval | 9 h 30 min ± 1 h | 10 h 50 min ± 2 h 19 min | <0.0001 |

**Table 3.2.** Characteristics of the multiparous Friesian and Holstein cows at Farm 2 used in the 21 d postpartum period (least square means ± SE).

| | Friesian | Holstein | *P* values |
|---|---|---|---|
| Number of Cows | 5 | 9 | |
| Parity | 2.00 ± 0.00 | 3.1 ± 0.26 | 0.0088 |
| BW (kg) | 474.60 ± 11.84 | 617.66 ± 17.72 | <0.0001 |
| Milk yield (kg)/d | 25.33 ± 3.78 | 25.51 ± 2.43 | NS |
| Total milkings in 21 d | 48.20 ± 5.85 | 64.78 ± 7.33 | NS |
| No. milkings/Cow/d | 2.27 ± 0.38 | 2.36 ± 0.27 | NS |
| Refusals | 14.2 ± 6.40 | 32.44 ± 8.70 | NS |
| Failures | 1.60 ± 0.60 | 7.89 ± 2.55 | NS |
| Milking Interval | 10 h 50 min ± 2 h 19 min | 10 h 7 min ± 1 hr 45 min | 0.0140 |

**Table 3.3** The r values, as determined by a Spearman correlation, between either variability of milking interval or milking frequency and response variables of interest on 7, 14, and 21 d and overall for the 21 d postpartum study period using the data from Friesian cows from both farms.

| | Milking interval variability | | | | Milking frequency | | | |
|---|---|---|---|---|---|---|---|---|
| DRTC[1] | 7 d | 14 d | 21 d | Overall | 7 d | 14 d | 21 d | Overall |
| BCS[2] | 0.32 | 0.03 | -0.12 | 0.06 | -0.43 | 0.33 | 0.10 | 0.005 |
| Weight (kg) | -0.12 | 0.05 | -0.23 | -0.10 | 0.17 | 0.31 | 0.10 | 0.16 |
| Milk yield/ milking (kg) | 0.69 | 0.66 | 0.78 | 0.65 | -0.42 | -0.37 | -0.74 | -0.55 |
| Gait score | -0.09 | -0.17 | 0.08 | -0.01 | -0.07 | 0.46 | -0.26 | 0.02 |
| Lying time (s) | 0.18 | -0.05 | -0.12 | 0.02 | 0.16 | -0.54 | 0.36 | -0.002 |

[1]Days relative to calving

[2]Body condition scores (BCS) were evaluated by at least two trained individuals on each sampling day using a 5-point (0.25 increments; 1 = emaciated, 5 = obese) based on the methods of Wildman et al. (1982) and Ferguson et al. (1994).

**Table 3.4** The r values, as determined by a Spearman correlation between either variability of milking interval or milking frequency and response variables of interest on 7, 14, and 21 d and overall for the 21 d postpartum study period using the data from Friesian and Holstein cows at Farm 2.

| | Milking interval variability | | | | Milking frequency | | | |
|---|---|---|---|---|---|---|---|---|
| DRTC[1] | 7 d | 14 d | 21 d | Overall | 7 d | 14 d | 21 d | Overall |
| BCS[2] | -0.10 | 0.36 | -0.53 | -0.10 | -0.17 | 0.47 | 0.05 | 0.11 |
| Weight (kg) | -0.20 | -0.21 | -.016 | -0.13 | 0.32 | 0.41 | 0.43 | 0.40 |
| Milk yield/ milking (kg) | -0.02 | 0.40 | 0.43 | 0.10 | -0.35 | -0.40 | -0.24 | -0.31 |
| Gait score | -0.10 | -0.23 | 0.40 | 0.01 | -0.01 | -0.11 | 0.14 | -0.02 |
| Lying time (s) | 0.19 | -0.33 | 0.02 | 0.05 | 0.51 | -0.12 | -0.45 | -0.04 |

[1]Days relative to calving

[2]Body condition scores (BCS) were evaluated by at least two trained individuals on each sampling day using a 5-point (0.25 increments; 1 = emaciated, 5 = obese) based on the methods of Wildman et al. (1982) and Ferguson et al. (1994).

**Figure 3.1.** The association between time condition score over the entire experimental period (A), milk yield (B), and body weight (C) for Farm 1 (●) and Farm 2 (▲). Results are graphed with asterisks (*) indicating significant differences between DRTC. There was a significant effect of DRTC on BCS () and milk yield per milking (C), but no significant differences were found for DTRC relative to body weight (D).

