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PRECISION DAIRY FARMING TECHNOLOGY SOLUTIONS FOR DETECTING DAIRY COW DISEASE TO IMPROVE DAIRY COW WELL-BEING

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PRECISION DAIRY FARMING TECHNOLOGY SOLUTIONS FOR DETECTING DAIRY COW DISEASE TO IMPROVE DAIRY COW WELL-BEING

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food, and Environment
at the University of Kentucky

By
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Lexington, Kentucky

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ABSTRACT OF DISSERTATION

PRECISION DAIRY FARMING TECHNOLOGY SOLUTIONS FOR DETECTING DAIRY COW DISEASE TO IMPROVE DAIRY COW WELL-BEING

Dairy cow health is multifactorial and complex. High producing dairy cows have been described as metabolic athletes, but metabolic and infectious diseases around calving affect many cows. These diseases have drastic negative effects on dairy cow well-being, milk production, and dairy farm economics. Early disease detection could potentially improve disease management, treatment, and future prevention techniques. The first objective of this research was to evaluate the use of activity, lying behavior, reticulorumen temperature, and rumination time determined by precision dairy farming technologies to detect transition cow diseases including hypocalcemia, ketosis, and metritis. The second objective was to evaluate the ability of activity, body weight, feeding behavior, lying behavior, milking order, milk yield and components, reticulorumen temperature, and rumination time determined by precision dairy farming technologies to predict clinical mastitis cases. The last objective of this research was to evaluate the precision dairy farming technologies used in Objective 3 to predict subclinical cases.

KEYWORDS: mastitis, ketosis, hypocalcemia, metritis, precision dairy farming

Amanda Stone
August 8, 2016

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DETECTING DAIRY COW DISEASE TO IMPROVE DAIRY COW WELL-BEING

By

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I. Disease Overview

Dairy cow health is multifactorial and complex. High producing dairy cows have been described as "metabolic athletes," but 30 to 50% of cows are affected by a metabolic or infectious disease around calving (LeBlanc, 2010). Cows are highly susceptible to metabolic and infectious disease during the transition period, or the period from 3 weeks before to 3 weeks after calving (Huzzey et al., 2007, Mulligan and Doherty, 2008). The transition period is marked by a series of adaptations to the demands of lactation. These adaptations are described as homeorhetic, or long term physiological adaptations to changes in state (i.e. the transition from dry to lactating) (DeGaris and Lean, 2009). Transition dairy cows are immunosuppressed and often have to deal with sudden dietary changes that cause metabolic problems. This fragile group of cows is also likely to experience environmental stressors, like routine group changes that are associated with dairy farm management of dry and lactating cows. These effects combined with the stress of parturition lead to a period of great risk for production diseases right after parturition. Dairy cow diseases signify a cow's inability to cope with the metabolic demands of high production. Unfortunately, these diseases cause economic losses to the dairy industry and are an animal welfare concern (Mulligan and Doherty, 2008).

Ketosis, fatty liver, hypocalcaemia, retained placenta, metritis, and displaced abomasums (discussed in more detail below) are linked etiologically. Unfortunately,

this interrelationship regularly results in “cascade effects” that increase the incidence of infectious and production diseases, reduce fertility, reduce milk production, and increase lameness incidence. The complex interaction of transition cow diseases, their relationship with nutrition, and their effects on social behavior and attitude make prevention and control of these diseases difficult (Mulligan and Doherty, 2008).

Metabolic events starting two weeks before calving have effects on reproductive performance months later (LeBlanc, 2010). Therefore, early identification of disease may be especially useful during this time (Huzzey et al., 2007, LeBlanc, 2010).

The probability of death is highest in the first month of lactation for both primiparous and multiparous cows. Cows are under great metabolic stress during this time and may be more vulnerable to disease. Risk factors for death in this period include retained placenta, milk fever, displaced abomasum, and mastitis for multiparous cows. Risk factors for death in the first month of lactation in primiparous cows include mastitis, retained placenta, and displaced abomasum. Milk fever, ketosis, and displaced abomasum increased the risk of culling while, interestingly, retained placenta decreased the risk (Hertl et al., 2011).

a. Mastitis

i. Cause

Mastitis is the inflammation of the mammary gland (Harmon, 1994, Bramley et al., 1996). Mastitis can occur as a result from physical trauma and chemical irritants (Bramley et al., 1996), but most often it occurs when microorganisms enter the teat opening into the udder (Bramley et al., 1996, Janzekovic et al., 2009). Because this route

of inflammation is almost always the cause of mastitis in dairy cows, the term mastitis implies the presence of a microorganism (Bramley et al., 1996). This inflammation is the cow's way of attempting to destroy or neutralize the infectious agents and their toxins in order to heal (Bramley et al., 1996).

Mastitis is a complex disease (Harmon, 1994, Hertl et al., 2011). The three major factors involved in mastitis include the cow as the host, microorganisms as the causative agent, and the environment, which influences the cow and the microorganisms (Bramley et al., 1996). The severity and consequences of mastitis are a result of the pathogenicity of the pathogen involved and the host's response. Therefore, identifying the causative pathogen is helpful in understanding treatment, culling, and other management decisions (Hertl et al., 2011).

Mastitis-causing bacteria can be categorized as major or minor pathogens. Major pathogens commonly isolated from cows with mastitis include *Staphylococcus aureus*, coliforms, and streptococci (Erskine et al., 1987). Infections by these organisms cause only moderate inflammation and SCC increases. Mastitis caused by minor pathogen infections do not commonly cause clinical mastitis or major milk yield decreases (Harmon, 1994). High SCC herds ($\geq$ 700,000 cells/mL) had a higher prevalence of contagious pathogens than low SCC herds ($\leq$ 150,000 cells/mL) (Erskine et al., 1987).

1. Environmental

Environmental mastitis is caused by pathogens that primarily reside in the cow's environment, not in other infected mammary glands. Unfortunately environmental mastitis presents some complex problems for dairy producers (Smith et al., 1985). The

cow's environment influences the type and number of bacteria to which they are exposed, but also their ability to resist mastitis. Management of the cow's environment can reduce pathogen exposure and increase mastitis resistance (Bramley et al., 1996), particularly since bedding often serves as an exposure point to these pathogens (Rowbotham and Ruegg, 2016b). Herds with environmental mastitis problems may be able to control the problem with better sanitation or correcting a poorly functioning milking system (Smith et al., 1985).

However, environmental mastitis is a multifaceted disease with risk factors associated with both the environment and the cow's immune system (Rowbotham and Ruegg, 2016a). One-third of clinical mastitis cases caused by environmental pathogens were severe, accounting for 75% of severe cases. Clinical mastitis cases caused by Gram-positive bacteria were mostly mild to moderate and did not typically cause a severe reaction (Oliveira et al., 2013).

Environmental pathogens include coliforms and environmental streptococci (Smith et al., 1985, Erskine et al., 1987, Bramley et al., 1996). Commonly isolated coliform bacteria include *E. coli*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, and species of Citrobacter, Serratia, and Proteus (Smith et al., 1985).

Environmental pathogens are the predominant cause of clinical mastitis cases on modern dairy farms (Pinzón-Sánchez and Ruegg, 2011, Oliveira et al., 2013, Rowbotham and Ruegg, 2016b). The most prevalent pathogens isolated in a study evaluating treatment outcomes were environmental streptococci (18%), followed by *Escherichia coli* (10%), and *Klebsiella spp*. (8%) (Pinzón-Sánchez and Ruegg, 2011).

Escherichia coli caused 62% of new Gram-negative mastitis cases in one study. Mastitis caused by environmental streptococci were typically caused by *Streptococcus uberis* (44%) and *Streptococcus dysgalactiae* (35%) (Oliver et al., 1993).

The most commonly isolated pathogens in primiparous cows were *E. coli* and *Streptococcus spp*. The same held true for multiparous cows, but Gram-negative infections outweighed Gram-positive infections (Hertl et al., 2011). Diagnosis of mastitis caused by environmental pathogens is difficult because of the short duration (Smith et al., 1985). Gram-negative pathogens release endotoxins, increasing the risk of death in cows with mastitis caused by this pathogen group (Hertl et al., 2011).

Current mastitis control methods are more effective against contagious pathogens than environmental. In well managed herds without contagious pathogen problems, environmental mastitis may still continue to be a problem (Bramley et al., 1996).

2. Contagious

Cows are exposed to contagious mastitis pathogens during milking when teats of healthy cows are exposed to bacteria present in milk from previously milked cows with infected quarters (Rowbotham and Ruegg, 2016b). Contagious mastitis-causing bacteria include *Streptococcus agalactiae*, mycoplasma species, and *Staphylococcus aureus*. Infected udders are the main reservoir for both bacteria, but *Staph. aureus* also colonizes the teat canal and chapped teat skin. *Staphylococcus aureus* has been isolated from heifer quarters before and after calving, creating a source of new infections to the herd. Contagious pathogens survive readily in the udder and usually present themselves as subclinical and chronic infections (Bramley et al., 1996).

Some strains of *Staphylococcus aureus* may produce enterotoxins that cause nausea, vomiting, and abdominal cramps when ingested by humans. However, if milk is cooled properly, pasteurized, and handled correctly during processing, this danger is trivial (Bramley et al., 1996).

All three contagious pathogen groups are transmitted mostly during milking time. Mycoplasma is difficult to treat and is often underdiagnosed because it is difficult to identify in many mastitis diagnostic laboratories. Bulk tank culturing has been used to screen herds to determine mycoplasma presence. If a positive culture occurs, cows with clinical and subclinical mastitis should be individually cultured and removed from the bulk tank. If removal of these cows' milk from the bulk tank create a bulk tank negative sample, then the producer can suspect that the mycoplasma cows were identified correctly. However, frequent bulk tank cultures should continue to occur for mycoplasma to ensure all infected cows have been identified (Fox et al., 2005).

3. Opportunistic

Coagulase-negative-staphylococci (**CNS**) and *Corynebacterium bovis* cause a two- to three-fold increase in SCC. This relatively small increase in SCC may protect the gland from more pathogenic pathogens (Bramley et al., 1996). Udder infection interference seems to be a common phenomenon. Resisting new naturally occurring infections occurs in already-infected quarters. Colonization by *C. bovis* reduced the risk of infection by other bacteria (though the effect was small). Minor pathogens in general were less able to establish an infection when a major pathogen was already colonized in the gland. Coagulase-negative staphylococci (**CNS**) species create resistance to major pathogen infection in the gland (Rainard and Poutrel, 1988).

Common coagulase-negative-staphylococci isolated from udders include: *Staphylococcus chromogenes*, *Staphyloccocus hyicus*, *Staphyloccocus warneri*, *Staphyloccocus epidermidis*, *Staphyloccocus simulans*, *Staphyloccocus xylosus*, and *Staphyloccocus sciuri* (Bramley et al., 1996).

ii. Effects

1. General

During mastitis, milk lactose, fat, and protein content decrease while salt, somatic cells, fatty acids, whey proteins, and bacterial load increase. Mastitis negatively affects product quality (Bramley et al., 1996, Chagunda et al., 2006b, Bansal et al., 2007) and thus may harm the image of the dairy industry to consumers (Hogeveen et al., 2010a). Bramley et al. (1996) explained that the dairy industry must supply milk that is free of antibiotics or adulterants, low in bacteria and SCC, and excellent in quality and flavor to maintain a positive consumer image. Mastitis also compromises animal welfare (Chagunda et al., 2006b, Hogeveen et al., 2010a).

2. Subclinical

Subclinical mastitis constitutes an animal with an udder infection but no visible health changes. Because it cannot be detected by the human eye, cytological and bacteriological and biochemical milk tests are the only way to detect it (Bramley et al., 1996, Janzekovic et al., 2009). Smith et al. (1985) explained that only 40% of intramammary infections caused by contagious pathogens resulted in clinical mastitis while the rest remained in a subclinical state. Subclinical mastitis is the most prevalent form of mastitis most herds experience, but many producers are unaware of the grave

consequences of this disease because there are no outward signs. Subclinical mastitis causes the greatest overall loss to dairy producers because of decreased production resulting from undetected infections (Bramley et al., 1996).

The level of subclinical mastitis in a herd can be monitored through individual cow and bulk tank somatic cell count, particularly in a herd dealing with contagious mastitis (Smith et al., 1985). Somatic cell count (**SCC**) represents the number of white blood cells or leukocytes in milk, although some other cells are included in this count in small numbers. Polymorphonuclear neutrophils (**PMN**) are the predominant leukocyte present in milk from infected quarters. Their purpose is to phagocytize the causative pathogens (Bramley et al., 1996). Subclinical mastitis involves milk yield decrease and increased SCC. Somatic cell count is the most common measurement of milk quality and udder health because it is most affected by intramammary infections (Harmon, 1994, Bramley et al., 1996). Cows with no mastitis have only slight fluctuations in SCC throughout their lactation (Janzekovic et al., 2009) so a drastic change is indicative of mastitis. Polymorphonuclear neutrophils may result in mammary secretory tissue damage. In an in-vitro study, Capuco et al. (1986) treated tissue cultures with intact, lysed, and phagocytising PMNs. All PMN types caused mammary epithelial damage, but phagocytising PMNs caused the greatest damage.

Milk from uninfected quarters will typically have a SCC < 200,000 cells/mL (Bramley et al., 1996). In a study evaluating dairy herds using Dairy Herd Improvement Association monthly SCC testing, 16 herds were considered low SCC herds (≤ 150,000 cells/mL) and 16 were considered high SCC herds (≥ 700,000 cells/mL) based on their

12-month mean SCC. The 365-day rolling herd average was 8,164 and 5,900 kg for the low and high SCC groups, respectively (Erskine et al., 1987).

High SCC milk will have decreased lactose and fat content. Casein, the primary protein used in cheese production, is reduced in high SCC milk. However, total milk protein content changes only slightly during subclinical mastitis because whey proteins (serum albumin, immunoglobulins, transferrin, and lactoferrin) increase when the membranes that normally prevent blood serum proteins from entering the milk are destroyed. This destruction also allows sodium and chloride increase in high SCC milk, but potassium decreases as it passes to lymph between secretory cells. Calcium in milk is mostly tied to casein micelles, thus calcium content is also decreased in high SCC milk (Bramley et al., 1996).

N-acetyl-β-D-glucosaminidase (**NAGase**) is released into milk in response to mammary epithelial injury, which occurs during mastitis. However, PMNs also release NAGase. If PMN are in the process of lysing, 22% of milk NAGase could be attributed to PMNs (Capuco et al., 1986).

Subclinical mastitis is also related to clinical mastitis occurrence (Lam et al., 2009). However, cows able to resist the pathogens may not develop clinical mastitis (Janzekovic et al., 2009).

3. Clinical

Subacute clinical mastitis includes udder or milk abnormalities, or both. Although subacute clinical mastitis can vary in severity, flakes, clots, and watery milk are the most obvious abnormalities. Unlike in subacute clinical mastitis, heat, swelling, and

pain occur in acute mastitis. Acute mastitis is the sudden onset of these signs plus grossly abnormal milk, decreased milk yield. Cows may also experience fever, anorexia, reduced rumen function, rapid pulse, dehydration, weakness, and depression. Peracute mastitis means the onset of mastitis is rapid and the signs are severe. Chronic mastitis has a long duration and may remain in a subclinical state indefinitely or it may alternate between subclinical and clinical states (Bramley et al., 1996). When mastitis becomes toxic and affects the whole animal, death can occur.

Approximately 50% of lactating cows have pathogenic bacteria in an average of two quarters and 1 to 3% of cows will show symptoms of mastitis at any point in time (Janzekovic et al., 2009). Within a 90 day period, 21% of cows had a clinical mastitis recurrence (Oliveira et al., 2013).

Clinical mastitis significantly decreases milk yield for a prolonged duration (Rajala-Schultz et al., 1999b, Gröhn et al., 2004). Daily milk loss during the first two weeks after a clinical mastitis varied from 1.0 to 2.5 kg, but overall loss was between 110 and 552 kg, depending on parity and DIM. The reduction in 305-day milk from clinical mastitis was 1.8 to 7.4%. When clinical mastitis occurs in late lactation, milk yield losses begin two to four weeks before the clinical signs appear, implying that subclinical mastitis occurs for a few weeks before clinical signs appear. Even after a clinical mastitis case is cleared, a cow will likely not be able to reach her pre-mastitis yield for the rest of her lactation (Rajala-Schultz et al., 1999b). Gröhn et al. (2004) cited that milk losses were greatest soon after clinical mastitis detection but started weeks before clinical signs appeared.

Milk yield loss from clinical mastitis varies by causative pathogen. In first lactation cows, *Staph aureus*, *E. coli*, and Klebsiella spp. caused the greatest declines in yield. In cows ≥ 2 parities, Streptococcus spp., *Staph. aureus*, *E. coli*, Klebsiella spp., and *A. pyogenes* caused the greatest milk yield losses (Gröhn et al., 2004).

However, measuring clinical mastitis' effects on milk yield are complex because cows with mastitis tend to have greater milk yields than those who do not get mastitis (Rajala-Schultz et al., 1999b, Gröhn et al., 2004). The pre-mastitis daily milk yield of cows with mastitis was 0.7 to 1.9 kg more than the yield of cows without mastitis. Therefore, interpreting lower milk yield in cows with mastitis simply as a loss caused by clinical mastitis would likely underestimate the actual effect (Rajala-Schultz et al., 1999b).

Odds ratio for pregnancy risk in cows with mastitis ≤ 21 days before AI, ≤ 30 days before AI, 31 to 60 days before AI, and ≥ 61 days before AI were 0.48, 0.81, 0.88, and 0.96, respectively (Loeffler et al., 1999). Hertl et al. (2010) explained that clinical mastitis occurrence around the time of artificial insemination (AI) decreases the probability of conception with the interval from 14 days pre-AI to 35 days post-AI being the most sensitive. Clinical mastitis occurring 15 or more days before or 36 or more days after AI was not associated with the probability of conception. However, clinical mastitis caused by gram-negative pathogens occurring between 8 and 14 days pre-AI was associated with a 32% lower conception probability compared to cows with no clinical mastitis in that same time period. Gram-positive or Gram-negative clinical mastitis occurring from 1 to 7 days pre-AI was associated with a 50% reduction in conception probability. Additionally, clinical mastitis caused by Gram-negative pathogens between

0 and 7 days post-AI was associated with a probability of conception decrease of 80% whereas Gram-positive infections decreased conception probability by 47%. These results imply that clinical mastitis, particularly cases caused by Gram-negative pathogens, interfere with oocyte fertilization or embryonic development. While a decreased probability of conception at first AI is an important finding, mastitis may also may affect future breedings because cows that fail to conceive on their first breeding often have a more difficult time conceiving in subsequent breedings also. Cows with clinical mastitis, severe lameness, or pneumonia during the first month after calving were 5.4 times more at risk of having delayed resumption of ovarian activity after calving (Opsomer et al., 2000).