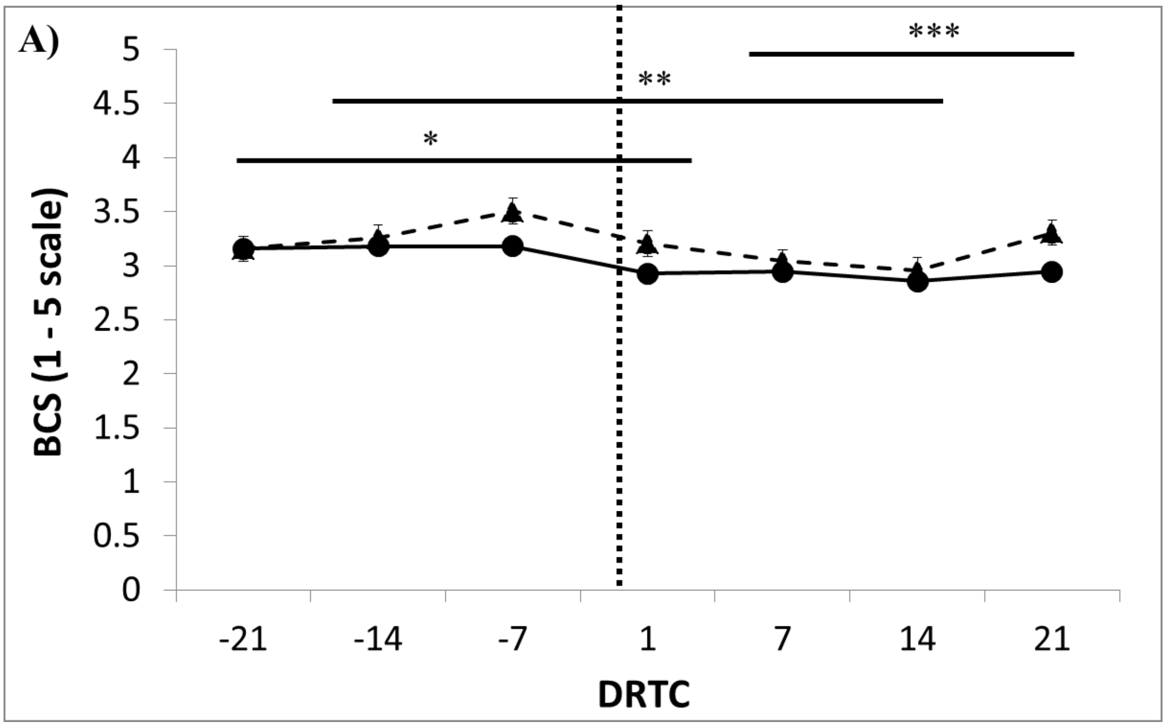


**Figure 3.1 (cont'd)**

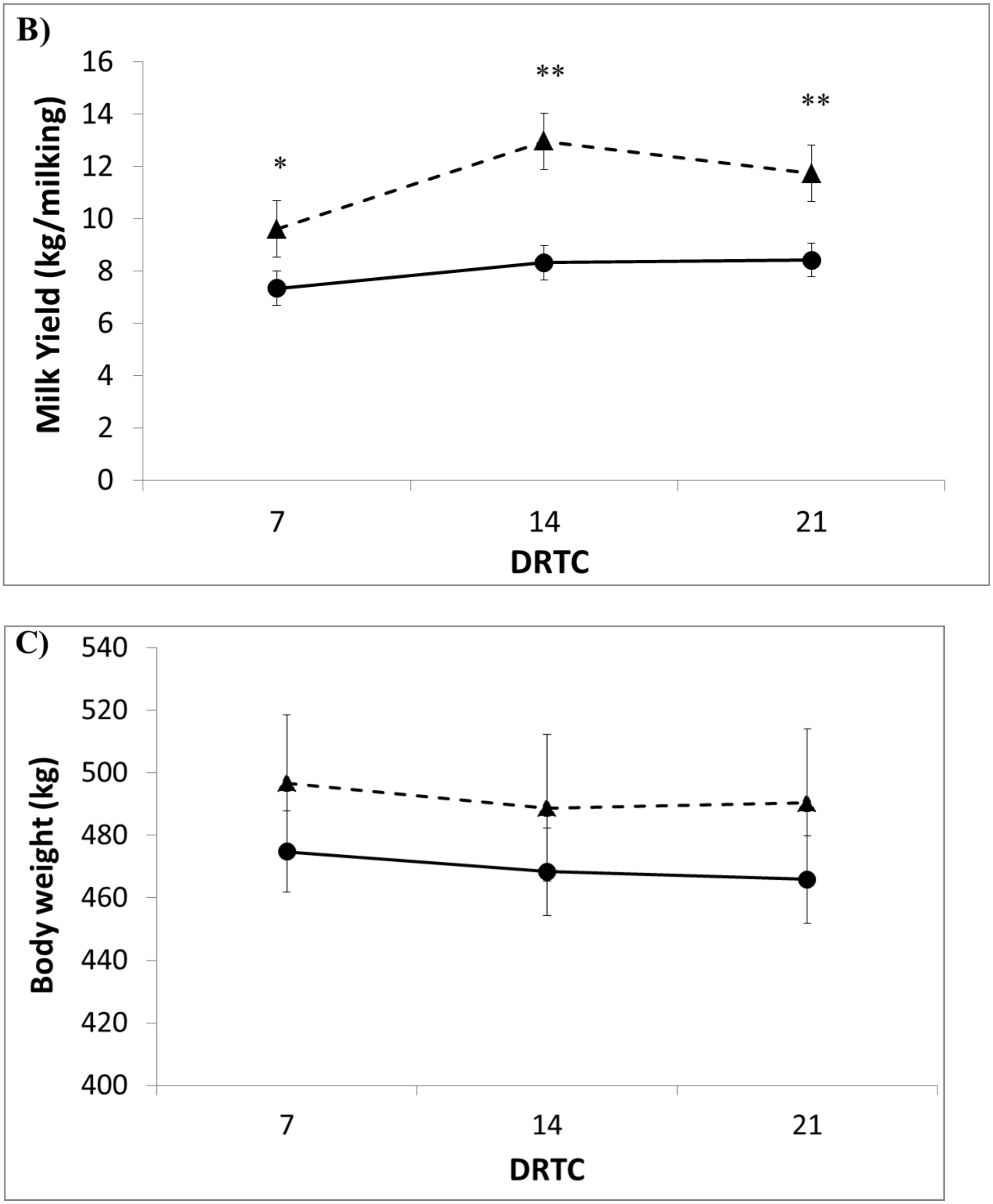


**Figure 3.2.** The association between time and gait score during the entire experimental period (A), and time spent lying (B) Farm 1 (●) and Farm 2 (▲). Results are graphed with asterisks (*) indicating significant differences between DRTC. There were no significant differences found by DRTC or the interaction of farm and DRTC relative to gait score (A and B) or time spent lying (C).

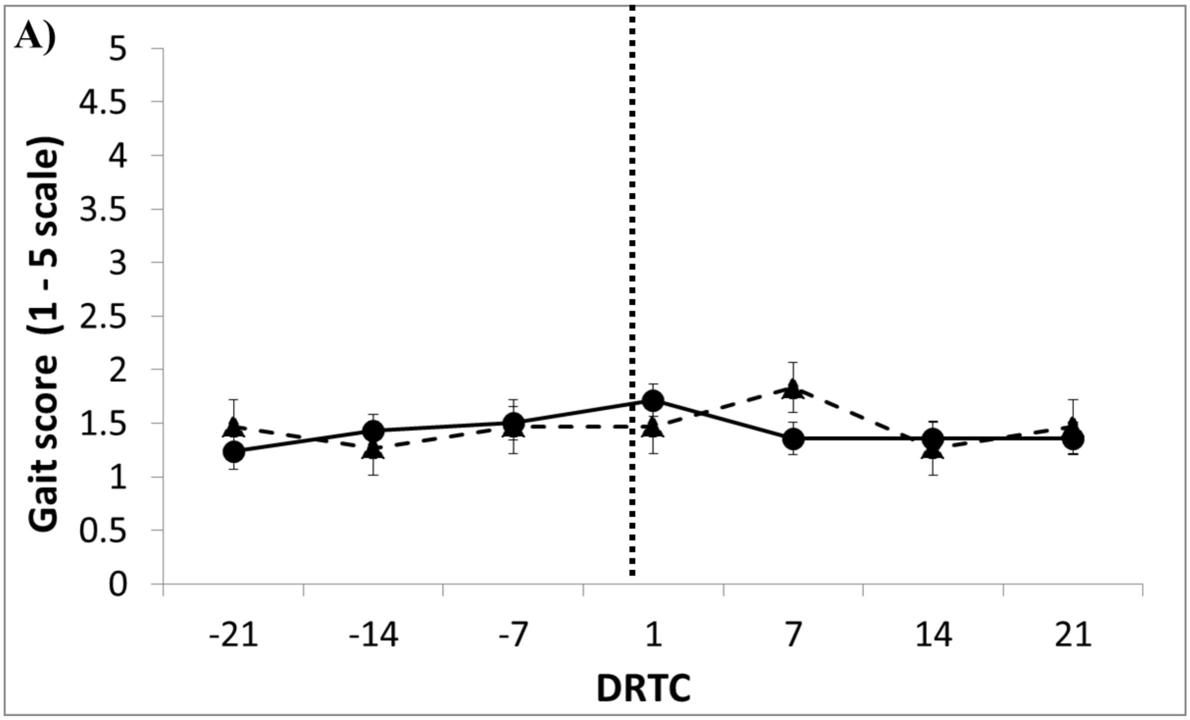


**Figure 3.2 (cont'd)**

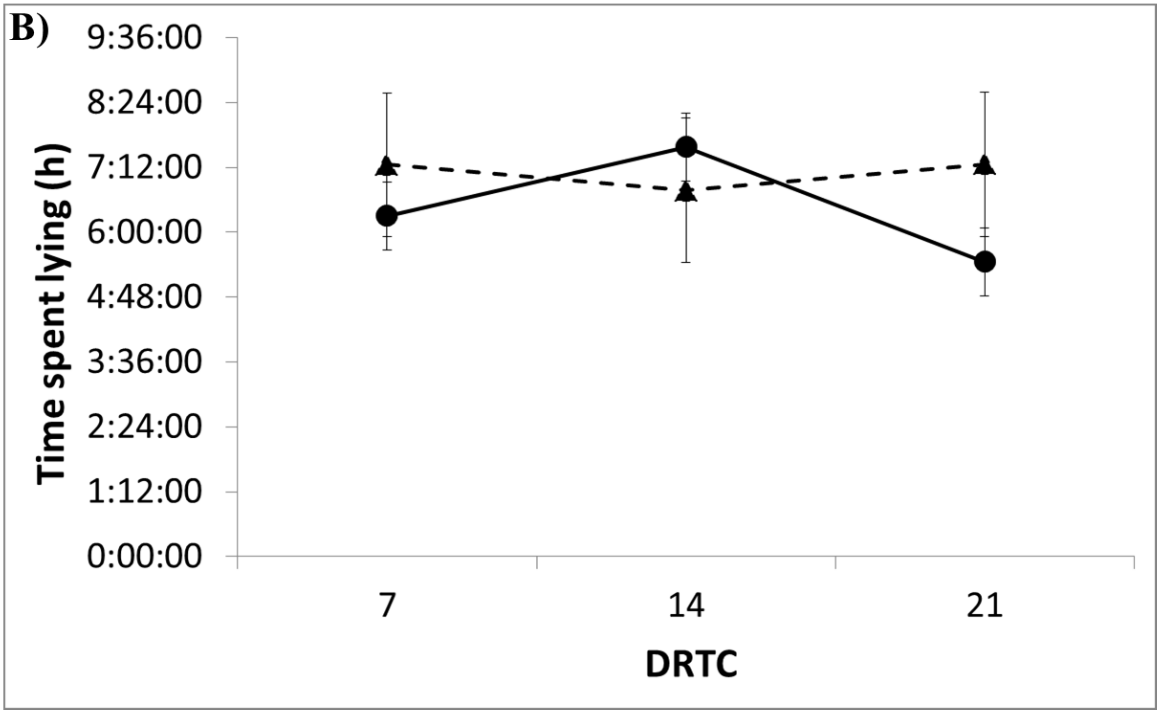


**Figure 3.3.** The association between time (DRTC) and body condition score during the entire experimental period (A), milk yield (B), and body weight (C) for Friesian cows (□) and Holstein cows (◇) at Farm 2. Results are graphed with asterisks (*) indicating differences between DRTC. There was a significant interaction of DRTC and breed during the entire experimental period on BCS (A), milk yield per milking (B), and body weight (C).