The probability of mortality in both primiparous and multiparous cows with clinical mastitis was greatest in the first month of lactation. Risk of death in primiparous cows was greater in the month of the clinical mastitis case and tapered off after the mastitis case ended. However, each subsequent case increased the risk of mortality. Cows with their first clinical mastitis case were 3.9 times more likely to die that month than a cow without mastitis, cows with their second clinical mastitis case were 8.2 times more likely to die in the same month than a cow without clinical mastitis. This result implies a cumulative effect of clinical mastitis on cow's ability to survive in the herd. In multiparous cows, clinical mastitis caused by Gram-negative pathogens throughout lactation increased the risk of mortality whereas mastitis caused by other organisms did not affect the risk of mortality (Hertl et al., 2011).

Clinical mastitis increased the risk of culling regardless of how many cases a cow had or at what point they occurred during her lactation. A producer may choose to

replace a cow with several clinical mastitis cases with a healthier first lactation animal (Hertl et al., 2011).

iii. Economic Impact

Maintaining good udder health is important for the entire production chain - from producer to consumer (Hogeveen et al., 2010a). Mastitis is the most costly disease on dairy farms worldwide. Even though exact costs differ between countries and regions, the same economic principles apply. However, producers underestimate its cost and do not perceive it as expensive. Economic damage is spread throughout the year and the most important costs (decreased milk production and increased risk of culling) are not directly visible to the producer (Hogeveen et al., 2010a). Additionally, opportunity costs are perceived at less value compared to out-of-pocket expenses (Thaler, 1981). Loss aversion, where people tend to prefer avoiding losses to acquiring gains, likely applies to producers in relation to deciding milk quality measures to implement (Hogeveen et al., 2010a)

Some differences in udder health from farm to farm can be explained by climate, age of barn, or breed of cow, but much of it depends on producer behavior. Choosing to implement a behavior and the precision with which the behavior is executed are important parts of the puzzle. Not all measures to reduce mastitis losses are cost-effective. However, when farmers are shown a positive net benefit of one or more mastitis prevention methods, the expectation by industry representatives is that the producer will do it. However, that is not always the case. Producers have scarce resources to distribute among suggested improvements and udder health improvements may not always top the list. They also may have different goals, economic behaviors, or

cost evaluations. Producers may avoid minimizing avoidable losses because their goals include more than maximizing profit like job satisfaction or increasing herd size. Lower milk prices may influence motivation to make changes (Hogeveen et al., 2010a).

In 1996, the cost of mastitis was estimated at $185 per cow annually, which totaled $1.8 billion in costs to the United States dairy industry annually. The average production loss per lactation for one infected quarter was considered to be 725 kg, making milk loss the largest economic loss related to mastitis. However, other losses are caused by discarded abnormal milk, milk withheld from cows treated with antibiotics, replacement cow costs, reduced cull cow value, increased labor, and the costs of drugs and veterinary services. Costs associated with antibiotic residues in human foods, milk quality control, dairy manufacturing, nutritional effects in milk, milk degradation, and the interference of genetic progress in the dairy industry are more difficult to account for (Bramley et al., 1996).

Huijps et al. (2008) cited the cost of a case of mastitis for a cow on a farm with an average production of 8,500 kg/cow at € 210. Fifty-five percent of this cost was attributed to subclinical mastitis. However, mastitis prevention costs were not included in this model under the assumption that they would be beneficial for the whole herd. Producers in this same study estimated the losses resulting from mastitis at € 78/cow/year, but also attributed subclinical mastitis to be the largest cost.

However, “disease costing” estimates like that of Bramley et al. (1996) fail to provide information to guide action, then the computation of an aggregate financial sum does not in itself represent useful information. Instead, economic disease analyses need to focus on the relationship between the variables about which decisions have to be made

(output losses and control expenditures) (McInerney et al., 1992). Also, discount rates for each individual may not equal the interest rate and tend to vary with the size and required wait of the reward (Thaler, 1981). Most disease estimates, mastitis in particular, use average economic losses from a clinical case and only look at the herd's recorded clinical mastitis cases, which may not paint the whole picture on each individual farm (Huijps et al., 2008).

Huijps (2009) cited that producers underestimated the economic losses from mastitis when asked about their own farm. Five producers estimated their economic losses closely to the calculated losses from the researchers. However, 33 farmers underestimated the economic losses by > 25%. No one overestimated the economic losses by > 25%. In a similar survey study, only 8% of producers estimated their losses from mastitis correctly, while 20% overestimated and 72% underestimated the losses (Huijps et al., 2008). Some producers value their opportunity cost at 0. Even in research-based models, some factors are difficult to account for, e.g. labor (Huijps et al., 2008).

In a stochastic model, Bewley et al. (2010a) estimated the cost of a case of mastitis to range between $112 and $316 with a mean of $206 and $163 for primiparous and multiparous cows, respectively. The most recent estimate for mastitis was $310 for primiparous cows and $340 for multiparous cows. For both groups, decreased milk production comprised the majority of the losses ($136 and 138 for primiparous and multiparous, respectively) (Liang, 2013).

iv. Prevention and treatment

A healthy udder always produces milk free of pathogens so pathogen identification means it came from a source outside of the udder (Janzekovic et al., 2009). Understanding historical bacteriological culture results can help producers optimize treatment of future mastitis cases. However, 10 to 50% of (Oliver et al., 1993, Lam et al., 2009, Rowbotham and Ruegg, 2016a) quarter milk samples from cows with clinical mastitis yield no growth. No growths can occur because there are too few bacteria present, pathogens are present but require special media to grow (e.g. Mycoplasma spp.) (Lam et al., 2009), or because of latent infections or shedding cycles (Sears et al., 1990).

Solely basing mastitis control on treatment of clinical mastitis is ineffective (Neave et al., 1966), but effective treatment and efficient prevention measures could be sustainable (Chagunda et al., 2006b). Eliminating existing infections and preventing or greatly reducing the rate of new infections are the two main factors that should be accounted for in a mastitis control program (Neave et al., 1966, Janzekovic et al., 2009). Good control measures include reducing the animal's susceptibility of infection and reducing her exposure to pathogens. Cows should be housed in a clean and comfortable environment and should be milked in a parlor with well-functioning and maintained equipment (Neave et al., 1966). Even an effective mastitis control program may still allow 15 to 20% of the herd to be infected, though (Janzekovic et al., 2009) because complete mastitis eradication is currently not feasible.

Most new mastitis cases occur during the first month of lactation when cows are more susceptible to infection (Janzekovic et al., 2009), particularly those caused by environmental pathogens (Bramley et al., 1996, Dosogne et al., 2002). The susceptibility of individual cows to severe coliform mastitis has been associated with the impairment of

PMN function (Dosogne et al., 2002). Susceptibility is greatest during the two weeks after dry off and during the two weeks before calving. After drying off, milk removal is terminated, udder pressure increases, teat dipping is discontinued, and phagocyte function is impaired. As calving nears, colostrum forms and can leak, non-specific immune factors in mammary secretions are reduced, physiological stress occurs, and accumulation of colostral components that interfere with leukocyte function occurs (Bramley et al., 1996).

Treating all quarters of all cows at dry off is one method to reduce established infections from lactation and prevent new dry period infections. Penicillin-streptomycin mixtures and cloxacillin in slow release bases eliminated greater than 90% of staphylococcal infections present at dry off (Neave et al., 1966). However, *Staph. aureus* is notoriously resistant to penicillin (Erskine et al., 1987).

Coliform mastitis vaccines are commercially available to producers and use gram-negative core antigens to produce non-specific immunity against endotoxic mastitis (Ruegg, 2005). Using a J5 vaccine can protect against severe coliform mastitis, likely through inducing a hyper-responsiveness in the mammary gland that is mediated by local memory cells (Dosogne et al., 2002). Researchers have presented successful results in both challenge (Hogan et al., 1999, Wilson et al., 2007) and naturally occurring mastitis studies (González et al., 1989). Wilson et al. (2007) cited that cows vaccinated with a J5 bacterin before an *E. coli* intramammary challenge cleared the *E. coli* from their milk "almost immediately," while control cows shed *E. coli* in milk for 24 hours. Milk from vaccinated cows was approximately 10% of the SCC in controls following challenge. At 21, 36, 48, 60, 72, 84, 96, 108, and 132 hours post-challenge, SCC in challenged control

quarter milk was significantly greater than that of vaccinates. Although milk production losses between the groups were only significantly different for one day post-challenge (-7.7 kg versus + 0.5 kg in controls and vaccinates, respectively), vaccinates lost about 3 kg/day less milk than controls.

Right before an intramammary *E. coli* challenge, J5-specific serum IgG1 ($P < 0.01$) and IgG2 ($P = 0.07$) responses were greater in cows that received subcutaneous J5 bacterin vaccination compared to controls. Twelve hours post-challenge, J5-specific serum IgM response in controls was greater than that in vaccinates ($P = 0.07$), but serum IgG1 and IgG2 were not statistically different among treatment groups during this time (Wilson et al., 2007). Intramammary immunization with a J5 vaccinate enhanced immunoglobulin G and M titers in serum and whey on the first day of lactation compared with cows that only received subcutaneous immunizations. Immunoglobulin G titers in serum were also greater at 30 days dry and at 14 and 21 DIM for cows that received intramammary immunization than for cows that were vaccinated by subcutaneous injections only (Hogan et al., 1997). In an *E. coli* 727 intramammary challenge study, Hogan et al. (1999) also found elevated serum immunoglobulin G titers against whole-cell *E. coli* J5 antigen at calving in heifers vaccinated with an *E. coli* J5 bacterin compared to those who were not. Clinical mastitis severity and duration were reduced in heifers vaccinated with an *E. coli* J5 bacterin compared with placebo-injected heifers. Bacteria counts were also less in milk from challenged quarters from vaccinated heifers than in control heifers at 12, 15, and 48 hours post-challenge. Researchers used a prospective cohort study to establish that cows vaccinated with the *E. coli* J5 vaccine

were five times less likely to suffer from clinical coliform mastitis than unvaccinated cows during the first 90 DIM.

Reducing teat end exposure to mastitis-causing pathogens, through good bedding management and milking hygiene, can reduce mastitis incidence (Bey et al., 1999). Rates of environmental mastitis increase during periods of hot and humid weather, which can be associated with increased bedding bacteria numbers and possible increased susceptibility in heat-stressed cows (Bramley et al., 1996).

Fortunately, *Strep ag* can now be eradicated from herds with mastitis management and *Staph. aureus* can be eradicated or reduced to low levels. Contaminated milking machines, udder clothes, and milkers' hands are common routes of contagious pathogen transmission (Bramley et al., 1996).

Although parlor hygiene will not cure existing mastitis infections, it can prevent the spread of contagious pathogens from cow to cow. Sufficient parlor hygiene includes wearing rubber gloves rinsed in disinfectant between cows, examining foremilk with a strip cup, and post-dipping with an effective disinfectant that is gentle on teat skin (Neave et al., 1966). Low SCC herds ($\leq$ 150,000 cells/mL) were more likely to use post-dip and dry cow treat all quarters of all cows than high SCC herds ($\geq$ 700,000 cells/mL) (Erskine et al., 1987).

Culling cows with chronic mastitis is recommended (Neave et al., 1966). However, van Asseldonk et al. (2010) discovered that producers actually viewed culling cows with consistently high SCC as a last resort, but agreed that it was an effective way to avoid SCC penalties.

Cow cleanliness has been associated with bulk tank SCC (Bey et al., 1999, Schreiner and Ruegg, 2003, Ellis et al., 2007), implying that cow hygiene is more than a cosmetic issue on dairy farms and is actually related to mastitis (Ellis et al., 2007). Logically, cleaner cows should have lower SCC, but "clean" is a subjective term (Reneau et al., 2005). Reneau et al. (2005) worked to develop and evaluate a simple scoring system for dairy cow hygiene and evaluate if the scores were associated with somatic cell score (**SCS**). These researchers cited that udder-hind limb hygiene was positively and significantly associated with SCS, but tail head, lateral aspect of the thigh, and ventral aspect of the abdomen were not. For each standard deviation increase in herd mean udder, hind limb, or udder-hind limb composite score, mean herd SCS increased by 0.13, 0.17, and 0.17, respectively. Each one-unit increase in udder-hind limb composite hygiene score was associated with a 40,000 to 50,000 cells/mL bulk tank SCC increase. Similarly, Schreiner and Ruegg (2003) found that dirtier udders and hind limbs were positively associated with SCS, but udder hygiene was more strongly associated with SCS. A positive relationship between herd bulk tank SCC and cow cleanliness score was discovered in both organic and conventional herds (Ellis et al., 2007).

Hygiene scores increased with increasing parity, likely because udders in older cows are closer to the ground providing more of an opportunity to contact manure. In order to prevent this, cows should not be rushed when being moved and alleys should be kept clean. Hygiene scores improved as DIM increased (Reneau et al., 2005).

In a study evaluating hygiene differences between seasons and between organic and conventional farming systems, increasing cow hygiene was more strongly associated with bulk tank SCC in organic herds than conventional. Cows became dirtier when going

from summer grazing to winter housing, likely because they have greater space and lying restrictions. High- and mid-production cows were less likely to be clean than low-yielding or all lactation groups. Cows housed on bedded packs with straw were more likely to be dirty than those housed in freestalls. However, cows in organic herds were more likely to be clean when housed in bedded packs with straw than conventional cows. Dry cows were cleaner than lactating cows in August and October, but this result was lessened when cows were not on pasture (Ellis et al., 2007).

Dairy professionals commonly use the "knee test" to evaluate the comfort of a lying surface, but it can also be used to evaluate cleanliness. Upon standing, if there is manure or wetness on the evaluator's knees, the bed base is too wet so more bedding is needed. The ideal bedding is cheap, dry, comfortable, clean, does not support bacterial growth, is compatible with the existing manure handling system, and never has to be changed. Although meeting all these criteria is not feasible, every producer's goal must be to meet as many as possible at all times. Bacteria need moisture, organic nutrients, and appropriate temperature to survive and grow. One major disadvantage of organic bedding material is that it can support bacterial growth well because they contain more nutrients. In addition, fine bedding particle size supports faster bacterial growth and sticks to udders and teats more readily. Bedding that looks and feels clean may still have high bacterial counts. During the summer, the ambient temperature is warm enough to allow bacterial growth even before manure contamination. Bedding with greater bacterial counts increases the risk of mastitis (Bey et al., 1999).

Sand is the ideal bedding from a bacteriologic standpoint because bacteria numbers are lower than in organic bedding and sand can tolerate greater bacterial

numbers but not increase mastitis. However, sand needs to be well managed by ensuring it is deep and smoothed over for cow comfort. Sand can be washed and re-used (Bey et al., 1999).

Teat disinfection is one of the most important preventive measures in mastitis control, but has no control on mastitis cases that already exist. Teat dip should cover the whole teat, not just the teat end. Dipping uses less dip than spraying and has a better chance of covering the entire teat when applied. Teat dip cups should be cleaned regularly to prevent contamination. Chemical compounds used in teat dips include iodophors, quaternary ammonium compounds, chlorhexidine, hypochlorite, and dodecyl benzene sulphonic acid (Blowey and Edmondson, 1996).

When a cow with mastitis is milked, mastitis-causing bacteria remain on the liners and can be transmitted to cows (Blowey and Edmondson, 1996). Post-dipping is recommended by dairy advisors, is simple to perform, and is economical (Oliver et al., 1993). Post-dipping controls contagious mastitis (Oliver et al., 1993, Blowey and Edmondson, 1996) while pre-dipping controls environmental mastitis. Pre-dip needs to remain on the teats for at least 30 seconds. Post-dip removes bacteria transmitted during the milking practice and should be applied as soon as possible after cluster removal (Blowey and Edmondson, 1996). Oliver et al. (1993) cited 49% less new mastitis cases caused by major pathogens in cows that were pre- and post-dipped versus those who were only post-dipped ($P < 0.01$).

Rough or chapped teat skin can be a reservoir for mastitis-causing pathogens. Emollients like lanolin and glycerin are added to disinfectants to protect and heal cracked teats (Blowey and Edmondson, 1996).

b. Metritis

i. Cause

Metritis is a severe inflammatory reaction involving all layers of the uterus including the endometrial mucosa and submucosa, muscularis, and serosa (BonDurant, 1999). Metritis usually occurs in the first week postpartum and is often associated with retained placenta (Sheldon, 2004) and Caesarian sections (Hussain et al., 1990). Clinical signs of metritis include pyrexia, fetid pus within the uterine lumen, vagina, or discharging from the vulva (Sheldon and Dobson, 2004), and delayed uterine involution (Sheldon, 2004, Sheldon and Dobson, 2004).

Uterine bacterial infections disrupt the function of the uterus, ovaries, and higher control centers in the hypothalamus and pituitary glands. These infections also compromise animal welfare and can cause sub-fertility or even infertility (Sheldon and Dobson, 2004). Mahnani et al. (2015) explained that a case of metritis increased days open and number of inseminations per conception by 16.4 and 0.1 per cow per lactation, respectively. Metritis cost a mean of $162.3 per case in that same study.

LeBlanc et al. (2002) explained that cows in their third or greater lactation were at greater risk of clinical endometritis than cows in their second or first lactation (prevalence of 21, 13, and 12%, respectively, $P < 0.001$). Twins, retained placenta, and metritis were all associated with increased risk of endometritis (odds ratios of 8.6, 4.9, and 4.6, respectively; $P < 0.001$).

The incidence of clinical metritis with the definition of a fetid, reddish-brown vaginal discharge and a rectal temperature $\geq 39.5°C$ was 18.5% (Drillich et al., 2001),

similar to the 18% cited by Bartlett et al. (1986). Etherington et al. (1984) cited a clinical metritis incidence rate of 25.9% and Markusfeld (1987) cited an even greater incidence at 36%.

Although metritis and endometritis are often named interchangeably, each has a clear definition (Sheldon, 2004). Endometritis is a superficial inflammation of the endometrium only, extending only to the stratum spongiosum (BonDurant, 1999). The clinical sign of endometritis is mucopurulent vulvar discharge 21 DIM or later (Sheldon and Noakes, 1998). LeBlanc et al. (2002) defined clinical endometritis as the presence of purulent or foul discharge, or cervical diameter greater than 7.5 cm between 20 and 33 DIM, or mucopurulent discharge after 26 DIM, when cows are examined between 20 and 33 DIM and vaginoscopy is performed. The prevalence of endometritis was 16.9% using this definition. However, when vaginoscopy was not performed and diagnosis was based on history, inspection and palpation, presence of mucopurulent or purulent discharge on the perineum, cervical diameter > 7.5 cm, and presence of a uterine horn ≥ 8 cm in diameter, endometritis prevalence was 14.6%.

The uterus is sterile throughout pregnancy because the vulva, vestibule, vagina, and cervix act as physical barriers to bacteria ascending the genital tract (BonDurant, 1999, Sheldon, 2004, Sheldon and Dobson, 2004). When the vulva is relaxed and the cervix is dilated during and just after parturition, bacteria from the animal's environment can contaminate the uterine lumen. The amount of bacterial contamination a cow has post-partum is dependent on bacterial numbers and the animal's defense mechanisms (Sheldon, 2004). Neutrophils are the primary phagocytic barrier in response to bacterial invasion, and the inflammatory barriers include the non-specific defense molecules like

lactoferrin and acute phase proteins. Neutrophils are the earliest and most important phagocytic cell recruited from the peripheral circulation to the uterine lumen, killing internalized bacteria and contributing to the formation of pus when the phagocytes die (Sheldon and Dobson, 2004).

Pathogens most often associated with clinical disease are *Arcanobacterium pyogenes*, *Escherichia coli*, Fusobacterium necrophorum and *Prevotella melaninogenicus*. *Arcanobacterium pyogenes* produces a growth factor for *F. necrophorum*, *F. necrophorum* produces a leukotoxin, and *P. melaninogenicus* produces a substance that inhibits phagocytosis (Sheldon, 2004, Sheldon and Dobson, 2004).

ii. Effects

1. Clinical

The most useful signs of uterine infection are the presence of fetid pus within or discharging from the vulva and delayed uterine involution. The routine method for examining the contents of the vagina is to withdraw the discharge for inspection manually. This technique is fast, cheap, and allows for volume quantification and odor detection. The secretion can be scored, where 0 represents clear or translucent mucus; 1 represents clear mucus containing flecks of white pus; 2 represents < 50 mL exudate containing ≤ 50% white or cream pus; and 3 represents > 50 mL exudate containing ≥ 50% white, cream, or bloody pus. The vaginal mucus odor can be scored 0 for no odor and 3 if a putrid odor is present. The character and odor scores can then be summed to give a score ranging from 0 to 6 (Sheldon, 2004)

Cows with clinical endometritis had a reduced overall relative pregnancy rate of 27% and a decrease in 21-d pregnancy rate from 20 to 14.6%. The median time to pregnancy was 32 days longer in cows with clinical endometritis than in cows without it. Cows with clinical endometritis were also 1.7 times more likely to be culled for reproductive failure than cows without clinical endometritis (LeBlanc et al., 2002).

Odds ratios for pregnancy risk in cows with metritis 2 to 21 days after AI, 0 to 1 day after AI, ≤ 30 days before AI, and ≥ 31 days before AI were 0.17, 0.93, 0.52, and 0.82, respectively (Loeffler et al., 1999).

Metritis was protective against culling while displaced abomasums and clinical mastitis increased the risks of culling (Hertl et al., 2011).

2. Subclinical

Monitoring rectal temperatures post-partum alone to diagnose metritis is less reliable than including an examination for abnormal uterine discharge because pyrexia is not consistently associated with pathogenic bacteria in the uterine lumen. Rectal temperature is often greater than the accepted normal range during the first 10 days after parturition (Sheldon, 2004). Fever occurs in response to detection of pro-inflammatory cytokines by receptors in the brain, which stimulate a coordinated neural response in the hypothalamus and brainstem to reset the thermostatic body temperature set point (Saper and Breder, 1994). Although fever indicates inflammation, additional clinical signs are necessary to identify a uterine bacterial infection (Sheldon et al., 2004). In a study evaluating the rectal temperature and uterine health of 90 dairy cows during the first 10 DIM, the greatest mean daily temperature was two days post-calving. The authors contributed this

spike in temperature to the tissue damage associated with parturition because a uterine bacterial infection was not likely to have become established that quickly (Sheldon et al., 2004).

In 90 cows with rectal temperatures monitored daily, 26% had temperatures greater than 39.4°C, which the authors considered a fever, within the first 10 days postpartum. The mean or maximum rectal temperature during the first 10 days postpartum was not a good indicator of the number of bacteria in the uterus, or the presence of recognized pathogens, although fever was more common in cows that had Prevotella spp. isolated from their uterus (Sheldon et al., 2004).

iii. Prevention and treatment

Unfortunately, even well-managed farms have enough bacteria for uterine contamination, and factors other than the environment determine whether or not a cow will get a uterine infection (Sheldon, 2004). Using historical data of risk factors for uterine disease to select cows for further examination at routine fertility visits may be beneficial so that treatment can be targeted and unnecessary use of antibiotics can be avoided (Sheldon, 2004).

Systemic antibiotic treatment of toxic puerperal metritis is important (Smith et al., 1998). Ideally, therapy for uterine infections should: 1) eliminate bacteria from the uterus, 2) not inhibit the normal uterine defense mechanism, and 3) not cause further adulteration of milk or meat for human consumption. Most intra-uterine treatments available fail to meet one or more of these criteria, therefore systemic antibiotic treatment may be more effective. Negative interactions between antibiotics and the uterine

environment, the inhibition of the uterine defense mechanism by irritating drugs, and a questionable efficacy of antibiotics within the inflamed uterine wall are why intrauterine antibiotic treatment is ineffective (Paisley et al., 1986). Tetracycline (intrauterine infusion of 1500 mg oxytetracycline hydrochloride solution), prostaglandin (intramuscular injection of 500 µg of cloprostenol), and estrogen (intramuscular injection of 3 mg estradiol benzoate per 500 kg estimated bodyweight) treatments had clinical success rates of 73, 68 and 62%, respectively, after the first treatment (Sheldon and Noakes, 1998).

iv. Economic impact

In a stochastic model, Bewley et al. (2010a) estimated the cost of a case of metritis to range between $169 and $441 with a mean of $210 and $295 for primiparous and multiparous cows, respectively. The most recent estimates for a case of metritis were $176 for primiparous cows and $186 for multiparous cows. For both groups, veterinary and treatment costs comprised the majority of the losses at $89 per case (Liang, 2013).

c. Hypocalcemia

i. Cause

Hypocalcemia, or milk fever, is a metabolic disorder in which homeostatic mechanisms fail to maintain normal blood calcium (**Ca**) concentrations at the onset of lactation (Goff and Horst, 1997). Blood Ca in the adult cow is maintained between 2.0 and 2.5 mmol/L (8.5 and 10 mg/dL) (Jorgensen, 1974, Goff, 2008). Typically, the lowest blood Ca concentration occurs between 12 and 24 h after calving (Goff, 2008).

Clinical milk fever incidence in the United States is 4% (McLaren et al., 2006) to 6% and increases with increasing parity (Rajala-Schultz et al., 1999a). However, nearly 25% of heifers and 50% of older cows will have blood Ca concentration < 2 mmol/L (Goff, 2008). Subclinical hypocalcemia (< 2.0 mmol/L serum within 48 h post-partum) occurred in 25%, 41%, 49%, 51%, 54%, and 42% of first, second, third, fourth, fifth, and sixth parity cows, respectively in a study conducted in Iowa (Reinhardt et al., 2011). Hypocalcemia is associated with older cattle (Jorgensen, 1974, DeGaris and Lean, 2009) that absorb less dietary Ca and may have less exchangeable bone calcium, cattle who ingest high Ca dry cow rations, cattle with reduced feed intake at parturition, over-conditioned cows, high producing cows, and cows with increased estrogen and glucocorticoids at parturition that may reduce serum Ca (Jorgensen, 1974).

Over-conditioned cows (body condition score >3.5, on a scale of 1–5) are at increased risk of hypocalcaemia (Heuer et al., 1999). Dystocia is also an important cause of peri-parturient recumbency (DeGaris and Lean, 2009).

Cows may lose more than 50 g of blood Ca per day to milk at the onset of lactation. Before calving, cows only require about 30 g of Ca, which equates to 15 g in fecal and urinary loss and 15 g to fetal growth. Cows can only afford to lose about half of their circulating blood Ca reserves before hypocalcaemia occurs. To meet the increased demands, the cow must increase absorption from the rumen or intestines and increase mobilization from tissue, especially bone reserves of Ca, as circulating blood Ca reserves are limited. “Most” cows have some degree of hypocalcaemia at calving (DeGaris and Lean, 2009).

Clinical hypocalcemia, or milk fever, can be defined as a total blood Ca level < 1.4 mmol/L. Subclinical hypocalcemia can be defined as a total blood Ca between 1.4 and 2.0 mmol/L. Both are risk factors for other diseases including mastitis, ketosis, retained placenta, displaced abomasum, and uterine prolapse (DeGaris and Lean, 2009).

ii. Effects

1. Clinical

Clinical and subclinical hypocalcemia are considered "gateway diseases" and greatly reduce the chance for full productivity during that lactation (Goff, 2008, Mulligan and Doherty, 2008). Hypocalcemia reduces rumen and abomasal motility, increasing the risk of displaced abomasums. Hypocalcemia reduces feed intake so that greater body fat mobilization occurs in early lactation. Hypocalcemia also reduces muscle contraction, including the teat sphincter muscle which is responsible for teat closure after milking, increasing the risk of mastitis (Goff, 2008). Clinical hypocalcaemia (total blood Ca < 1.4 mmol/L) and subclinical hypocalcaemia (total blood Ca 1.4–2.0 mmol/L) are risk factors for many of the important diseases of lactation including mastitis, ketosis, retained placenta, displaced abomasum, and uterine prolapse (DeGaris and Lean, 2009).

2. Subclinical

iii. Prevention and treatment

Extracellular Ca will be lost to milk at the start of lactation, which must be replaced to prevent blood Ca from decreasing also. A healthy cow will withdraw Ca from bone and increase the absorption efficiency of dietary Ca, forcing her into a state of lactational osteoporosis. Bone Ca mobilization is regulated by parathyroid hormone,

which is produced any time there is a decrease in blood Ca (Goff, 2008). Cows are at an increased risk of hypocalcemia post-calving when pre-calving diets are Ca-deficient because of the gradual loss of Ca stores. Although data is lacking, DeGaris and Lean (2009) explained that post-calving diets high in Ca combined with increased passive absorption from Ca stores are protective against milk fever.

Milk fever prevention depends partly on nutrition. Recumbency can be caused by hypocalcemia, hypomagnesemia, hypophosphatemia, ketosis associated with twins, musculo-skeletal injury predisposed by calving and hypocalcemia, and, less frequently, peracute mastitis or other infections (DeGaris and Lean, 2009).

Reducing the number of cations (sodium, potassium, calcium, and magnesium) present in feeds may help reduce hypocalcemia post-partum (Goff, 2008). Cows fed a low Ca diet (< 20 g of Ca/d) during the dry period cannot meet Ca maintenance and fetal skeletal development requirements. A negative Ca balance stimulates the secretion of parathyroid hormone before calving, which activates bone osteoclasts to stimulate bone Ca resorption, and activates renal tubules to resorb urinary Ca and to begin producing calcitriol before calving. When these Ca homeostatic mechanisms are active post-calving, they prevent a severe decline in plasma Ca concentration in the lactating cow (Goff and Horst, 1997, Goff, 2008). In order to benefit from this prophylactic effect, close up dry cow diets should be low in calcium and high in phosphorous to successfully prevent hypocalcemia (Boda and Cole, 1954, Jorgensen, 1974). Reducing Ca concentration in a dry cow ration means that high Ca forages like alfalfa should be removed and replaced with low Ca forages like corn silage or grass hays (Goff and Horst, 1997).

Hypocalcaemia cannot be entirely prevented by ration formulation. Recumbency is often caused by hypocalcaemia, but other significant causes include hypomagnesaemia, musculo-skeletal injury predisposed by calving and hypocalcaemia, ketosis associated with twinning, hypophosphataemia and a number of less frequent problems such as peracute mastitis and other infections. Dystocia is a major cause of periparturient recumbency (DeGaris and Lean, 2009).

Keeping potassium as close to the dry cow NRC requirement as possible (about 10 g/kg or 1.0% diet K) is a good preventive practice for hypocalcemia. Adding chloride to the ration to counteract the effects of even low dietary K on blood alkalinity can help reduce subclinical hypocalcemia. Chloride concentration should be about 5 g/kg (0.5%) less than the concentration of K in the diet (Goff, 2008).

Hypocalcemia can make a cow unable to stand up because Ca is necessary for nerve and muscle function (Goff, 2008). "Crush syndrome" can occur on the appendages under the weight of the cow when recumbent in just 4 hours. The cow's weight cuts off the blood supply to the muscles and nerves, followed by necrosis of these tissues resulting in the downer cow syndrome. The fastest way to restore plasma Ca concentration is to administer Ca salts (commonly Ca borogluconate) intravenously. Commercial preparations for intravenous use supply from 8.5 to 11.5 g Ca per 500 mL and may also contain sources of magnesium, phosphorous (often as ineffective phosphite), and glucose (dextrose). The most effective intravenous Ca dose is 2 g Ca per 100 kg body weight. If administered too rapidly, fatal arrhythmia of the heart can occur and the heart may stop, so Ca should be administered at a rate of 1 g/min. Intravenous Ca treatments elevate blood Ca above normal for about 4 h (Goff, 2008).

Oral Ca supplementation can force Ca across the intestinal tract through passive diffusion between intestinal epithelial cells. The assumption when using oral Ca supplementation is that the cow's ability to use active Ca transport across intestinal cells is inadequate to maintain normal blood Ca concentrations. The best results are obtained with Ca doses between 50 and 125 g (Goff, 2008).

iv. Economic impact

In a stochastic model, Bewley et al. (2010a) estimated the cost of a case of milk fever to range between $72 and $172 with a mean of $114 for multiparous cows (with the assumption that primiparous cows are not likely to have milk fever). The most recent estimate for milk fever was $166 for multiparous cows. Veterinary and treatment costs comprise the largest portion of the loss at $85 per case (Liang, 2013).

d. Ketosis

i. Cause

Ketosis is a disease related to carbohydrate and fat metabolism and is characterized by increased concentrations of ketone bodies in blood (ketonemia), urine (ketonuria), and milk (ketolactia). The major ketone bodies are betahydroxybutyrate (**BHBA**), acetoacetate, and acetone. Ketosis can be classified as subclinical or clinical and as a primary or secondary disease (Geishauser et al., 1998). Higher producing cows are at greater risk of ketosis, which comes with a temporary milk yield decrease, so if they do not develop ketosis their milk yield would be even greater (Detilleux et al., 1994, Rajala-Schultz et al., 1999a).

Clinical ketosis incidence in the United States is between 2 and 3% (McLaren et al., 2006, Seifi et al., 2011), but is greater in third or greater parity cows (6%, P = 0.02) (Seifi et al., 2011). However, subclinical ketosis incidence is 54% in week one and 47% in week two post-partum (McLaren et al., 2006).

ii. Effects

1. Clinical

The gold standard of determining subclinical ketosis status is the measurement of BHBA in blood plasma or serum (McLaren et al., 2006). The optimum BHBA cut-point based on maximum total sensitivity and specificity for clinical ketosis was 1200 μmol/L in the first week post-partum (Seifi et al., 2011).

Postpartum BHBA increases have been associated with decreased milk production and milk protein content (Duffield et al., 2009) and increased risk for culling, clinical ketosis, and displaced abomasum (Seifi et al., 2011). LeBlanc et al. (2005) cited the odds of a left displaced abomasum were 8 times greater in cows with serum BHBA ≥1200 μmol/L. Cows with milk BHBA concentration ≥ 1200 μmol/L were 3.4 times more likely to develop a left displaced abomasum. Walsh et al. (2007) explained that the herd prevalence of anovulation increased by 2.1% for every 10% increase in the herd prevalence of subclinical ketosis in the first week postpartum. Cows with BHBA concentrations ≥ 1200 μmol/L were also 4.7 times more likely to develop clinical ketosis (Seifi et al., 2011).

Ketolac® BHB strips (Hoechst, Unterschleißheim, Germany) were 92 and 72% sensitive at detecting subclinical ketosis using 500 and 100 mmol BHBA/L milk thresholds, respectively (Geishauser et al., 1998).

Cows with clinical ketosis were 11 times more at risk of developing delayed ovarian function (Opsomer et al., 2000).

2. Subclinical

Subclinical ketosis develops in response to a poor adaptive response to negative energy balance and the liver is overwhelmed with non-esterified-fatty-acids (**NEFA**). Cows that experience subclinical ketosis after the first seven DIM may have better adapted to the effects of decreased dry matter intake in the periparturient period but are not able to sustain using energy stores for increased milk production in early lactation. However, cows that develop subclinical ketosis within the first week postpartum likely experienced poor adaptation to negative energy balance through calving and into lactation (McArt et al., 2012).

Subclinical ketosis starts at serum or plasma BHBA concentrations greater than 1,000 μmol/L. However, setting an appropriate subclinical threshold using serum or plasma BHBA is somewhat arbitrary (Duffield et al., 2009). Duffield et al. (2009) explained that defining hyperketonemia in order to predict health risk in early lactation dairy cows begins at a BHBA serum concentration ≥1,200 μmol/L.