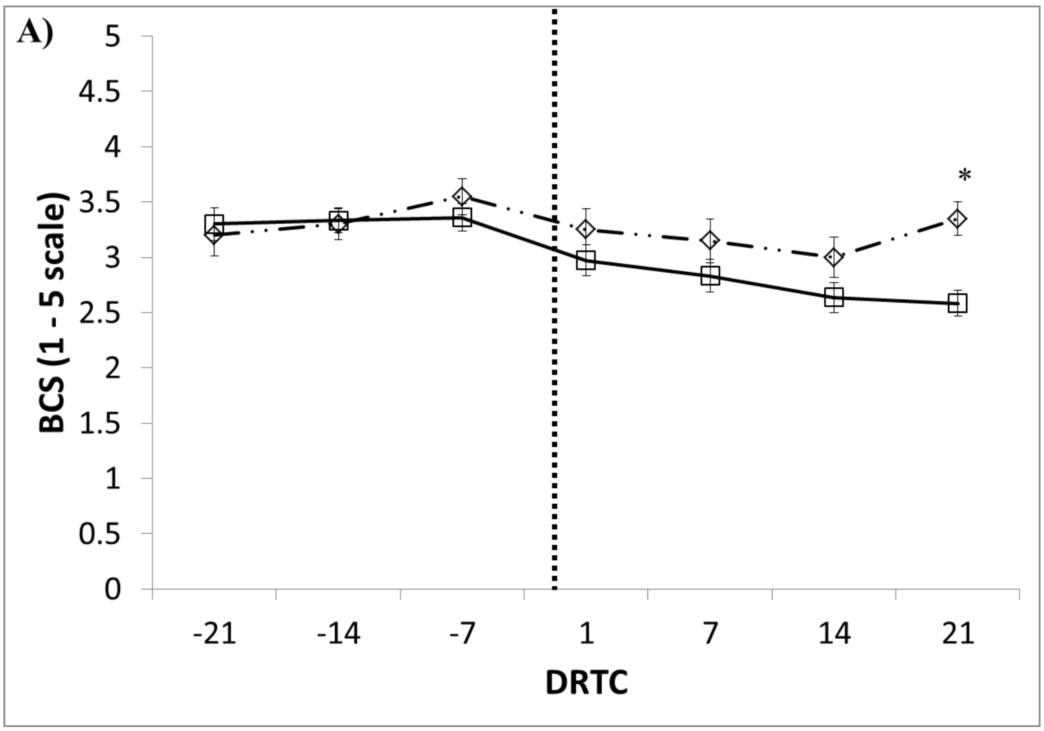
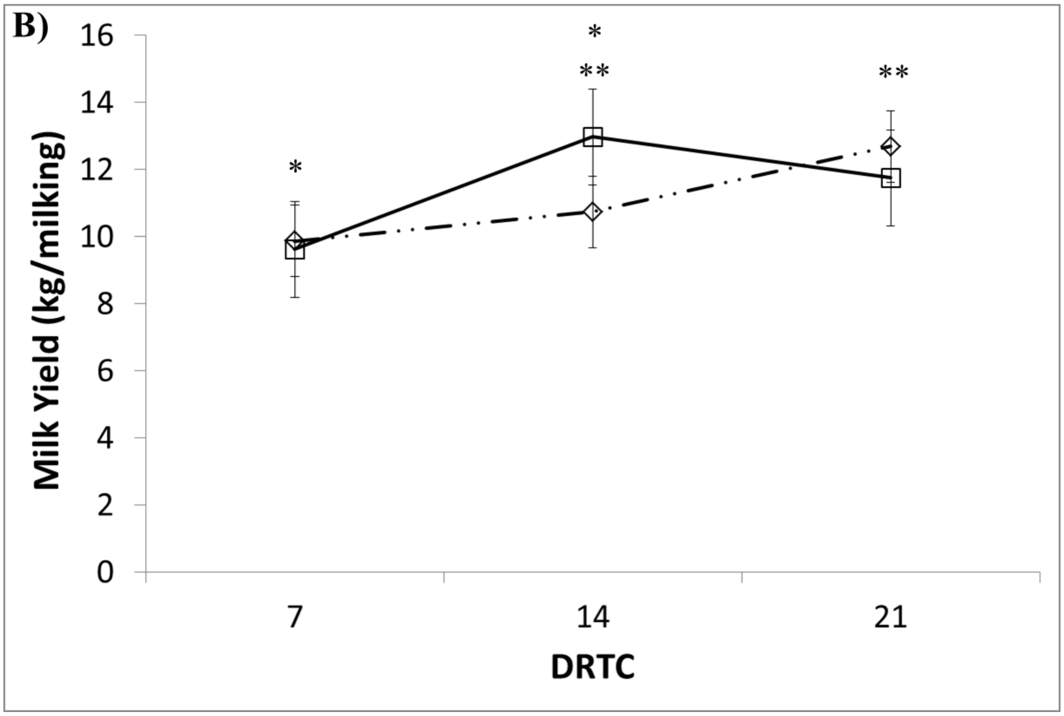