Subclinical ketosis incidence has been cited at 43% (McArt et al., 2012) and ranged from 26 to 56% with peak subclinical ketosis incidence at 5 DIM (McArt et al., 2011). Cumulative subclinical ketosis incidence ranged from 46 to 59% (Duffield et al.,

1998), but likely underestimated the true incidence because cows were only tested once weekly.

Subclinical ketosis increases the risk of displaced abomasum (**DA**) and metritis (Duffield et al., 2009, Ospina et al., 2010), which could increase culling risk. In the first 30 DIM, 0.3% of cows without ketosis developed a DA while 6.5% of cows with subclinical ketosis developed a DA. Cows that tested positive for subclinical ketosis were 19.3 times more likely to develop a DA than cows without ketosis. Of the cows that developed a DA, cows diagnosed with subclinical ketosis for the first time between 3 and 5 DIM were 6.1 times more likely to develop the DA compared to cows first testing positive at 6 or more DIM. Each 0.1 mmol/L increase in BHBA was associated with an increased risk of developing a DA by 30 DIM by a factor of 1.1 ($P < 0.01$) (McArt et al., 2012).

Also in the first 30 DIM, 5.4% of cows with subclinical ketosis were culled or died compared to only 1.8% for cows without subclinical ketosis. Cows with subclinical ketosis were 3 times more likely to die or be culled than cows without ketosis. The median time from first positive subclinical ketosis diagnosis to removal from the herd was 9 days, but ranged from 2 to 24 days. Cows diagnosed with subclinical ketosis for the first time from 3 to 7 DIM were 4.5 times more likely to be removed from the herd than the cows first testing positive at 8 or later DIM ($P < 0.01$) (McArt et al., 2012).

Cows without ketosis produced 1.2 kg/cow/day more than cows with subclinical ketosis in the first 30 days of lactation ($P < 0.01$). Cows diagnosed with subclinical ketosis for the first time between 3 and 7 DIM produced 0.7 kg/cow/milking more during

the first 30 DIM compared to cows diagnosed for the first time between 8 and 16 DIM ($P = 0.04$) (McArt et al., 2012).

Cows with a BHBA of 2.4 mmol/L, where a BHBA of 1.2 to 2.9 represented subclinical ketosis were 3 times more likely to develop a DA, > 50 times more likely to be culled, and was expected to produce 180 kg less milk in the first 30 DIM compared to a cow with a BHBA of 1.2 mmol/L (McArt et al., 2012).

iii. Prevention and treatment

Seifi et al. (2011) explained that the risks of clinical ketosis in cows with BCS of ≥ 3.75, between 3.25 and 3.5, and ≤ 3.0 were 10.7%, 3.1% and 1.3%, respectively ($P < 0.01$).

Administering a monensin controlled-release capsule (Duffield et al., 1998, Petersson-Wolfe et al., 2007) or supplementing cows three weeks before expected calving with a monensin premix (Petersson-Wolfe et al., 2007) significantly decreased serum BHBA concentrations in early lactation when compared to control cows. Duffield et al. (1998) explained that using a controlled-release monensin capsule reduced BHBA concentrations by 20% in the first 3 weeks post-partum. Petersson-Wolfe et al. (2007) cited similar results with a reduction of BHBA by 17% and 28% for weeks one and two post-partum, respectively.

iv. Economic impact

In a stochastic model, Bewley et al. (2010a) estimated the cost of a case of ketosis to range between $55 and $167 with a mean of $78 and $106 for primiparous and multiparous cows, respectively. The most recent estimate for ketosis was $80 for

primiparous cows and $92 for multiparous cows. For both groups, veterinary and treatment costs comprised the majority of the losses at $52 per case (Liang, 2013).

II. Disease detection using precision dairy farming technologies

a. Background

As average herd size increases, time producers can devote to each animal decreases (Schulze et al., 2007, Ipema et al., 2008, Bewley, 2010, Brandt et al., 2010) as the administrative, technical, organizational, and logistic workload for the producer increases (Berckmans, 2004). Livestock production today requires the desire to look beyond economic goals (Frost et al., 2003, Berckmans, 2004). Consumer pressure and concern for animal well-being and health, efficient and sustainable farming, food safety and quality, and control of zoonotic diseases, pathogens, and medical treatments has altered decision-making processes on farms (Berckmans, 2004, Schukken et al., 2008, Bewley, 2010). Dairy operations also have narrower profit margins than in the past because the government is less involved in regulating agricultural commodity prices. In turn, dairy producers need to increase efficiency, which can increase profit (Bewley, 2010, 2012). Because of the aforementioned major industry shifts, on-farm decision making is changing and dairy cow monitoring tools will likely increase in importance (Berckmans, 2004, Schulze et al., 2007, Ipema et al., 2008, Bewley, 2010) to help make decisions that previously were based solely on producer experience and judgement. Unfortunately, on-farm decisions are riddled with complexities, many of which the effects have to be estimated or guessed at by the producers (Frost et al., 2003). One way to counteract these problems is through the use of automated monitoring systems (Chagunda et al., 2006b).

Throughout history, agricultural techniques have advanced to support larger populations, With the growth of the non-farming population alongside an increase in living standards, agriculture's role and function has been transformed (Marchesi, 2012). Precision agriculture refers to the use of technologies to increase efficiency and reduce environmental damage in crop farming. Precision livestock farming applies the precision agriculture principles to animals, focusing on individual animal production and environmental impact (Laca, 2009). One goal of precision livestock farming to develop in-line systems that monitor animals objectively, continuously, and automatically, without adding stress on the animals (Berckmans, 2004). Precision dairy farming (**PDF**) is the use of technologies to measure physiological, behavioral, and production indicators on individual animals to improve management and farm performance (Bewley, 2010, 2012). This type of management system relies on the observation that the animal herself is the important part of the biological production process at hand (Berckmans, 2004).

Objective physiological measures of animal responses to environmental stressors can be used to evaluate the degree of stress and consequent adaptations to that stress (Hahn et al., 1990). Animals are complex and respond differently at different moments of time compared to their herdmates. Outside of precision livestock farming, animals are commonly considered an "average of a population" thus creating a steady-state system. Within precision livestock farming, however, each animal can be treated as its own CIT system (Complex, Individual, and Time-variant) (Berckmans, 2004). Real-time data from PDF technologies could be incorporated into decision support systems to facilitate decision making when multiple data sources are necessary (Bewley, 2010).

The goals of PDF are maximizing individual animal potential, early disease detection, and maximizing preventive care instead of medical treatments. Perceived benefits of PDF technologies include increased efficiency, reduced costs, improved milk quality, minimized environmental impacts, and improved animal health and well-being. Additionally, information from PDF technologies could potentially be incorporated into genetic evaluations for traits targeted at improving subsequent generations' health, well-being, and longevity (Bewley, 2010). Marchesi (2012) explained that implementing an animal monitoring system is both a moral and commercial interest to producers because it helps them satisfy the animal's needs.

To date, PDF evaluations have focused mainly on automated estrus detection, aimed to supplement or replace visual estrus detection (Dolecheck et al., 2015). Precision Dairy Farming technologies also have the potential to detect disease early, maximizing individual animal potential. Disease detection in the past has relied on producers observing clinical signs, but once clinical signs are displayed, it is often too late to act effectively. Clinical signs are often preceded by physiological changes that are undetectable with human senses, but may be possible with PDF and could allow producers to intervene sooner (Bewley, 2012). Technologies may alert producers to cows at risk for a disease instead of the existing disease detection method of identifying cows that are already sick (Itle et al., 2015).

Many disease cases go unnoticed because veterinary examination is the gold standard of disease detection, are conducted relatively infrequently on most dairy farms (Urton et al., 2005). Instead, dairy producers often rely on their experience and judgement to identify sick animals, but human perception of a cow's condition is limited

(Bewley, 2010). Additionally, some diseases do not present obvious signs (Weary et al., 2009). Even worse, sometimes, by the time an animal does display outward signs of illness or stress, it is too late to intervene. Physiological changes typically occur before clinical symptoms, though. If a producer were able to detect these physiological changes, interventions could occur sooner (Bewley, 2010). Even when individual monitoring is employed on farms, behavioral indicators used to detect illness are often based simply on the experience and intuition of the producer and tend to be unreliable (Weary et al., 2009).

Producers can examine real time data and reports to identify abnormal deviations from a baseline (Bewley, 2010). However, the data itself is meaningless unless it is transformed into a good decision management program. Thus, the producer remains a critical factor in good animal management and technologies will only support, not replace, the producer (DeGaris and Lean, 2009, Bewley, 2010, Marchesi, 2012). The ability to combine computer systems with the strengths and abilities of the producer is where the potential benefits of PDF systems lie (Marchesi, 2012).

However, to achieve success using precision livestock farming processes, three conditions apply. First, animal variables should be monitored continuously and the data should be analyzed consistently. The definition of "continuously" depends on the animal variable of interest, like weight, activity, drinking and feeding behavior, feed intake, body temperature, etc. Second, a reliable prediction or expectation on how the animal will respond to the change must be available constantly. Lastly, this prediction should be coupled with the technology measurements in an algorithm to monitor or manage the

animals automatically, and to monitor animal health or welfare or make desired system changes (Berckmans, 2004).

Often, each individual process involved in livestock production is controlled separately. Integrated management systems can control multiple, and ideally all, the interrelated processes involved in production. Each of the various processes within a dairy is usually controlled by one or more open-loop control systems, which has limited consideration for the effects that it has on other parts of the process. Management systems where various processes are integrated so that the production system is managed as a whole closed-loop system is a solution to the problems current systems being used on-farm create (Frost et al., 2003).

Daily milk yield recording, milk component monitoring, pedometers, automatic temperature recording devices, milk electrical conductivity monitors, and automatic estrus detection monitors, and daily body weight systems are currently available for producers to implement on-farm (Bewley, 2010). Bewley (2010) explained that other "theoretical" PDF systems may be able to measure: jaw movements, ruminal pH, reticular contractions, heart rate, animal positioning and activity, vaginal mucus and electrical resistance, feeding behavior, lying behavior, odor, glucose, acoustics, progesterone, individual milk components, color, infrared udder surface temperatures, and respiration rates. Excitingly, just six years later, many of these technologies are already available and being researched. Because the rapid development and availability of new PDF continues to grow, they are becoming more feasible for producers to implement in their own herds (Bewley et al., 2010b, Bewley, 2012).

Although the technology required to achieve fully automated dairy systems is available, multidisciplinary and innovative research is required to achieve its application. The bottleneck for application is the availability of reliable sensor systems because the required algorithms to go along with them can be developed (Berckmans, 2004). Unfortunately, the dairy industry is relatively small, which limits corporate willingness to invest in developing technologies exclusively for dairy farms. Thus, technology development is instead driven by the availability of a technology in other industries and then transferred to the dairy industry, regardless of the actual needs (Bewley, 2010, 2012).

Precision dairy farming technologies provide great opportunities to improve dairy herd management systems and may improve individual animal management (Bewley, 2010, Singh et al., 2014). However, the data itself is not useful unless it is interpreted and used effectively in decision making (Bewley, 2012, Singh et al., 2014). Bewley (2012) explained that the "majority" of data management systems currently available are not used to their full potential. Other PDF limitations include: slow adoption rates, erroneous animal reads, equipment failure, the amount of data may overwhelm systems during data transfer, a lack of validated research results, and cows are normally housed in a restricted spacial area (Singh et al., 2014).

b. Economics

On-farm decision-making tool adoption rates have been scarce in the dairy industry as of yet. Still, the dairy industry allows for great success using decision science because: 1) it is characterized by considerable price, weather, and biological variation and uncertainty; 2) PDF technologies designed to collect data for decision making

abound; and 3) fluid milk is difficult to differentiate, increasing the need for producers to differentiate themselves through their business models (Bewley et al., 2010a).

Precision dairy farming technology adoption may be more feasible as larger dairy operations rely more on less skilled labor and can take advantage of discounts related to economies of size (Bewley, 2010). Increasing labor costs relative to capital costs may drive adoption of PDF (Rutten et al., 2013). Interestingly, (Bewley et al., 2010b) showed that the profitability of investment in an automated body condition scoring system largely depended on what happens with the technology once it has been purchased.

Before investing in a PDF technology, a farm-specific economic analysis is recommended to ensure that the investment is sound. Decision support tools allow producers to make better investment decisions by considering these decisions at a systems level (Bewley, 2012). Stochastic simulation models account for more of the risk and uncertainty inherent to dairy farming. The results will therefore represent that there is uncertainty in the profitability of some investments. Although results from this type of economic analysis can be useful, the producer's level of risk aversion will ultimately determine whether the investment should occur (Bewley et al., 2010b).

Even though major production and economic losses result from increased SCC, adoption of better SCC control practices may be difficult to achieve. Adopting and implementing management practices to control SCC requires a behavioral change, which requires awareness, intention, and action. Producers may already understand inefficiencies in their farms, but quantifying the effect may motivate them to make changes. Farmers with high bulk tank SCC were aware of their situation and, therefore, the authors deemed them unlikely to change their actions even if they were provided with

specific economic consequences. However, the concept of the "value of information" implies that additional information adds to knowledge which allows for a more informed decision making process (van Asseldonk et al., 2010). Precision dairy farming technologies may provide this added information.

c. Parameters measured by precision dairy farming technologies

i. Temperature

Body temperature is influenced by health, environment, ambient temperature, eating behavior, drinking behavior, estrus, and the pregnancy status of an animal (Bewley et al., 2008). Fever, or a body temperature over a predefined threshold, is an indicator of disease (Leon, 2002, Burfeind et al., 2010). Fever is a complex physiological response to infection and inflammation. Once the body recognizes a pathogen invasion, macrophages and other immune cells release cytokines which signal the hypothalamus to increase the thermal set point. Although the mechanism of cytokine action remains unclear through studies in mice, this reaction causes body temperature to increase to match the increased thermal set point (Leon, 2002).

Producers often implement rectal temperature recording into their disease detection system (Schutz and Bewley, 2009, Burfeind et al., 2010, Vickers et al., 2010). The accuracy of commercially available electronic rectal thermometers is within 0.1°C (Vickers et al., 2010). However, several limitations to rectal temperature recording do exist. The first is that the presence of the recorder may affect temperature by making the animal nervous (Simmons et al., 1965, Bewley and Schutz, 2010). Other limitations include air in the rectum, failure to insert the probe deeply, and the creation of ulcers in

the rectum from forceful insertion. Ambient temperature also has an effect and accuracy is related to the competency of the recorder (Aalseth, 2005).

Fever is described as a rise in body temperature above the "normal" range. Fever is a common, but complex, physiological response to infection, inflammation, and trauma aimed at the host's survival (Leon, 2002). Generally, average daily body temperatures for cattle fall within a range of 38 to 39.4°C (Lefcourt et al., 1999, Aalseth, 2005, Benzaquen et al., 2007). Temperatures can vary between individual cows in the same conditions and can vary within cows throughout a day (Simmons et al., 1965, Lefcourt et al., 1999).

Manual collection of rectal temperatures is the most common method of obtaining body temperatures in practice because of the ease of measurement and low purchase costs of rectal thermometers (Aalseth, 2005). Furthermore, because restraining animals to collect temperature data by manual means may cause stress that alters temperature, a reliable method of collecting temperatures without human intervention is likely to provide a more accurate measure of temperature in dairy cattle (Hahn et al., 1990).

Pararectal temperature rose when the four study cows stood and decreased when they laid down. The opposite occurred in subcutaneous temperature where a thermometer was placed under the skin behind the shoulder (Simmons et al., 1965).

Firk et al. (2002) suggested that the value of a temperature monitor is highly dependent on its location. Body temperature has been monitored in dairy cattle in several anatomical locations including the rectum, tympanic and skin portion of the ear, vagina, reticulorumen, intraperitoneal cavity, udder skin, and milk. Internal temperature

measurement sites may be more useful indicators of body temperature because they are not as readily affected by ambient conditions (Hahn et al., 1990). However, water consumption temporarily, but dramatically, decreases reticulorumen temperatures (Simmons et al., 1965, Brod et al., 1982, Bewley et al., 2008). In fistulated sheep, microbial activity decreased when injected intra-ruminally with 2 liters of 0°C water, which did not occur for the 10, 20, and 30°C water treatments. For the 0, 10, 20 and 30°C water treatments, temperatures did not return within ± 0.5°C to baseline rumen temperature for 108, 96, 96 and 72 minutes (Brod et al., 1982).

Simmons et al. (1965) cited that the mean pararectal, subcutaneous, and reticular ternperatures over four days were 38.4 ± 0.3°C, 35.6 ± .8°C, and 38.8 ± 1.2°C., respectively. Pararectal and subcutaneous temperatures consistently dropped between 6 pm and 7:30 pm, likely related to water ingestion. One cow on the study showed greater variation in her pararectal and subcutaneous temperatures than the other cows. Observationally, she drank more often throughout the day and had a more nervous temperament than the other three, which the authors stated as a reason for her temperature variation.

In a Canadian study evaluating rectal temperature measurements to determine intra- and inter-investigator variability and to determine the effects of penetration depth into the rectum and defecation on measured body temperature, repeated rectal temperatures by a single researcher were consistent (39.5 ± 0.1°C). Correlation between two researchers was high ($r = 0.98$; $P < 0.001$). However, temperatures were 0.4°C ± 0.2°C greater when the probe was inserted deeper into the rectum ($P < 0.001$). Temperature around defecation varied, with some cows having a difference of ≥ 3.0°C

after defecation while others had a difference of ≥ 3.0°C before defecation and some had no difference before or after defecation (Burfeind et al., 2010).

Reticular temperatures decrease when cows drink water and take 1.5 (Simmons et al., 1965) to 3.5 hours (Bewley et al., 2008) to return to the pre-drinking temperature. Simmons et al. (1965) observed reticular temperatures as low as 32°C after water consumption.

Automatic temperature recording may allow producers to detect disease, estrus, heat stress, and the onset of calving earlier than currently possible (Bewley et al., 2008). Body temperature has commonly been used to detect fever, heat stress, and the onset of calving for many years. However, core body temperature is desired, but is fundamentally difficult to obtain and rectal temperature only approximates core body temperature. Taking rectal temperatures may cause stress that alters the temperatures so a reliable method with no human intervention may be a more accurate measure. Attempts to measure body temperature of cattle have been made at various anatomical locations including rectum, ear (tympanic), vagina, reticulum-rumen, and milk (Bewley and Schutz, 2010).

Adams et al. (2013) explained that cows with clinical mastitis had 6.7 times higher odds of having a reticulorumen temperature 0.8°C above their baseline within 4 days of diagnosis compared to control cows (76.9% specificity and 67.0% sensitivity). However, reticulorumen temperature was not different for cows diagnosed with metritis compared to control cows.

Cows with retained placentas averaged 0.1°C greater temperature than matched control cows ($P < 0.001$) (Vickers et al., 2010). Cows with puerperal metritis underwent a significant rectal temperature increase 24 hours before clinical signs (reaching 39.2 ± 0.05°C on the day of clinical diagnosis) (Benzaquen et al., 2007).

In a Canadian study, rectal and vaginal temperatures were highly correlated ($r = 0.81$; $P < 0.01$) in the 1,393 temperatures recorded for 29 fresh cows. However, rectal and vaginal temperatures were only moderately correlated ($r = 0.46$; $P < 0.01$) for the 556 temperatures recorded from the 13 peak lactation cows in this study. The correlation difference may have been because the fresh cows exhibited a larger temperature range (37.7 to 40.5°C) compared with peak-lactation cows (37.9 to 39.6°C) (Vickers et al., 2010).

Healthy cows and cows with retained placentas both showed diurnal rhythms in their vaginal and rectal temperatures, with increases in the afternoon and decreases during the morning (Vickers et al., 2010). Diurnal variations in temperatures may be attributed to individual cow or breed characteristics and ambient weather conditions (Bewley et al., 2008). Some limitations to vaginal temperature monitoring are logger movement (particularly around calving when the vaginal cavity was enlarged), influx of ambient air, expulsion from the vagina (Vickers et al., 2010).

Reticular temperatures were lowest between noon and 4:00 PM (39.4°C) and between 8:00 AM and noon (39.5°C). In contrast, reticular temperatures were greatest between 8:00 PM and midnight (40.2°C) and between midnight and 4:00 AM (40.3°C) (Ipema et al., 2008).

In an *E. coli* intramammary mastitis challenge, ruminal temperature peaked between 40.5°C and 41.0°C and remained above 40.0°C for two hours (AlZahal et al., 2011). Reticular temperature of cows diagnosed with mastitis deviated more than 3 standard deviations from baseline temperature in 45.7% of cows in another study (Bewley and Schutz, 2010).

ii. Lying time and activity

Accelerometers measure three different movements: side-to-side, up and down, and front to back, and are thus provide more information than pedometers. A decrease in activity could be a sign of illness (Marchesi, 2012).

In dairy cattle, lying down is a high-priority behavior, which ensures that the necessary time to rest and ruminate is achieved. Danish researchers restricted time to feed access and explained that this restriction decreased time spent on all activities, but the proportion of time spent feeding and time spent on social contact remained constant. Yet the proportion of time spent lying increased. Therefore, the authors concluded that the priority for the behaviors studied were lying, followed by eating and social contact (Munksgaard et al., 2005). Lying time has been referenced between 10.5 and 11 hours per cow per day (Ito et al., 2009, Bewley et al., 2010c, Cyples et al., 2012, Medrano-Galarza et al., 2012).

Changes in lying behavior may be related to a state of chronic stress (Ladewig and Smidt, 1989). Reduced mobility and increased rest may be strategy of energy conservation in order to allow more energy to be spent on fighting the infection and to allow the full development of a fever, which may help the animal recover (Aubert, 1999).

Cook et al. (2007) video recorded lying behavior of 14 dairy cows over all seasons and discovered that mean lying time decreased from 10.9 to 7.9 hours/day from the coolest to the hottest session recorded because of heat stress ($P < 0.01$). Additionally, cows with greater locomotion scores (using a 1 to 4 scale where 1 represents non-lame and 4 represents severely lame) lied down more (2.9, 4.0, and 4.41 hours/day for locomotion scores 1, 2, and 3, respectively; $P < 0.01$ between 1 and 2; $P = 0.02$ between 1 and 3), indicating that pain may increase lying time.

Canadian researchers challenged 19 cows with an *E. coli* lipopolysaccharide and cited that baseline lying time (averaged from the two days before mastitis induction; 707.0 minutes/day) was higher than the day of induction (633.3 minutes/day; $P = 0.005$). Lying time increased on the two days after infusion (743.1 and 726.3 minutes/day for days one and two after infusion, respectively), but not significantly (Cyples et al., 2012). In a behavioral study of cows with naturally-occurring clinical mastitis, cows with clinical mastitis laid down more than control cows on the day after mastitis detection (707.5 versus 742.5 minutes/day, $P = 0.04$). However, no difference was observed in lying times of animals with mastitis that had been treated with antibiotics and control animals (Medrano-Galarza et al., 2012).

While physical discomfort may decrease dairy cow lying time, lying on hard surfaces may also exacerbate pain caused by mastitis, causing lying time to decrease during mastitis (Cyples et al., 2012). Chapinal et al. (2013) explained that lying down at the time when the most severe signs of local inflammation occur causes pain, forcing cows to stand for longer periods during mastitis.

Total daily standing time was 20% longer for cows later diagnosed with clinical ketosis during the week before calving (14.3 ± 0.6 vs. 12.0 ± 0.7 h/d) and 35% longer on the day of calving (17.2 ± 0.9 vs. 12.7 ± 0.9 h/d) compared to those without ketosis, but no differences were observed postpartum. Cows later diagnosed with clinical ketosis also stood up fewer times (14.6 ± 1.9 vs. 20.9 ± 1.8 bouts/d) and stood for longer periods (71.3 min/bout vs. 35.8 min/bout) than cows without clinical ketosis on the day of calving (Itle et al., 2014). Cows with ketosis behave in a subordinate fashion (Itle et al., 2014), causing them to be less motivated to engage in behaviors that are energetically expensive like changing position from lying to standing (Susenbeth et al., 2004) or competing for feed (Goldhawk et al., 2009). Ketosis is a progressive disease associated with gradual changes in non-esterified fatty acids and blood glucose, starting in the prepartum period and progressing toward the more severe fatty liver disease (Bobe et al., 2004). Other researchers cited that postpartum activity was reduced among cows that were diagnosed with subclinical ketosis (502.20 ± 16.5 vs. 536.6 ± 6.2) (Liboreiro et al., 2015).

Cows diagnosed with metritis had reduced postpartum activity (512.5 ± 11.5 vs. 539.2 ± 6.0 arbitrary unit) (Liboreiro et al., 2015).

iii. Feeding time

Edwards and Tozer (2004) explained that cows with ketosis had lower activity ($P < 0.01$) than healthy cows up to 5 DIM, but then actually became more active after 12 DIM. The difference in activity may have been due to sick cows having lower appetites, spending less time at the feed bunk, and spending more time lying down.

During the week before, week after, and two weeks after calving, the dry matter intake (DMI) of cows with subclinical ketosis was 18, 26, and 20% lower that the DMI of cows without subclinical ketosis after calving ($P < 0.01$). Cows with subclinical ketosis also visited the feeder 18, 27, 28, and 16% fewer times during two weeks before, one week before, one week after, and two weeks after calving and spent less time at the feeder the same weeks (Goldhawk et al., 2009).

Cows with severe metritis consumed less feed than healthy cows beginning 2 weeks before calving and continued to consume less dry matter through three weeks post-partum. Cows with mild metritis ate less dry matter compared with healthy animals during the week before calving and throughout the 3-wk postpartum period. The odds of severe metritis increased by 2.87 for every 1 kg decrease in DMI during the week before calving. The odds of severe metritis increased by 1.72 for every 10-min decrease in feeding time during the week before calving. During the two weeks before calving, healthy cows displaced others from the feed bins 16.8 ± 1.74 times/d compared with severely metritic cows who only displaced others on average 12.2 ± 1.58 times/d ($P = 0.06$) (Huzzey et al., 2007). Urton et al. (2005) also explained that cows with acute metritis spent 24 minutes less at the feed bunk compared to those without acute metritis between 12 days pre-calving to 19 days post-calving ($P < 0.01$). In this study, the odds of a cow having metritis increased by 1.97 for every 10-minute decrease in average daily feeding time.

Hansen et al. (2003) cited a linearly negative relationship between feed intake and plasma calcium level in cows with induced hypocalcaemia.

iv. Rumination time

Rumination is defined as the regurgitation of fibrous ingesta from the rumen to the mouth, re-mastication, followed by swallowing and returning of the material to the rumen. Dairy cows normally ruminate for eight to nine hours a day when measured by visual observation. Researchers in a Vermont study fitted steers with a facemask that restricted all jaw movement for ten hours a day during the study period. When the facemask was removed, the steers were offered hay, but the animals instead chose to ruminate (Welch, 1982). A more recent study using rumination collars by Kaufman et al. (2016) cited rumination times of 7 and 8 hours for primiparous and multiparous cows, respectively.

Rumination is affected by diet, including feed digestibility, neutral detergent fiber intake, forage quality (Welch and Smith, 1970), and particle size (Welch, 1982). Rumination time decreases with acute stress (Herskin et al., 2004) and disease (Welch, 1982, Hansen et al., 2003).

Researchers have estimated rumination based on direct visual observations, but systems now exist to automate this process (Schirmann et al., 2009). Automated rumination-monitoring system was validated by comparing values from a rumination logging device with those from a human observer for 51 two-hour observation periods on 27 Holstein cows. Rumination times from the electronic system were highly correlated with those from human observation ($R = 0.93$), indicating that the automated system accurately monitored rumination in dairy cows (Schirmann et al., 2009).

Kansas researchers studied nine Angus-Hereford cows and observed that high cortisol levels (above 22 ng/mL, the mean of the group) were highly correlated with less time spent ruminating ($r = -0.85$, $P < 0.01$). Cortisol is released when an animal is

stressed, therefore an association between stress and decreased rumination may exist (Bristow and Holmes, 2007). However, decreases in rumination may not always occur around stress. A study examining behavioral changes related to increased stocking density reported that at 100% stocking density, 95.1% of rumination occurred within a stall, but as stocking density increased to 142%, only 87.3% of rumination occurred within a stall. However, overall rumination time did not decrease between any of the stocking densities ($P > 0.05$) (Krawczel et al., 2012).

In an *E. coli* challenge with 20 cows, rumination decreased ($P < 0.05$) on the day of mastitis induction and gradually increased to levels before the induction during the following two days (Fogsgaard et al., 2012). Canadian researchers evaluated the effects of a non-steroidal anti-inflammatory drug on the pain mitigation of mastitis and discovered that an *E. coli* lipopolysaccharide challenge did not affect daily rumination time, recorded by neck-mounted rumination loggers. However, when diurnal patterns were taken into account, an interaction between time and rumination recorded in two-hour intervals was significant ($P < 0.01$) where cows spent less time ruminating after the challenge, but made up for it later in the day (Fitzpatrick et al., 2013). Siivonen et al. (2011) also conducted an *E. coli* challenge to evaluate rumination behavioral changes around mastitis and concluded that the mean time spent ruminating decreased between four and eight hours post-challenge compared to the control day (222 versus 252 for control and induction days, respectively).

Cows diagnosed with metritis had reduced postpartum daily rumination time (416 vs. 441 minutes/day) (Liboreiro et al., 2015). Induced hypocalcaemia resulted in reduced rumination time, possibly related to the anti-peristaltic esophageal movements during

rumination (Hansen et al., 2003) or decreased ruminal contractions (Jorgensen et al., 1998) because Ca is required for muscle contractions (Hansen et al., 2003).

Kaufman et al. (2016) explained that cows with greater rumination time the week before calving was associated with decreased odds of ketosis. The odds of a cow getting ketosis and another health problem increased when rumination time decreased from 1 week before calving to one week after. Rumination time decreased in primiparous and multiparous cows from two weeks prepartum and began to increase from weeks 1 to 2 postpartum. The increase postpartum may represent changes in dry matter intake. Clément et al. (2014) explained that rumination was a small, but significant, contributor in dry matter prediction. However, rumination time within weeks and cows are variable, making it difficult to use rumination time to predict dry matter intake.

Primiparous cows ruminated less than multiparous cows 3 and 4 weeks postpartum (Kaufman et al., 2016). Maekawa et al. (2002) visually observed rumination times and explained that primiparous cows ruminated 52 minutes per day less than multiparous in mid-lactation.

v. Milk bacteriology, yield, and components

Dairy cattle economic efficiency is closely related to milk production (Dohoo and Martin, 1984) because production losses decrease producer revenue. Unfortunately, mastitis has a long lasting effect on milk yield (Rajala-Schultz et al., 1999b). Bar et al. (2008) explained that even after an infection was cured, milk yield remained depressed for two months. Additionally, cows may be unable to reach their pre-mastitis milk yield

after a clinical mastitis case throughout their entire lactation (Rajala-Schultz et al., 1999b).

Canadian researchers examined the effects of subclinical mastitis on milk yield and discovered that each unit increase in log SCC was associated with a 6.2% milk yield loss (Dohoo and Martin, 1984). Cobo-Abreu et al. (1979) concluded that cows with mastitis produced significantly less milk in the lactation when the mastitis occurred compared with their lifetime average milk production ($P < 0.05$).

French researchers developed a mastitis simulation model using data from three herds and determined that overall losses amounted to 49,000 kg per 100 Holstein cows and 35,000 kg per 100 Friesian cows with clinical mastitis, which was 8 and 7% of total projected production. The model did not include discarded milk loss. The authors concluded that one-third of cows experienced no significant response relative to control cows (a loss of 22 kg for cows with clinical mastitis). However, the other two-thirds of study cows experienced substantial milk losses between the week of mastitis occurrence and the five weeks following (144 kg) or experienced substantial milk loss extended throughout their lactation (911 kg) (Lescourret and Coulon, 1994).

A Finnish study examined milk yield changes around clinical mastitis and observed that milk yield began to decline four weeks before clinical mastitis detection. Milk yield of cows with clinical mastitis dropped below that of the healthy cows in the first two weeks after diagnosis. After this two-week period, yield gradually increased, but it did not reach the level it was at more than four weeks before the onset of mastitis during the rest of the lactation. However, the yield decrease of the cows with clinical mastitis was not significantly different from the healthy cows. Overall, total lactation

milk yield loss caused by mastitis varied between 294 and 552 kg under the assumption of a 305-day lactation with clinical mastitis occurrence on day seven. Milk loss increased with increasing parity with older cows suffering greater losses. Milk loss among parity 1, 2, 3, and 4 or higher cows was 4.6, 4.1, 6.9, and 7.4% of the overall lactation yield, respectively (Rajala-Schultz et al., 1999b).

Milk loss resulting from clinical mastitis may depend on the number of affected quarters and the number of clinical mastitis occurrences throughout the lactation. A Dutch study reported that first parity cows with clinical mastitis in only one quarter lost 40 kg as opposed to second parity cows that lost 140 kg. Milk loss in first and second parity cows infected in only one quarter did not change with increased months in lactation. In second parity cows, milk yield was more significantly reduced when three or more cases of clinical mastitis were observed compared with two cases. Milk loss in month eight of the second lactation was 527 kg (8.1%) and 214 kg (3.3%) for three or two cases, respectively (Houben et al., 1993).

In a 6-month study on a 1700-cow Michigan Holstein dairy farm, total milk loss over all clinical cases of mastitis was 341 kg. Of that loss, decreased production accounted for 92 kg and milk withheld accounted for 249 kg. First lactation cows maintained a significantly lower milk loss than ≥ 2 parity (177 kg versus 369 kg for first and ≥ 2 parity cows, respectively; $P < 0.01$). Milk withheld from first parity cows was also significantly less than milk withheld from ≥ 2 parity cows (102 versus 269 for first and ≥ 2 parity cows, respectively; $P < 0.01$) (Bartlett et al., 1991).

Using bacteriological culturing as a mastitis diagnostic method requires samples to be taken correctly. Samples collected at the quarter level are ideal. Selecting cows to

culture can be done through cow-level SCC information and then quarters can be further determined through CMT or quarter-level SCC. Selecting cows with at least two consecutive SCC samples > 200,000 cells/mL based on monthly SCC testing enables more cows harboring *Staph. aureus* to be detected (Lam et al., 2009).

Eighty-seven percent of 113 cases had the same CMT score for milk strippings and foremilk fractions while 11% had a greater value in stripping, and only 3% had a lower value (Peris et al., 1991).

Using molecular methods of pathogen detection is becoming increasingly common, mostly through polymerase chain reaction (**PCR**) technology. A particular bacterial species is determined through DNA amplification and visualization. Molecular methods are expensive and labor-intensive. Real-time PCR assays may be developed for both detection and quantification of mastitis pathogens in milk. Beyond pathogen identification, molecular methods can differentiate bacterial strains within a species, allowing researchers to understand differences in virulence, epidemiology, and cure rates. Using PCR for mastitis detection is not readily available in all laboratories and is costly. Other molecular methods could also be used to genotype mastitis-causing pathogens, including pulse field gel electrophoresis, ribotyping, random amplified polymorphic DNA, amplified fragment length polymorphism, and multi locus sequence typing (Lam et al., 2009).

Even slightly abnormal milk means there is a problem within that udder quarter so it should be detected in order for appropriate action to be taken (Lam et al., 2009).

A simple, inexpensive, and fast way to estimate SCC cow-side is the California Mastitis Test (**CMT**). The CMT is a semi-quantitative SCC measure using 3% sodium lauryl sulphate to form a viscous mixture with the DNA of disrupted cells in milk. Practically, a CMT could be used to detect the affected quarter of a cow with a high SCC or to evaluate treatment success (Lam et al., 2009).

Direct SCC measurement is more accurate than a CMT, but is more expensive and not always available cow-side (Lam et al., 2009). Evaluating SCC by the DeLaval cell counter (DeLaval International AB, Tumba, Sweden) produced similar results to counting cells via microscopy and thus may be effectively employed as a mastitis detection tool at least in buffaloes (Bansal et al., 2007).