**Figure 3.3 (cont'd)**



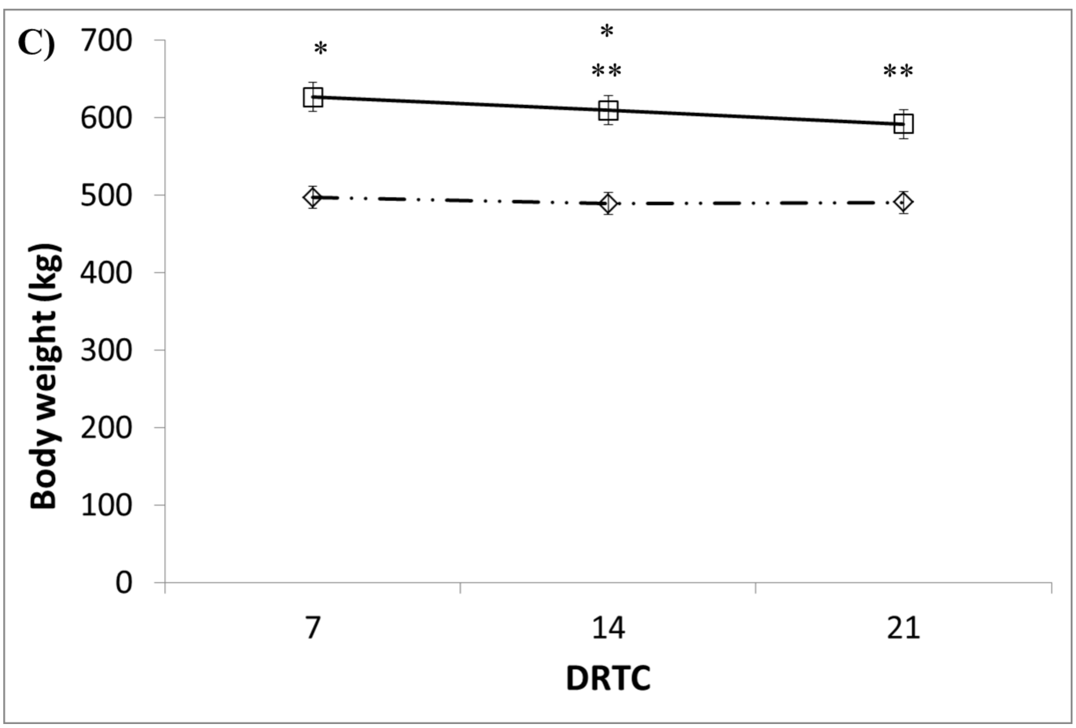



**Figure 3.4.** The association between time (DTRC) and gait score gait score during the entire experimental period (A), and time spent lying during the postpartum period (B) for Friesian cows (□) and Holstein cows (◇) at Farm 2. Results are graphed with asterisks (*) indicating differences between DRTC. Gait score during the entire experimental period was significantly affected by day relative to calving and the interaction of breed and DRTC ($P < 0.05$; A). There were no differences found for breed or breed by day interaction relative time spent lying (B).