Mastitis is not the only disease affecting milk yield or components, however. Milk yield began to decrease 6 d before clinical ketosis diagnosis and remained lower ($P < 0.01$) than that of healthy cows (cows without ketosis, displaced abomasums, or digestive disorders) until at least d 10 after diagnosis (Edwards and Tozer, 2004). Cornell researchers cited that milk loss started 4 weeks before and continued for at least 2 weeks after a clinical ketosis diagnosis. The daily milk loss was greatest within the first 2 weeks after diagnosis: 3, 4, 3, and 5 kg/d for parities 1, 2, 3, and ≥ 4, respectively. The overall production loss during lactation was between 126 and 535 kg per cow. Cows without clinical ketosis in parity 1 yielded 1 kg less milk/day and cows in parity 4 or greater yielded 2 kg less milk/day than cows with clinical ketosis in the same parity (Rajala-Schultz et al., 1999a). In another study, cows with clinical ketosis produced 141.1 kg more 305-d yield than cows without clinical ketosis, but production was 44.3 kg less over 17 d following diagnosis (Detilleux et al., 1994). However, Rowlands and

Lucey (1986) cited a 7% decrease in peak milk yield but overall no difference in 305-d yield. In contrast, Dohoo and Martin (1984) explained that a case of clinical ketosis increased milk production by 2.5%. The authors contributed this beneficial effect to the initial treatment of cows with clinical ketosis with malt or propylene glycol. However, it is likely that the cows with ketosis were higher yielding and were able to continue milking more even after ketosis, which was also the case in (Rajala-Schultz et al., 1999a).

Average daily milk production during the first 21 d after calving did not differ between cows with subclinical ketosis compared to those without (Goldhawk et al., 2009). Higher producing cows are at greater risk of ketosis, which comes with a temporary milk yield decrease, so if they do not develop ketosis their milk yield would be even greater (Detilleux et al., 1994, Rajala-Schultz et al., 1999a). Milk yield began to decrease 6 d before clinical ketosis diagnosis and remained lower ($P < 0.01$) than that of healthy cows (cows without ketosis, displaced abomasums, or digestive disorders) until at least d 10 after diagnosis (Edwards and Tozer, 2004). Cornell researchers cited that milk loss started 4 weeks before and continued for at least 2 weeks after a clinical ketosis diagnosis. The daily milk loss was greatest within the first 2 weeks after diagnosis: 3, 4, 3, and 5 kg/d for parities 1, 2, 3, and ≥ 4, respectively. The overall production loss during lactation was between 126 and 535 kg per cow. Cows without clinical ketosis in parity 1 yielded 1 kg less milk/day and cows in parity 4 or greater yielded 2 kg less milk/day than cows with clinical ketosis in the same parity (Rajala-Schultz et al., 1999a). Detilleux et al. (1994) explained that cows with clinical ketosis produced 141.1 kg more 305-d yield than cows without clinical ketosis, but production was 44.3 kg less over 17 d following diagnosis. However, Rowlands and Lucey (1986) cited a 7% decrease in peak milk yield

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Canadian researchers discovered that milk production was less in cows identified with severe or mild metritis during the first three weeks after calving. The decreased yield is likely a consequence of the decreased dry matter and water intake observed after calving in the cows with severe and mild metritis (Huzzey et al., 2007). Mahnani et al. (2015) explained that a case of metritis reduced 305-d milk yield by 129.8 ± 41.5 kg per cow per lactation. In contrast, Wittrock et al. (2011) cited no difference in milk yield between cows with metritis and those without.

vi. Lactose

Lactose concentration decreases with mastitis, mainly because of the reduced synthesis capacity of damaged tissue. Because lactose is a milk osmotic regulator, decreased milk yield follows decreased lactose concentration. Conversely, fat and casein appear to increase during mastitis, but this change mostly occurs because the milk yield decrease is greater than the decrease in fat and casein synthesis (Burriel, 1997).

Vanlandingham et al. (1941) explained that mastitis changes the synthetic and secretory mechanism of milk secretion and increases the permeability of cell membranes. Chlorine, lactose, chlorine-lactose number, and casein number changes occurred

simultaneously during mastitis. The chlorine-lactose number was preferred over either chlorine or lactose alone to determine mastitis. Evaluating changes in the percentage of chlorine and lactose with corresponding changes in the chlorine-lactose number based on quarter differences in the same udder were recommended as valid methods for detecting chronic mastitis.

Lactose percent in buffalos with mastitis was a better indicator of mastitis than electrical conductivity in a study on buffaloes. Using electrical conductivity to detect mastitis was 84% effective in discriminating milk from cows with mastitis (Bansal et al., 2007).

Kester et al. (2014) explained that lactose concentration decreased in cows challenged with *Strep. uberis* on days 3, 4, 5, and 6 post-challenge compared to controls, but was significantly different only on day 3 ($P < 0.05$). After intramammary antibiotic therapy, lactose concentration from challenged cows returned to levels comparable to control cows. In an intravenous endotoxin-induced mastitis challenge, milk lactose concentration was depressed for three milkings post-challenge ($P < 0.05$) (Shuster et al., 1991).

Although lactose concentration decreased with increasing mastitis severity, Berning and Shook (1992) explained that lactose was “not useful for mastitis detection.” Lactose was least responsive to changes in bacterial status. Somatic cell count and NAGase were more responsive than lactose.

vii. Lactate dehydrogenase and N-acetyl-β-D-glucosaminidase

Biosensor assays for milk enzymes allow for improved, automated, real-time, in-line mastitis detection (Chagunda et al., 2006a). Milk's heterogeneous composition limits the use of spectrophotometry, particularly because composition may vary considerably between samples. Milk is also an opaque and colloidal solution of proteins that both scatter and absorb light. However, enzyme analyses have been performed using spectrophotometry with success in determining mastitis (Larsen, 2005).

Lactate dehydrogenase (**LDH**) is an enzyme that is released in the milk when epithelial cells in the udder are destroyed during mastitis (Marchesi, 2012). Lactate dehydrogenase is part of the glycolytic pathway and mediates the oxidative and reductive connection between pyruvate and lactic acid (Larsen, 2005). Because LDH level increases with mastitis, it has attracted attention as being a mastitis detection indicator (Larsen, 2005). However, great variation in LDH, N-acetyl-β-D-glucosaminidase (**NAGase**), and SCC exists between cows (Chagunda et al., 2006a).

Chagunda et al. (2006a) evaluated the relationship between LDH, NAGase, and SCC during periods of mastitis. In healthy animals, Jersey cows had a 38% and 55% greater NAGase activity than Danish Holsteins and Danish Reds, respectively ($P < 0.01$). Parity also had little effect on LDH, NAGase, and SCC in healthy cows. In cows with clinical mastitis, however, Danish Holsteins had a 22% and 28% greater LDH activity than Danish Reds and Jerseys, respectively ($P < 0.05$). Cows with clinical mastitis had the greatest SCC, NAGase, and LDH in parity three. From first to third parity, LDH activity and NAGase activity increased by 51% and 38%, respectively. In general, SCC, LDH, and NAGase were greatest at calving then decreased until 30 to 40 DIM. After reaching its nadir level at 30 DIM, SCC gradually increased for the duration of the

lactation. However, LDH and NAGase reached their nadir levels at 34 and 40 DIM, respectively, but remained almost constant after 50 and 60 DIM, respectively. Eight days before clinical mastitis signs appeared, LDH, NAGase, and SCC increased by 56%, 30%, and 8%, respectively. Three days after clinical mastitis was treated, LDH, NAGase, and SCC decreased by 32%, 19%, and 7%, respectively.

In cows with clinical mastitis, the correlation between LDH and SCC was greater than in healthy cows (r = 0.76 and 0.48, respectively). Somatic cell count and NAGase were lower in healthy cows than in cows with clinical mastitis also (r = 0.41 and 0.59, respectively). The sensitivity and specificity of using LDH and NAGase to detect clinical mastitis was between 73 and 95%, depending on the thresholds used. The specificity for classifying cows without clinical mastitis was between 92 and 99% using LDH and NAGase. Using LDH as a mastitis indicator was better than NAGase, but both enzymes were similarly capable in classifying healthy cows as healthy (Chagunda et al., 2006a).

viii. Electrical conductivity

Electrical conductivity is a solution's ability to conduct an electric current between two electrodes. The concentration of anions and cations, specifically Na^+, K^+, and Cl^-, determines the electrical conductivity in milk. In the mammary alveoli, the sodium pumps in the basolateral membrane of the cells, pump Na into the extracellular fluid and K into the cells. Na and K are transported passively between the milk and alveoli through the apical membrane. Sodium and Cl move into the milk and K and lactose move into the extracellular fluid through destruction of tight junctions (Janzekovic et al., 2009). When a cow has mastitis, the electrical conductivity of her milk increases because of the increased Na and Cl (Janzekovic et al., 2009), or salt

(Bansal et al., 2007), concentration. In dairy cattle, if electrical conductivity is greater than 6.5 mS/cm or if the difference between the quarters is higher than 1 mS/cm, mastitis is likely. In 44 cows with a milk electrical conductivity of less than 6.5 mS/cm, 80% had SCC less than 400,000 cells/mL (Janzekovic et al., 2009).

Electrical conductivity accuracy in determining mastitis has been estimated at 80% (Janzekovic et al., 2009). However, milk fraction can affect results even beyond the effects of mastitis on milk. In ewes, the foremilk fraction consistently had a 1.7 times greater electrical conductivity than the stripping fractions ($P < 0.01$). Because the stripping fraction has a greater fat content, electrical conductivity could be decreased. In order to avoid fat issues, producers could measure electrical conductivity in skimmed samples. The machine milk fraction was different from foremilk and stripping fractions ($P < 0.01$) (Peris et al., 1991).

Milk from buffaloes with mastitis had significantly higher electrical conductivity than buffaloes without mastitis. Electrical conductivity could correctly differentiate 63% of quarters with and without mastitis (Bansal et al., 2007).

ix. Milk leukocyte differential

Elevated bulk milk PMN proportion is hypothesized to indicate increased prevalence of mastitis. On-farm, however, high PMN milk may have different implications than those associated with milk of similar SCC (Kelly et al., 2000).

Kelly et al. (2000) cited a Pearson's correlation coefficient of 0.88 between SCC and PMN for 103 individual milk samples ($P < 0.001$). A regression coefficient of 0.69 between SCC and PMN was obtained from 203 individual bulk tanks SCC ($P < 0.001$).

However, bulk tank samples with a SCC between 450,000 to 550,000 cells/mL had sizable variation in PMN content. Somatic cell count and PMN levels increased at the end of lactation ($P < 0.01$).

Dosogne et al. (2003) evaluated lymphocytes and monocytes in milk and cited a coefficient of correlation of 0.81 ($P < 0.05$). For the PMN population, a coefficient of correlation of 0.90 ($P < 0.01$) was obtained between low cytometric and microscopic differential leukocyte count. In early lactation, the percentage of lymphocytes and monocytes was greater and the percentage of mature macrophages and PMN were lower than in the other stages of lactation ($P < 0.01$).

Pillai et al. (2001) explained that quarters with greater SCC also had greater total cell count, mononuclear leukocyte count, and PMN count. Interestingly, quarters with high SCC, mononuclear leukocyte count, total cell count, and PMN count also had almost double the rate of bacterial infection. The proportion of PMN ranged from 33 to 49% with a mean of 40% for infected quarters compared with 17 to 25% with a mean of 20% for uninfected quarters. Polymorphonuclear leukocyte count had the greatest correlation with SCC and thus may be a good marker for the presence of bacterial infection in bovine quarters. However, there was no correlation between the species of bacteria isolated and SCC, total cell count, mononuclear leukocyte count, or PMN count.

III. Statistical analyses

a. Sensitivity/specificity

Reneau (1986) outlined the ideal clinical test as being able to establish the presence or absence of disease in every case screened without any false positives or false

negatives. He also suggested that the ideal test would provide a correct diagnosis, data to aid in prognosis, an indication of subclinical disease, data that may indicate possible disease reoccurrence, and would also be able to monitor treatment effects.

Correctly identified events are considered true positives (**TP**), non-alerted events are false negatives (**FN**), non-alerted non-events are true negatives (**TN**), and alerted non-events are false positives (**FP**) (Firk et al., 2002). Specificity is the probability that a negative sample is from a disease-negative cow. Sensitivity is the probability that a positive alert is a true indicator of a disease (Hamann and Zecconi, 1998, Sherlock et al., 2008, Hogeveen et al., 2010b). Because sensitivity and specificity are interdependent, thresholds should be set to optimize both (Hogeveen et al., 2010b). Specificity is equal to TN / (TN + FP) x 100. Sensitivity is determined by the following equation: TP / (TP + FN) x 100 (Sherlock et al., 2008, Hogeveen et al., 2010b). Accuracy, which can account for the prevalence of a disease whereas sensitivity and specificity cannot, can be determined by: [(TP + TN) / (TP + TN + FP + FN) X 100]. Accuracy depends on how strongly and closely the measured parameters are associated with the event, how accurately the technology measures the parameters, and how well the manufacturer algorithm processes the data to create useful alerts (Dolecheck et al., 2015).

Positive predictive value is the proportion of true positives against the apparent positives (Hamann and Zecconi, 1998). A true positive occurs when the event occurs with an alert from the automated detection system (Hogeveen et al., 2010b). Negative predictive value is the proportion of true negatives against the apparent negatives (Hamann and Zecconi, 1998). A true negative occurs when the event does not occur and an alert is not produced (Hogeveen et al., 2010b). False positives, or type I errors, can

cause financial losses because healthy animals may be treated. Conversely, false negatives, or type II errors, may leave sick animals untreated causing animal welfare problems and decreased milk yield and health throughout the lactation (Burfeind et al., 2010). Therefore, although a 90% sensitivity may seem acceptable in a research setting, it would likely be inadequate when applied in a herd setting (Sherlock et al., 2008).

Steeneveld et al. (2010) explained that a general complaint of producers using robotic milking systems was the "relatively large" amount of false alerts. Even the most sensitive and specific test still needs to be available and affordable (Reneau, 1986). To be a valuable commercial management tool, cow performance should be related to the potential improvement in management of subclinical disease (Nielen et al., 1995).

Sensitivity and specificity of a disease detection tool depend on the disease definition (Nielen et al., 1995) and time window (Mollenhorst et al., 2012) in which alerts can be given. Wider time windows will produce a higher sensitivity and specificity (Hogeveen et al., 2010b, Kamphuis et al., 2010), but they will also lose their practicality in a commercial setting (Kamphuis et al., 2010).

The results of a survey of 139 Dutch producers that owned an automated milking system revealed that farmers preferred a clinical mastitis detection system that produced few false alerts and provided alerts for severe cases with enough time to take effective treatment action. Producers preferred that time windows were set at a maximum of 24 hours before clinical symptoms appear. However, variation in responses to the survey varied greatly, suggesting that detection systems should be adaptable to match the conditions of each farm (Mollenhorst et al., 2012). Kamphuis et al. (2010) used an alert time window < 24 hours, but the authors were not confident that it was the correct

window to use for other studies. The use of a decision tree and this narrow time window resulted in 40% sensitivity and 99% specificity. Rasmussen (2002) suggested that a clinical mastitis system should provide 80% sensitivity and 99% specificity and that time windows should be within 24 to 48 hours of a clinical mastitis event.

Sensitivity and specificity will be lower if a new test disagrees with the comparison to the gold standard. Disagreement between the gold standard and a new test is often interpreted as the test lacking capability. However, the test could be better at detecting negatives, causing true negatives to display as false negatives (Nielen et al., 1992). This problem is made even more complex by the circumstance that neither the new test nor the gold standard detection methods may be ideal (Vickers et al., 2010). A universally accepted gold standard does not exist, though. Another limitation of an automated disease detection method is that clinical infections are infrequent, causing statistical analyses to be "weak" (Mein and Rasmussen, 2008).

b. Logistic regression and artificial neural networks

Predictive models are built from "experience," which means data is acquired from actual cases. The data can be pre-processed and used to develop rules (in knowledge-based expert systems) or serve as training data for statistical and machine learning models. Two of the most popular machine learning methods are logistic regression and artificial neural networks (Dreiseitl and Ohno-Machado, 2002).

Dichotomous classification is commonly used, where y can be either 0 or 1. The x_i are m-dimensional vectors, in which the components are called covariates and independent variables in statistics community or input variables in the machine learning

community. The second approach to data classification is modeling P(y|x), which yields a class label for a data point, but also the probability of class membership (Dreiseitl and Ohno-Machado, 2002).

k-Nearest neighbor method does not require details of model construction. The number of nearest neighbors to include in the estimate of class membership is k and the model can be made more or less flexible by varying k. The value of P(y|x) is calculated as the ratio of members of class y among the k nearest neighbors of x. This method is advantageous because the neighbors can provide an explanation for the classification result. However, the researcher needs to define a metric that measures the distance between data items, which is not always clear (Dreiseitl and Ohno-Machado, 2002).

Support vector machines attempt to build consistent estimators from data. Performance of these systems have been shown to be equal or better than other machine learning algorithms (Dreiseitl and Ohno-Machado, 2002).

Decision trees separates data into tree-like structures which allow for information gain. The estimate of P(y|x) is the ratio of y class elements over all elements of the leaf node that contains data item x. At each step, the combination of single best variable and optimal split-point is selected, but a multi-step lookahead that considers combinations of variables may obtain different or better results. Decision trees are not black-box models and can easily be expressed as rules (Dreiseitl and Ohno-Machado, 2002).

Logistic regression and artificial neural network both provide a functional form f and parameter vector α to express P(y|x) as $P(y|x) = f(x,\alpha)$. The parameters α are based on dataset D usually by maximum-likelihood estimation. In logistic regression, f is

known as the parametric method. In artificial neural networks, f is considered non-parametric or semi-parametric. Coefficients and intercepts can be interpreted in logistic regression, but weights in neural networks cannot always be interpreted (Dreiseitl and Ohno-Machado, 2002).

Model complexity is low in logistic regression, particularly when few interaction terms are used. Performing variable selection is a way to reduce a model's complexity and decrease the chances of overfitting, but may decrease the model's flexibility. Neural network models are more flexible and are more susceptible to overfitting, but methods like regularization and weight decay exist to prevent overfitting (Dreiseitl and Ohno-Machado, 2002).

Discrimination measures how well the classes in the set are separated. Common measures of discrimination are sensitivity, specificity, accuracy, and area under the ROC curve. Calibration determines how accurate the model probability estimate $f(x,\alpha)$ is to the true probability $P(y|x)$. Calibration measures how close the predictions of a given model are to the real underlying probability, which is almost always unknown and can only be estimated retrospectively by verifying the true binary outcome of the dataset. Calibration measures the similarity between two different estimates of probability (Dreiseitl and Ohno-Machado, 2002).

White box models, including decision trees, k-nearest neighbor, and logistic regression, allow interpretation of model parameters. Black box models, including support vector machines and artificial neural networks, can only be verified externally (Dreiseitl and Ohno-Machado, 2002). Some machine learning techniques including random forest, linear discriminant analysis, and neural networks have shown promise for

using PDF technologies for estrus detection (Dolecheck et al., 2015). Thus these methods may also produce reasonable results for other PDF applications.

IV. Conclusions

Dairy cow diseases, particularly during the transition period, are expensive and compromise cow well-being and milk production. Current disease detection methods rely on visual observation. However, early disease detection may allow producer intervention (e.g. antibiotic treatment), thus decreasing the negative economic and well-being implications of the disease. Precision dairy technologies, or technologies that reside in and on cows to monitor individual cow physiology, production, and behavior, may be able to predict and detect disease and alert producers to cows with changes in the indicators monitored.

Chapter 2:

Evaluation of Precision Dairy Monitoring Technologies to Detect Postpartum Dairy Cow Diseases

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INTRODUCTION

Dairy cow health is multifactorial and complex. High producing dairy cows have been described as "metabolic athletes," but 30 to 50% of cows are affected by a metabolic or infectious disease around calving (LeBlanc, 2010). Cows are highly susceptible to metabolic and infectious disease during the postpartum period, or the period from 3 weeks before to 3 weeks after calving (Huzzey et al., 2007, Mulligan and Doherty, 2008). Postpartum dairy cows are immunosuppressed and often have to deal with sudden dietary changes and environmental stressors like routine group changes associated with moving from dry to lactating. These effects combined with the stress of parturition present great risk for production diseases right after parturition.

Metritis is a severe inflammatory reaction involving all uterine layers (BonDurant, 1999). Clinical signs of metritis include pyrexia, fetid pus within the uterine lumen, vagina, or discharging from the vulva (Sheldon and Dobson, 2004), and delayed uterine involution (Sheldon, 2004, Sheldon and Dobson, 2004). Uterine bacterial infections compromise animal welfare and can cause sub-fertility or infertility (Sheldon and Dobson, 2004). The most recent cost estimates for a case of metritis were $176 for primiparous cows and $186 for multiparous cows (Liang, 2013). Clinical metritis incidence has been cited from 18 to 36% (Etherington et al., 1984, Bartlett et al., 1986, Markusfeld, 1987, Drillich et al., 2001).

Hypocalcemia, commonly referred to as milk fever, is a metabolic disorder in which homeostatic mechanisms fail to maintain normal blood calcium (**Ca**) concentrations at the onset of lactation (Goff and Horst, 1997). Blood Ca in the adult

cow is maintained between 2.0 and 2.5 mmol/L (8.5 and 10 mg/dL) (Jorgensen, 1974, Goff, 2008). Typically, the lowest blood Ca concentration occurs between 12 and 24 h after calving (Goff, 2008).

Clinical hypocalcemia incidence in the United States ranges from 4% (McLaren et al., 2006) to 6% and increases with increasing parity (Rajala-Schultz et al., 1999). However, nearly 25% of heifers and 50% of older cows will have blood Ca concentration < 2 mmol/L (Goff, 2008). Subclinical hypocalcemia (< 2 mmol/L serum within 48 h post-partum) occurred in 25%, 41%, 49%, 51%, 54%, and 42% of first, second, third, fourth, fifth, and sixth parity cows, respectively in a study conducted by Reinhardt et al. (2011). The most recent cost estimate for hypocalcemia was $166 for multiparous cows (Liang, 2013).

Ketosis is a disease related to carbohydrate and fat metabolism and is characterized by increased concentrations of ketone bodies, including β-hydroxybutyrate (**BHBA**), in blood, urine, and milk. The gold standard for determining subclinical ketosis status is the measurement of BHBA in blood plasma or serum (McLaren et al., 2006). The optimum BHBA cut-point based on maximum total sensitivity and specificity for clinical ketosis was 1.2 mmol/L in the first week post-partum (Seifi et al., 2011). Postpartum BHBA increases have been associated with decreased milk production (Duffield et al., 2009, McArt et al., 2012a) and increased risk for culling and displaced abomasum (Seifi et al., 2011, McArt et al., 2012b, a). Cows with BHBA concentrations ≥ 1.2 mmol/L were also 4.7 times more likely to develop clinical ketosis (Seifi et al., 2011). Higher producing cows are at greater risk of ketosis, which comes with a temporary milk yield decrease (Detilleux et al., 1994, Rajala-Schultz et al., 1999).

Clinical ketosis incidence in the United States is between 2 and 3% (McLaren et al., 2006, Seifi et al., 2011), but is significantly more common in third or greater parity cows (6%) (Seifi et al., 2011). In McLaren et al. (2006), subclinical ketosis incidence was cited as 54% in week one and 47% in week two post-partum. Subclinical ketosis incidence has been cited at 43% (McArt et al., 2012b). The cost per case of hypocalcemia, based on blood BHBA concentration ≥ 1.2 mmol/L, was estimated at $134, $111, and $117 for primiparous, multiparous, and all animals, respectively (McArt et al., 2016).

As average herd size increases, time producers can devote to each animal decreases (Schulze et al., 2007, Ipema et al., 2008, Brandt et al., 2010). Consumer pressure and concern for animal well-being and health, efficient and sustainable farming, food safety and quality, and control of zoonotic diseases, pathogens, and medical treatments has altered decision-making processes on farms (Berckmans, 2004, Schukken et al., 2008, Bewley, 2010). Dairy operations also have narrower profit margins than in the past because the government is less involved in regulating agricultural commodity prices. In turn, dairy producers need to increase efficiency, which can increase profit (Bewley, 2012). Because of these major industry shifts, on-farm decision making is changing and dairy cow monitoring tools will likely increase in importance (Berckmans, 2004, Schulze et al., 2007, Ipema et al., 2008) to help make decisions that previously were based solely on producer experience and judgement.

Precision dairy monitoring is the use of technologies to measure physiological, behavioral, and production indicators on individual animals to improve management and farm performance (Bewley, 2010. This type of management system relies on the

observation that the animal herself is the most important part of the biological production process at hand (Berckmans, 2004).

To date, precision dairy monitoring technology (**PDMT**) evaluations have focused mainly on automated estrus detection, aimed to supplement or replace visual estrus detection (Dolecheck et al., 2015). Precision dairy monitoring technologies also have the potential to detect disease early, maximizing individual animal potential. Disease detection in the past has relied on producers observing clinical signs, but once clinical signs are displayed, it is often too late to act effectively. Clinical signs are often preceded by physiological changes that are undetectable with human senses, but may be possible with PDMT and could allow producers to intervene sooner (Bewley, 2012).

The objectives of this study were: 1) to quantify changes in rumination time (**HRRUM**), lying time (**IQLT**), standing time (**IQSTAND**), lying bouts (**IQLB**), motion index (**IQMI**), number of steps (**IQSTEPS**), reticulorumen temperature (**DVMRT**), neck activity (**HRACT**), and milk yield (**MY**) around subclinical hypocalcemia (**SHCA**) subclinical ketosis (**SKET**), and clinical hypocalcemia (**CHCA**), clinical ketosis (**CKET**), and clinical metritis (**CMET**) during the first 14 DIM; 2) to evaluate differences in RU, LT, LB, RT, NA, and MY between cows with no disease, subclinical disease, and clinical disease; and 3) to determine the sensitivity and specificity of alerts created from RU, LT, LB, RT, NA, and MY for identification of fresh cow disease.

MATERIALS AND METHODS

This study was conducted from September 13, 2011 to May 3, 2013 at the University of Kentucky Coldstream Dairy. Directly after calving, cows were moved into a tie-stall barn equipped with 10 dual chamber waterbeds (Advanced Comfort

technology, Inc., Reedsburg, WI), covered with sawdust. If more than 10 study cows were housed at the same time, the newest cows were housed on rubber-filled mattresses, also covered with sawdust. Cows were allowed access to an exercise lot for 1 h/d at 1430, weather permitting. Cows had ad libitum access to feed and water in each stall. Lactating cows were fed the lactating cow ration consisting of corn silage, alfalfa hay, concentrate mix, whole cottonseed, and alfalfa silage at 0600 and 1330 daily. Cows were milked before the rest of the lactating herd 2X at 0430 and 1530, in one of two double-two bypass parlors located in the same building.

General cow demographic information was obtained from PCDart (Dairy Records Management Systems, Raleigh, NC) records. An intensive health evaluation occurred at 0800 ± 2 hours from 1 to 14 DIM for every cow in the herd that calved during the study period. Four researchers and farm employees, trained to conduct the health exams, were the only personnel to carry out the exams. Cows with two lactations within the study period remained in the study for both lactations. Every cow in the herd remained in the study from calving until 14 DIM and no voluntary culling occurred until cows left the study. Cows were required to have 14 consecutive daily health checks to remain on the study and cows that died before 14 DIM were removed from the study (n = 4). After the last health exam at 14 DIM, cows were removed from the study and joined the lactating herd housed in freestall barns.

Disease diagnosis

Serum calcium levels were evaluated on days 3, 7, and 14 postpartum. Approximately 10 mL of blood was collected aseptically from the coccygeal vein using a vacuum-sealed blood collection tube (Blood Collection Tube Vacutainer Glass 10 mL,

red; Becton Dickinson Canada Inc., Mississauga, ON, Canada) and 20-gauge needle (Needle Vacutainer Multiple Sample 21G × 1 in, Becton Dickinson Canada Inc., Mississauga, ON, Canada). The blood was then centrifuged and refrigerated until analysis at the University of Kentucky Veterinary Diagnostic Laboratory within 1 d. Calcium was analyzed using a Calcium-Arsenazo assay (ACE Alera, Alfa Wassermann Diagnostic Technologies, LLC, West Caldwell NJ). Subclinical hypocalcemia was defined as a serum Ca level < 8.55 ng/dL (Liboreiro et al., 2015).

Clinical hypocalcemia was determined based on signs described by Kelton et al. (1998). These signs included mild excitement without recumbency, nervousness, anorexia, weakness, and rapid heart rate, sternal recumbency, depression, fine muscle tremors, rapid heart rate, cold ears, decreased gastrointestinal activity, and dilated pupils, or lateral recumbency progressing to loss of consciousness, severe bloat, profound gastrointestinal atony, rapid heart rate, and a pulse that was difficult to detect.

On days 3, 7, and 14, BHBA concentration was measured in a whole-blood sample left over from the blood drawn for the Ca test. A Precision Xtra electronic handheld device (Abbott Laboratories, Chicago, IL, USA), validated by Iwersen et al. (2009), was used with ketone test strips that drew in 1.5 μL of blood into a sample well. Cows with BHBA ≥ 1.2 mmol/L were classified as SKET (Geishauser et al., 1998, McArt et al., 2012a, Kaufman et al., 2016). Clinical ketosis was defined as a cow with any or all of the following symptoms: decreased feed intake, reduced milk production, lethargy, an empty-appearing abdomen, dehydration, abnormal licking, chewing incessantly on inanimate objects, incoordination, gait abnormalities, aggression, and bellowing (Merck Veterinary Manual, 2005).

Cows were classified as having clinical metritis if they had pyrexia, fetid pus within the uterine lumen, vagina, or discharging from the vulva (Sheldon and Dobson, 2004). Discharge, if excreted, was examined daily. On days 3, 5, 7, 9, 11, and 14, cows were rectally palpated and the discharge was manually expelled for examination.

The herd manager treated clinical diseases following farm protocol. However, because evaluating treatment efficacy was not a goal of this research, treatment was not accounted for as a co-variate in any models.

Precision dairy technologies

The Milpro P4C (Milkline, Gariga di Podenzano, Italy) milking system provided milk yields per cow per milking. The DVM Systems, LLC (Boulder, CO) bolus system monitored DVMRT using a passive RFID transponder (Phase IV Engineering, Inc., Boulder, CO) equipped with a temperature sensor queried twice daily by a panel reader placed in parlor entrances. Boluses were inserted orally with a bolus gun. HR Tags (SCR Engineers Ltd., Netanya, Israel) measured HRACT with a 3-axis accelerometer and rumination time HRRUM with a microphone and microprocessor, summarized into 2-h time blocks. Cows were fitted with an HR tag, snugly hung around their necks. IceQubes (IceRobotics Ltd., Edinburgh, Scotland) measured IQLT, IQLB, IQSTAND, and IQMI with a 3-axis accelerometer, summarized into 15 min time blocks. IceQubes were strapped to each cow's left rear leg just above the fetlock.

All cows were fitted with all PDMT $\geq$ 21 d before enrolling in this study. All PDMT were monitored and replaced promptly when failure occurred, including dead batteries and broken tags. Data that was not already missing from these time periods were deleted. All computer clocks were set to synchronize with NIST Internet Time

Service (NIST, Gaithersburg, MD, USA) automatically, and time was manually verified on all computers on a weekly basis.

Hourly temperature and relative humidity were obtained from Kentucky Climate Data, calculated through the University of Kentucky College of Agriculture via a Campbell Scientific Inc. (Logan, UT) 23× data logger, located 5.63 kilometers from the farm. Temperature humidity index (**THI**) was computed using Eq. 2.1. The maximum THI for each day was used in all analyses.

THI = Temperature (°F) - (0.55 - (0.55 × Relative Humidity / 100)) × (Temperature (°F) - 58.8) (**Eq. 2.1**; NOAA, 1976).

Data cleaning and statistical analysis

Statistical analyses were conducted using SAS Version 9.3 (SAS Institute Inc., Cary, NC). Milk yields < or > 4 standard deviations from the previous week's average milk yield were removed, presumably caused by technology error from cow misclassification. To account for decreased reticulorumen temperature caused by water bouts, DVMRT were removed if < 38.3ºC and if they were less than 4 standard deviations from the previous week's average temperature.

Milk yield, IQLB, IQBD, IQSTAND, IQMOT, IQLT, HRRUM, and HRACT, were each summed individually to create one value per variable per cow per day. Temperature humidity index and DVMRT were averaged to create one value per variable per cow per day. If any variable amounted to 0 for the day, that variable was set as missing for that cow day.

Cow days were removed if < 90% of each day's IQLT, IQSTAND, IQMOT, HRRUM, and HRACT was recorded, but if a cow had > 90% of each day's data, linear

interpolation was used to include the missing 10% from that day. In cases where less than 24 hours of data were available, the percentage lying for that time period was used to calculate the percentage lying within 24 hours. The UNIVARIATE procedure was used on these variables and the 1st and 99th percentile of all variables were removed.

The previous day's data was used for each PDMT variable to account for the timing of data availability to producers. Because SKET was only measured on days 3, 7, and 14, all other days were removed from the SKET model to avoid the assumption that cows did not have SKET just because they were not sampled that day. Although SHCA and CHCA were monitored separately throughout the study, these diseases were combined to create one hypocalcemia (**HCA**) variable because only 8 cases of CHCA were identified. Therefore, all days remained in the model because CHCA was monitored daily, even though SHCA was only monitored on days 3, 7, and 14.

The GENMOD procedure of SAS was used to evaluate the effects of breed, MY, PG, THI, DVMRT, HRRUM, HRACT, IQLT, IQLB, IQSTAND, and IQMI on disease status. Disease status included: model 1) cows with SHCA, CHCA, or no hypocalcemia; model 2) cows with SKET, CKET, or no ketosis; and model 3) cows with CMET and cows without CMET. Cows without HCA, SKET, CKET, or CMET each day were considered to be in the no HCA, SKET, CEKT, or CMET groups, respectively, and were treated as the reference group for all analyses. The individual general linear models were used to screen for variables to include in the three multi-variable models and non-significant variables ($P \geq 0.10$) were not accounted for as co-variates in any models. Variables significant in these individual models were then included in a multi-variable model for each of the five disease models.

The LOGISTIC procedure of SAS was used to calculate ROC curves and determine probabilities of disease at each sensitivity and 1 – specificity. Probabilities that represented approximately 80% sensitivity, 95% sensitivity, 80% specificity, and 95% specificity were then used to determine alert levels in GENMOD. All four probabilities were used for each of the HCA, SKET, CKET, and CMET final multi-variable models in order to determine alerts at each probability. If a probability was greater than the probability associated with the respective sensitivity or specificity, an alert was created. Alerts were then used alongside human-detected disease detection in order to calculate sensitivity and specificity at the desired probabilities and to include these points on the ROC curves.

Correctly identified events are considered true positives (**TP**), non-alerted events are false negatives (**FN**), non-alerted non-events are true negatives (**TN**), and alerted non-events are false positives (**FP**) (Firk et al., 2002). Specificity is the probability that a negative sample is from a disease-negative cow. Sensitivity is the probability that a positive alert is a true indicator of a disease (Hamann and Zecconi, 1998, Sherlock et al., 2008, Hogeveen et al., 2010). Because sensitivity and specificity are interdependent, thresholds should be set to optimize both (Hogeveen et al., 2010). Accuracy can account for the prevalence of a disease whereas sensitivity and specificity cannot. Accuracy depends on how strongly and closely the measured parameters are associated with the event, how accurately the technology measures the parameters, and how well the manufacturer algorithm processes the data to create useful alerts (Dolecheck et al., 2015). Sensitivity, specificity, and accuracy for each final model were determined using Eq. 2.2, 2.3 and 2.4 (Sherlock et al., 2008, Hogeveen et al., 2010).

Specificity = TN / (TN + FP) x 100 (**Eq. 2.2**).

Sensitivity = TP / (TP + FN) x 100 (**Eq. 2.3**).

Accuracy = [(TP + TN) / (TP + TN + FP + FN) X 100] (**Eq. 2.4**).