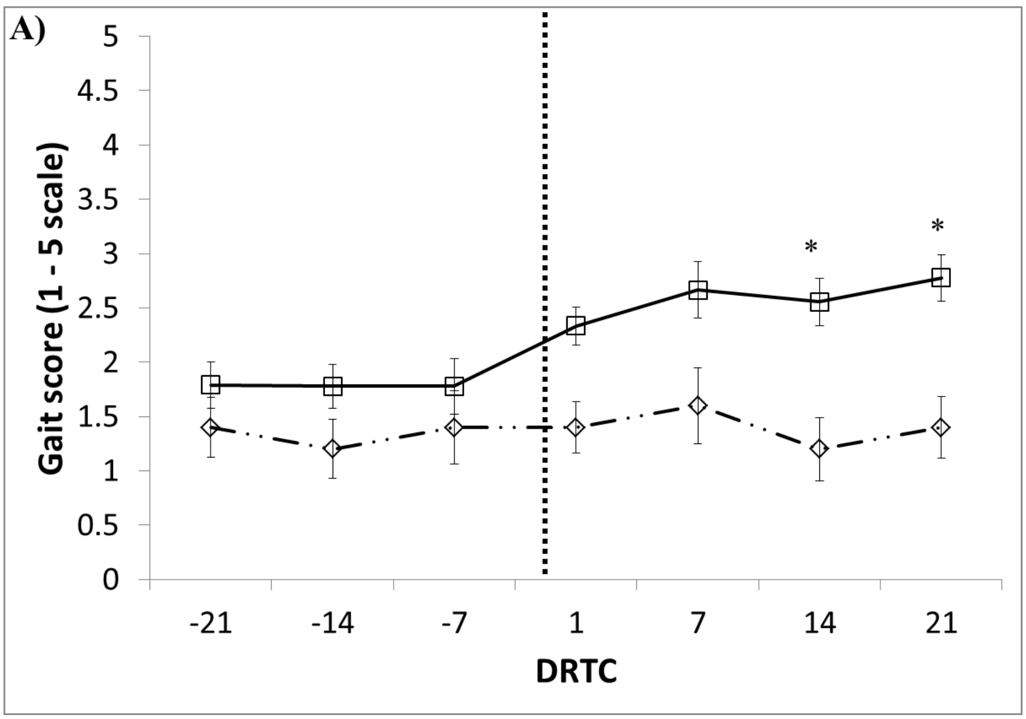


**Figure 3.4 (cont'd)**

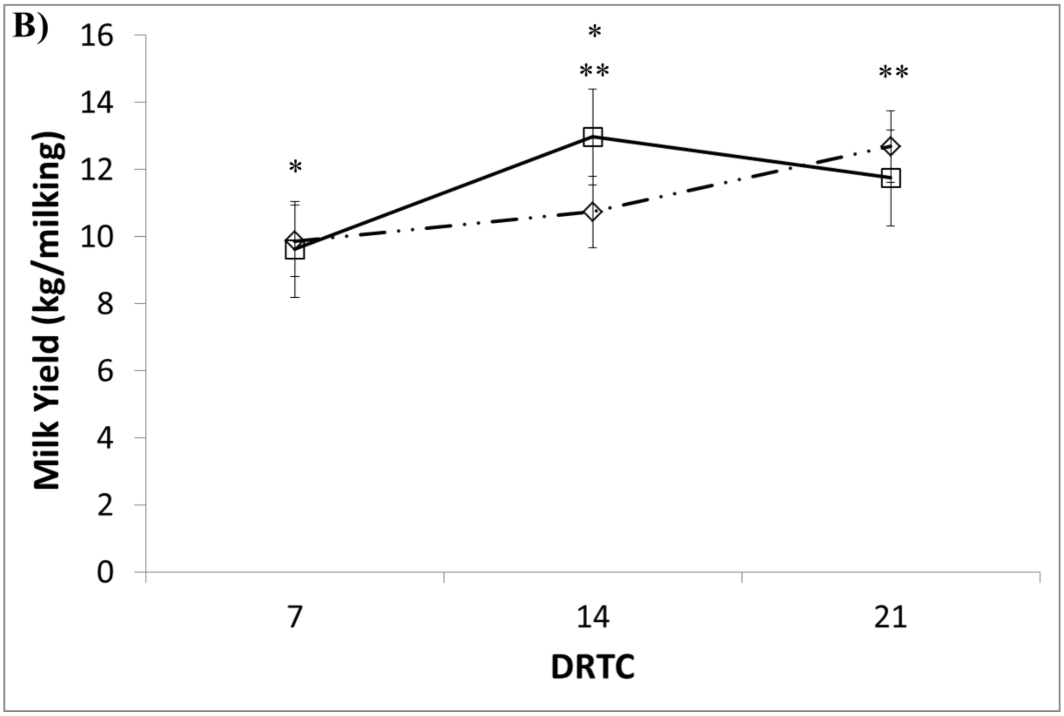
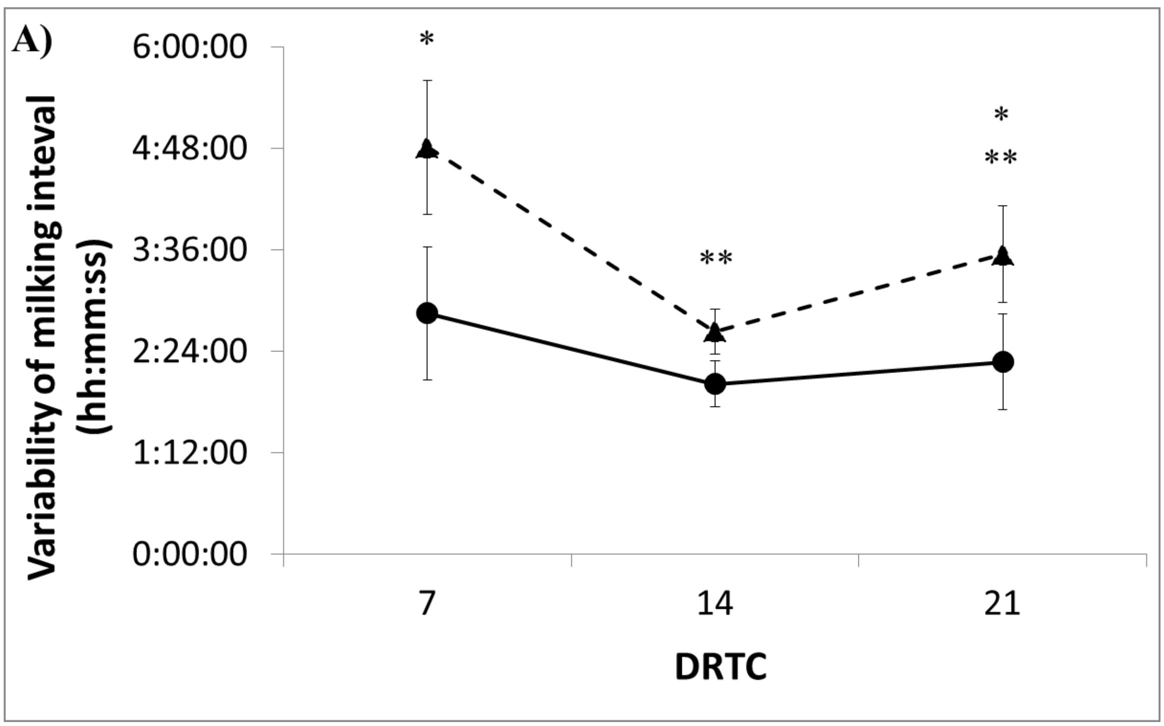


**Figure 3.5.** The association milking interval variability and milking frequency with time between Farm 1 (●) and Farm 2 (▲) and Friesian cows (☐) and Holstein cows (◇) at Farm 2. A significant effect of DRTC was observed for both the variability and frequency of milkings at the farms, with the greatest variability of interval on 7 d compared to 14 d (A; $P < 0.05$), with 21 d not differing from 7 d or 14 d. Milking frequency at Farms 1 and 2 was significantly lower on 1 d compared to all other days (B; $P < 0.05$). There was no interaction of farm and DRTC for Farms 1 and 2. There was no significant effect of DRTC or breed or their interaction at Farm 2 for milking interval variability (C) or milking frequency (D).



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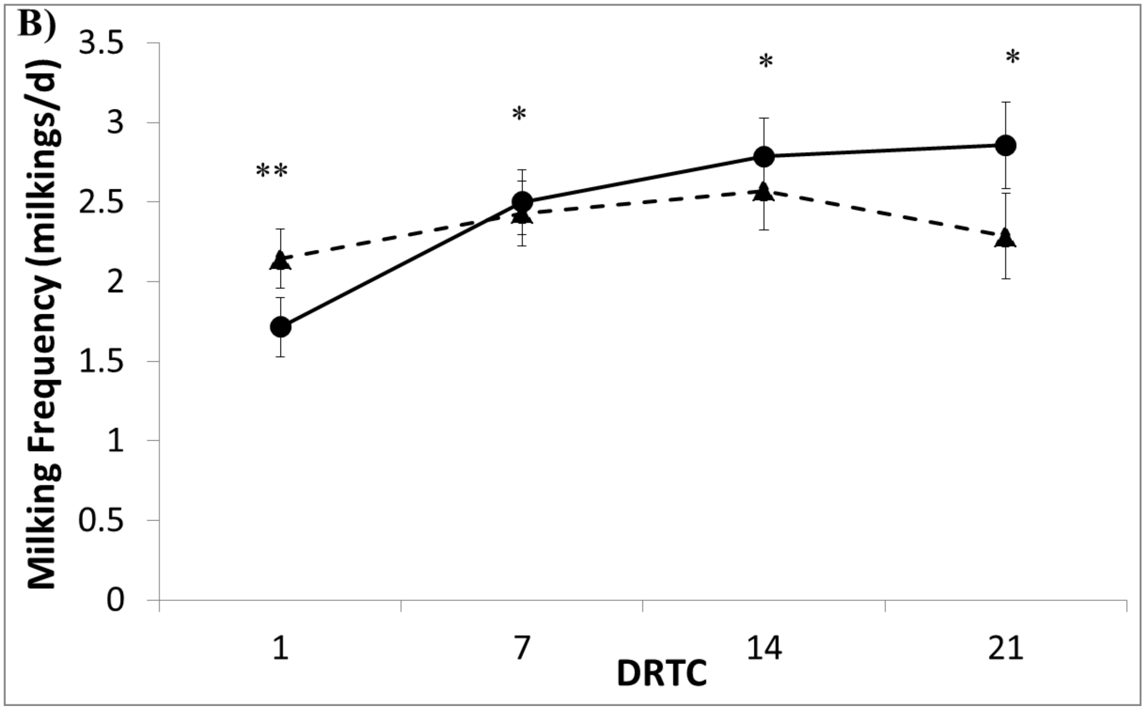


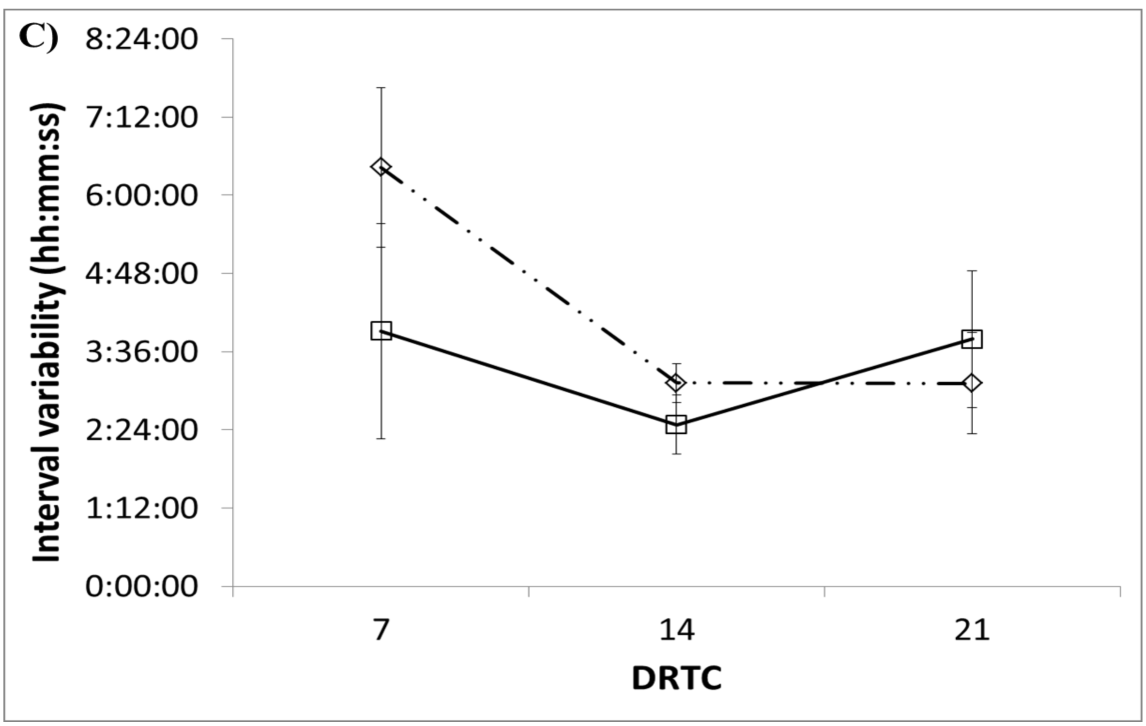

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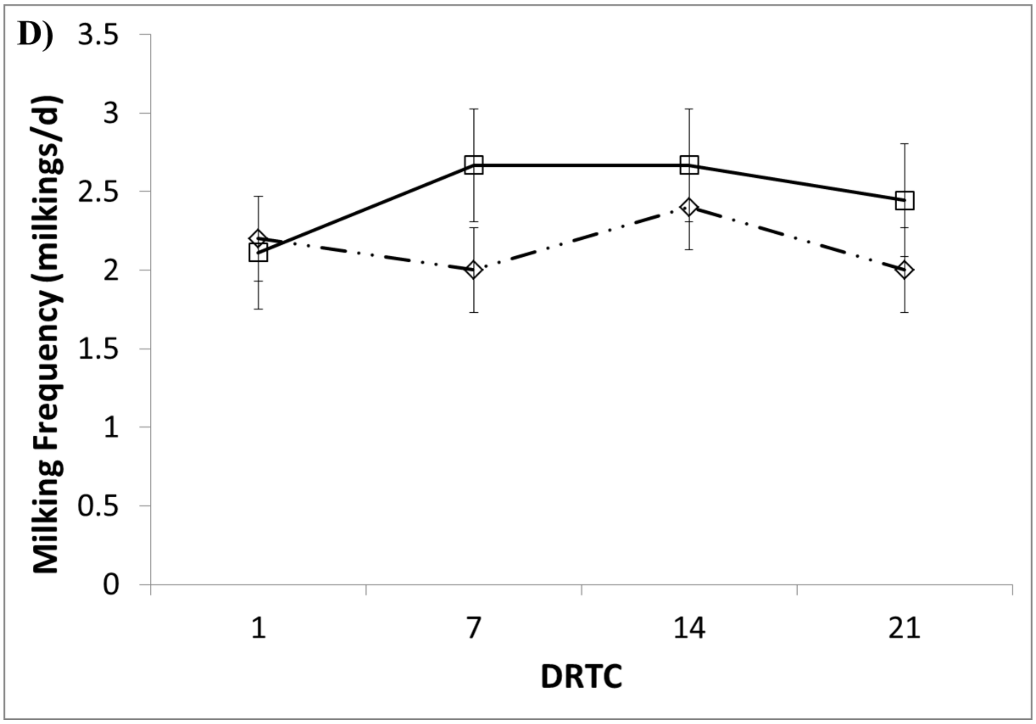

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# CHAPTER 4

## SUMMARY AND CONCLUSIONS

The overall goal of this research was to characterize the metabolic and oxidant status, pro-inflammatory cytokine expression, and behavioral and production responses of periparturient dairy cows at a pastured-AMS dairy. The aim of the first study was to evaluate the metabolic and immune parameters of multiparous and primiparous Friesian dairy cows as they transitioned from late gestation into lactation, a period that is known to be highly stressful for cows. We hypothesized that pastured-AMS cows would not experience high levels of metabolic and oxidant stress and would have lower expressions of pro-inflammatory cytokines because there would be lower nutritional inputs mirrored by lower milk yields. Additionally, we hypothesized that primiparous cows would have better metabolic and oxidant profiles, that is high values of glucose, insulin, GHS and antioxidant potential and lower concentrations of NEFA, BHBA, and relative expression of pro-inflammatory cytokines, than multiparous cows because of lower milk production, despite requirements for growth. As expected, the degree of metabolic and oxidant stress was relatively low, however, as expected, both multiparous and primiparous cows did experience a period of negative energy balance, marked by increased levels of circulating NEFA and BHBA accompanied by decreases in glucose and insulin concentrations. Further, immune status was impaired, as demonstrated by fluctuations in overall antioxidant potential and reduced glutathione, two important first lines of defense of the immune system. The changes in pro-inflammatory cytokine gene expression are consistent with the findings of metabolic and oxidative status: primiparous cows seem to experience the stress of parturition differently than multiparous cows. Contrary to what was expected, milking frequency and milk yield did not significantly impact any of the metabolic or immune parameters, rather day relative to calving and parity were the most influential factors.

Primiparous cows had better overall antioxidant defenses and seemed to be in a less-stressed metabolic state, however, their physiological responses seemed to be more acute than those of multiparous cows. This could be due to the fact that body of the primiparous cow is adapting for the first time to lactation and the challenges experienced during the periparturient period, offering her a different type of stress than a multiparous cow. Multiparous cows, on the other hand, have been through at least one previous lactation cycle, so the transition from pregnancy to lactation is not a new physiological adaption for them, rather, their body must prepare for a larger volume of milk production. The results of this study indicate that periparturient dairy cows still experienced metabolic and oxidative stress during the transition from gestation to lactation in a pasture-based AMS dairy, however the stress does not seem to be as severe as what has been reported elsewhere for some cows in TMR-parlor dairies. Further, primiparous and multiparous cows experienced different types and levels of metabolic and oxidative stress during the periparturient period, likely related to differences in milk yield and previous adaptions (or lack thereof) to lactation. Further research to parse out the precise causes of these observed differences between parities would be beneficial to provide even more accurate advice to producers on how to best manage their herd.

The aim of the second study was to characterize the lying and milking behavior, production, and physiological responses of multiparous cows of two different breeds at two different pasture-based AMS farms during the sensitive periparturient period. We hypothesized that pastured cows milking with an AMS, regardless of farm or breed, would maintain a healthy body condition score over the periparturient period, exhibit low weight loss after parturition, maintain a low gait score during the periparturient period, and have uniform lying times postpartum. Further, differences in dairy management would affect milking behavior and breed would influence all parameters of interest.

As expected, cows in the current study did show decreases in weight and body condition score as they entered lactation and milk yield increased, however, there was not a dramatic shift indicative of an extreme mismatch of nutritional level to volume of milk production. Body condition score remained near a 3.0 on a 5.0 scale, the recommended target for lactating cows and body weight did not exhibit large decreases. Agreeing with the milk frequency and yield data, interval between milkings in the AMS and the variability of time between milkings did not significantly impact either the health or behavior of periparturient cows. This disagrees with some reports in the literature that a highly variable milking interval with an AMS negatively impacts milk yield, however, those studies were conducted with cows in later stages of lactation.

As expected, gait scores also remained low during the periparturient period for cows at both farms and for both breeds, suggesting good foot and leg health, which contributed positively to the overall welfare of the cow. Lying times were lower than those reported for cows in other pasture-based dairies, however, differences in barn design and management strategies may account for this, as well as the ambient temperatures during the experimental period. A meta-analysis incorporating parameters describing variability in facilitates, herd characteristics, AMS model, and feeding management could be helpful to better understand the reasons for low lying time and possible impacts on health and welfare of AMS-milked cows. Technical difficulties with the pedometers that reduced the number of subjects in the data set may also have contributed to these lower than expected values, thus additional research to confirm these findings would be beneficial.

Differences in milk yield and milking interval were noted between farms, highlighting the fact that there are differences in management and facilities impacting dairy cows even when dairies appear to offer a very similar production setting. There were minimal differences between Friesian and Holsteins cows managed at the same farm, suggesting that different breeds are able to adapt to a pasture-based AMS system and maintain good health and welfare.

Overall, the findings of this research indicate that cows in a pasture-based AMS dairy experience metabolic and oxidative stress and shifts in immune function during the periparturient period in directions similar to those seen in cows managed in traditional systems. Further, these stresses impact multiparous and primiparous cows in different ways. This stress, however, does not appear to be severe, relative to some of the findings reported from TMR-parlor dairies. Milking frequency, milk production, and milking interval were not good predictors of the stress experienced by the cow during the transition from gestation to lactation in the current study, however parity and days since calving appeared to be important factors to consider. A well-managed pasture-based AMS dairy appear to be viable operations that do not place additional stress on the cows that has the potential to negatively impact the health or welfare of periparturient cows, yet variations from farm to farm, as were noted in this study, indicate that there is still room to improve periparturient cow welfare.