RESULTS AND DISCUSSION

This study included 90 Holstein, 19 crossbred, and 11 Jersey cows for 137 lactations (17 cows entered the study for two lactations). Fifty-four percent of cows calved in a bedded pack maternity barn bedded with straw while 46% calved in the dry cow pasture. Forty-five percent of calves were bulls, 48% were heifers, 4% were male-female twins, 1% were female-female twins, and 1% were male-male twins. Most calvings did not require human assistance (79% and 21% for no help needed and human assistance required, respectively). Eighty-seven percent of calves were born alive while 13% were stillborn.

Means for each PDMT variable within each disease status are displayed in Table 2.1. Dairy cattle lying time has been referenced between 10.5 and 11 hours per cow per day (Ito et al., 2009, Bewley et al., 2010c, Cyples et al., 2012, Medrano-Galarza et al., 2012), which is greater than the mean of 9.56 h/d for all cow days in this study. Cows in this study were housed in an outdated tie-stall facility and cow comfort was poor, which may have altered lying times. Also, cows monitored in the referenced studies were not fresh and fresh cows may have different lying times than the rest of the herd, possibly caused by the stress of a new environment.

Mean HRRUM in this study were between 5.99 and 6.31 for multiparous and primiparous cows, respectively. In a previous study with the same herd, mean HRRUM was 6.4 h/d (Stone et al., 2016 In Review). This result was within the range of 4.8 to 8.4

h/d cited by Krause et al. (2002) and the 6.3 to 6.5 h/d range reported by Moallem et al. (2010). However, HRRUM was less than the 8 to 9 h/d recommendation cited by Welch (1982) and Soriani et al. (2013). Kaufman et al. (2016) cited collar-derived rumination times of 7 and 8 h for primiparous and multiparous cows, respectively. Maekawa et al. (2002) observed that primiparous cows ruminated almost 1 h/d less than multiparous in mid-lactation. However, the opposite was true in this study, where multiparous cows ruminated less than primiparous cows.

Fresh cow HRRUM may differ from later-lactation HRRUM. Rumination is affected by diet, including feed digestibility, neutral detergent fiber intake, forage quality (Welch and Smith, 1970), and particle size (Welch, 1982). Fresh cows switch from a dry cow to a lactating cow ration, which may be the cause of the lower rumination times. Because diet information is not included in manuscripts, comparing rumination times between studies is difficult. Rumination time also decreases with acute stress (Herskin et al., 2004) and disease (Welch, 1982, Hansen et al., 2003), which may also play a role in why fresh cow rumination times would be less than that of later lactation cows. Dairy personnel usually associate a positive relationship between daily feed intake and RU because greater intakes may require more ruminal processing time (Schirmann et al., 2012). Krause et al. (2002) explained that a positive relationship between long particle DMI and rumination time also exists. However, Canadian researchers discovered a negative relationship between RU and DMI in dry cows ($r = -0.18$; $P < 0.01$), possibly because cows cannot eat and ruminate at the same time.

Hypocalcemia

Reticulorumen temperature (DVMRT), IQLT, HRRUM, MY, and PG were significant predictors of hypocalcemia ($P = 0.09$, $P < 0.01$, $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively). Because IQSTAND and IQLT are directly related (24 hours – IQSTAND should = IQLT), IQSTAND was not included in the multi-variable model. The HCA sample size was only 8 in the multi-variable model so MY was removed in order to include 25 HCA cases. Within the multi-variable model, HRRUM was a significant predictor of hypocalcemia ($P < 0.01$), but IQLT, DVMRT, and PG were not ($P = 0.08$, $P = 0.13$, and $P = 0.38$, respectively). Lying time was a significant predictor in the model ($P < 0.01$).

Cows with decreased HRRUM of 1 h/d were 0.64 times less likely to have HCA than cows without the increased lying time (Table 2.2). The area under the curve for this multi-variable model was 0.84 (Figure 2.1), implying that this model was a good fit for detecting HCA. This result is in agreement with a study where induced HCA resulted in reduced rumination time, possibly related to the anti-peristaltic esophageal movements during rumination (Hansen et al., 2003) or decreased ruminal contractions (Jorgensen et al., 1998) because Ca is required for muscle contractions (Hansen et al., 2003).

Sensitivities, specificities, and accuracies of each multi-variable model are displayed in Table 2.3. The threshold that produced the best accuracy in detecting HCA was still only 57.53%, with 93.88% sensitivity and 17.05% specificity. Although this sensitivity means only 6 out of 100 cows with HCA are missed, it also means only 17 of the 100 cows a producer checks actually have HCA.

Ketosis

In the SKET univariate models, IQMI, IQSTEPS, and THI were significant predictors ($P < 0.01$, $P < 0.01$, and $P = 0.03$, respectively). Only IQSTEPS was a significant predictor of subclinical ketosis in the multi-variable model ($P < 0.01$, $P = 0.29$, and $P = 0.16$, for IQSTEPS, THI, and IQMI, respectively). The area under the curve shown in Figure 2.2 was only 0.61, signifying that IQSTEPS alone was not much better than a coin toss to detect SKET.

In the CKET univariate models, IQSTEPS and HRACT were significant predictors of CKET ($P = 0.09$ and $P = 0.08$, respectively). However, only 2 cows with clinical ketosis remained in the model because HRACT data was missing compared to 27 cases when only IQSTEPS was included. When HRACT was removed from the model to leave only IQSTEPS, IQSTEPS was no longer considered significant at $P = 0.09$. The area under the curve shown in Figure 2.3 was only 0.60.

Higher producing cows have been referred to at greater risk of ketosis, which comes with a temporary MY decrease, so if they do not develop ketosis their milk yield would be even greater (Detilleux et al., 1994, Rajala-Schultz et al., 1999a). In this study, MY was not a predictor of CKET or SKET, however.

Itle et al. (2014) explained that daily standing time was 20% longer for cows later diagnosed with CKET during the week before calving (14.3 ± 0.6 vs. 12.0 ± 0.7 h/d), but no differences were observed postpartum. Cows later diagnosed with CKET also stood up fewer times (14.6 vs. 20.9 bouts/d) and stood for longer periods (71.3 min/bout vs. 35.8 min/bout) than cows without clinical ketosis on the day of calving (Itle et al., 2014). Cows with ketosis behave in a subordinate fashion (Itle et al., 2014), causing them to be less motivated to engage in behaviors that are energetically expensive like changing

position from lying to standing (Susenbeth et al., 2004) or competing for feed (Goldhawk et al., 2009). Other researchers cited that postpartum activity was reduced among cows that were diagnosed with subclinical ketosis (502.20 ± 16.5 vs. 536.6 ± 6.2) (Liboreiro et al., 2015), which may explain why IQSTEPS was significant in the screening model. However, HRACT was not significant in the screening model. Activity may have been hindered because the cows were housed in a tie-stall instead of a freestall or open pack facility.

Kaufman et al. (2016) explained that the odds of a cow getting ketosis and another health problem increased when rumination time decreased from 1 week before calving to one week after. However, the authors explained that the increase postpartum may represent changes in dry matter intake. Rumination time (HRRUM) was not significant in the screening models for SKET or CKET, which may be because pre-partum monitoring was not conducted and it may be difficult to detect differences without having a longer baseline before calving.

The threshold that produced the best accuracy in detecting CKET was 96.55%, with 97.37% sensitivity and 6.06% specificity. The threshold that produced the best accuracy in detecting SKET was 93.56%, with 98.14% sensitivity and 5.77% specificity. Although these sensitivities mean only 3 and 2 out of 100 cows with CKET and SKET, respectively, are missed, it also means only 6 of the 100 cows a producer checks actually have CKET or SKET.

Metritis

Standing time (IQSTAND), IQLT, and HRACT were significant predictors of CMET ($P = 0.01$, $P < 0.01$, and $P < 0.01$, respectively. Because IQSTAND and IQLT

are similar, IQSTAND was not included in the multi-variable model. When the multi-variable model was evaluated with HRACT and IQLT, only 2 cases of metritis remained in the model, due to missing data from HRACT. Therefore, the final model left only IQLT so that 22 out of the 30 possible cases could be included. Lying time was a significant predictor in the model ($P < 0.01$). Cows with decreased IQLT of 1 h/d were 0.46 times more likely to have CMET. However, the area under the curve shown in Figure 2.4 was only 0.66, implying that IQLT was probably not the best predictor of metritis possible.

Cook et al. (2007) explained that cows with greater locomotion scores (using a 1 to 4 scale where 1 represents non-lame and 4 represents severely lame) lied down more, indicating that pain may increase lying time. To the author's knowledge, studies evaluating pain in cows with CMET have not been conducted, but there may be pain associated with this disease which would explain why IQLT was significant.

Liboreiro et al. (2015) explained that cows diagnosed with metritis had reduced postpartum activity (512.5 ± 11.5 vs. 539.2 ± 6.0 arbitrary unit). The results of the screening CMET model also show that HRACT was a significant predictor of CMET. Unfortunately, HRACT data was too sparse on days when cows had CMET to include it in the multi-variable model and thus a comparison cannot be made with this study. Contrary to the results of this study, Liboreiro et al. (2015) explained that cows diagnosed with metritis had reduced postpartum daily rumination time (6.93 vs. 7.35 h/d).

The threshold that produced the best accuracy in detecting CMET was 86.00%, with 86.24% sensitivity and 51.85% specificity. While these results are not ideal, they

unfortunately are the best combination of sensitivity, specificity, and accuracy determined through any of the fresh cow diseases monitored in this study.

Limitations

Sensitivity and specificity of a disease detection tool depend on the disease definition (Nielen et al., 1995) and time window (Mollenhorst et al., 2012) in which alerts can be given. The results of a survey of 139 Dutch producers who owned an automated milking system revealed that farmers preferred a clinical mastitis detection system that produced few false alerts and provided alerts for severe cases with enough time to take effective treatment action. Producers preferred that time windows were set at a maximum of 24 hours before clinical symptoms appeared (Mollenhorst et al., 2012). This timing was also agreed upon by Rasmussen (2002) who suggested time windows within 24 to 48 hours of the event. Wider time windows will produce a higher sensitivity and specificity (Hogeveen et al., 2010b, Kamphuis et al., 2010), but they will also lose their practicality in a commercial setting (Kamphuis et al., 2010). A 24-hour alert time window was chosen in this study for this reason.

Rasmussen (2002) suggested that a clinical mastitis system should provide 80% sensitivity and 99% specificity. Although the systems in this study were not used for clinical mastitis detection, similar expectations may be applied to other disease detection models also. All of the models reached at least 80% sensitivity, but none of the models used in this study reached the specificity goal along with the sensitivity goal. These goals may be too lofty at this point in the PDMT research stage, but there may be PDMT or algorithms that are able to reach them also.

Sensitivity and specificity will also be less if a new test disagrees with the comparison to the gold standard. Disagreement between the gold standard and a new test is often interpreted as the test lacking capability. However, the test could be better at detecting negatives, causing true negatives to display as false negatives (Nielen et al., 1992). This problem is made even more complex by the circumstance that neither the new test nor the gold standard detection methods may be ideal (Vickers et al., 2010). A universally accepted gold standard does not exist, though. Another limitation of an automated disease detection method is that clinical infections are infrequent, causing statistical analyses to be "weak" (Mein and Rasmussen, 2008).

Examples IQLT for a cow with no diagnosed fresh cow disease, with SKET, and with all the diseases monitored in this study (SKET, CKET, CMET, and HCA) throughout the fresh period are displayed in Figure 2.5. Differences in the three example cows are obvious in Figure 2.5a, but these are only one variable on three cows and do not take into account the cows who did not show as much variation around disease. The cow in Figure 2.5b had all the diseases monitored in this study (SKET, CKET, CMET, and HCA) at some point in her 14-day study period. This cascade of disease events is common in fresh cows and discerning which changes in each variable are a result of which disease difficult. Cows may be recovering from one disease while they spiral into the next, not allowing time to recover and establish a new baseline to deviate from in order to be detected by a technology. The cow in Figure 2.5d did not have any of the fresh cow diseases monitored in this study, yet still had variation in IQLT throughout her fresh period.

The cow in Figure 2.5c had SKET on days 3, 7, and 14, when BHBA was measured. However, it is plausible that she had SKET on at least some of the days between also. Blood samples for both SKET and SHCA were only obtained three times throughout the study for each cow, which was likely not often enough to detect all cows with these diseases. Because clinical disease was monitored daily, days that disease went undetected were considered non-disease days. However, some clinical diseases may have been overlooked or misclassified. Postpartum cow diseases tend to build upon each other and some share similar signs, making distinction difficult. Future studies should focus on a smaller time window within the fresh period and take more frequent diagnosis samples to ensure no diseases are missed.

Several cows were treated before they showed signs of HCA because the herd manager knew each cow's history and tried to be pro-active in disease prevention. Clinical diseases were also treated, which may have affected the rest of the fresh period for those cows. Although this may have skewed the data for those cows, the welfare of the animal was considered a higher priority.

CONCLUSIONS

Some of the variables evaluated in this paper may be useful in detecting hypocalcemia, ketosis, and metritis. However, the best area under the curve evaluated in this study was still only 0.84, implying that the best possible combination of variables was not achieved. The generalized linear models for HCA, SKET, CKET, and CMET all included at least one variable from IceQubes, indicating this PDMT may be useful in detecting the diseases evaluated. Technology manufacturers should continue to seek ways to monitor multiple variables at once and to improve upon the variables they

already monitor. The PDMT used in this study have progressed from the time of this study and may be better able to predict disease than they were at this time. Beyond refinements in what information is collected within each technology, actually collecting that data consistently is very important. Missing data from both technology and human error required variables to be excluded from models, which may have provided better results if those variables had been included. Overall, using PDMT to predict hypocalcemia, ketosis, and metritis is promising, but needs future work into evaluating the best variables and the best statistical methodology.

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Table 2.1. Health status summary of cows enrolled in the intensive health cow checks from 1 to 14 DIM during a study evaluating the associations of rumination time, activity, reticulorumen temperature, lying behavior, and milk yield of cows with subclinical and clinical ketosis, hypocalcemia, and mastitis, and clinical metritis.[1 - 12]

Variable	Subclinical ketosis[6]	Clinical ketosis[7]	Hypocalcemia[8]	Clinical metritis[9]	No disease[10]
n cows diagnosed, cow days	208	33	176	30	-
Mean ± SD d diagnosed, DIM	7.09 ± 4.07	6.52 ± 3.72	6.8 ± 3.68	5.96 ± 3.33	-
Mean ± SD rumination time, h/d[1]	5.49 ± 1.93	6.47 ± 2.04	5.20 ± 1.77	315 ± 133.15	6.08 ± 1.90
Mean ± SD neck activity, activity units/d[2]	205.68 ± 80.43	281.85 ± 25.10	273.52 ± 64.73	141.80 ± 0.00	254.90 ± 69.59
Mean ± SD reticulorumen temperature, °C[3]	39.04 ± 1.16	39.32 ± 0.69	38.99 ± 0.59	39.36 ± 0.56	39.05 ± 1.26
Mean ± SD lying time, h/d[4]	10.37 ± 3.43	9.49 ± 3.58	10.33 ± 3.94	11.41 ± 3.11	9.51 ± 3.13
Mean ± SD standing time, h/d	13.25 ± 3.37	14.25 ± 3.42	13.01 ± 3.94	12.39 ± 3.11	13.83 ± 3.44
Mean ± SD number of steps, steps/d	716.12 ±	704.04 ±	895.49 ±	718.54 ±	901.53 ±

	450.95	388.14	529.27	548.74	607.70
Mean ± SD motion index, units/d	3092.37 ± 2049.37	3819.44 ± 4524.06	3763.83 ± 2064.78	3196.83 ± 2398.78	3737.70 ± 2563.80
Mean ± SD milk yield, kg/d[5]	27.60 ± 8.20	31.63 ± 6.12	25.09 ± 8.67	25.45 ± 10.65	28.92 ± 7.39

[1]HR Tags (SCR Engineers Ltd., Netanya, Israel) measured rumination time with a microphone and microprocessor.

[2]HR Tags (SCR Engineers Ltd., Netanya, Israel) measured neck activity with a 3-axis accelerometer.

[3]The DVM Systems, LLC (Boulder, CO) bolus system monitored reticulorumen temperature using a passive RFID transponder (Phase IV Engineering, Inc., Boulder, CO) equipped with a temperature sensor queried twice daily by a panel reader placed in parlor entrances.

[4]IceQubes (IceRobotics Ltd., Edinburgh, Scotland) measured lying time, standing time, number of steps, and motion index with a 3-axis accelerometer.

[5]The Milpro P4C (Milkline, Gariga di Podenzano, Italy) milking system provided milk weights per cow per milking, which were summed to obtain the daily milk weight.

[6]Subclinical ketosis was defined as cows with BHBA ≥1.2 mmol/L, obtained on days 3, 7, and 14 post-partum.

[7]Clinical ketosis was defined as a cow with any or all of the following symptoms: decreased feed intake, reduced milk production, lethargy, an empty-appearing abdomen, dehydration, abnormal licking, chewing incessantly on inanimate objects, incoordination, gait abnormalities, aggression, and bellowing.

[8]Subclinical hypocalcemia was defined as a serum Ca level < 8.55 ng/dL, obtained on days 3, 7, and 14 post-partum. Clinical hypocalcemia represented a cow with mild excitement without recumbency, nervousness, anorexia, weakness, and rapid heart rate, a cow with sternal recumbency, depression, fine muscle tremors, rapid heart rate, cold ears, decreased gastrointestinal activity, and dilated pupils, or a cow with lateral recumbency progressing to loss of consciousness, severe bloat, profound gastrointestinal atony, rapid heart rate, and a pulse that was difficult to detect.

[9]Clinical metritis was defined as a cow with non-clear and thick uterine fluid, examined through rectal palpation and discharge expulsion.

[10]No disease represented a cow with no subclinical or clinical hypocalcemia, ketosis, or mastitis, or clinical metritis between 1 and 14 DIM.

[11]The previous day's data was used to account for the timing of data availability to producers.

[12]Cow day data included in each disease status column were included only on the day(s) they were categorized in that disease status. Cows could be included in multiple disease columns throughout their 14 d fresh period.

Table 2.2. Odds ratios of cows having clinical metritis, clinical ketosis, subclinical ketosis, or hypocalcemia based on precision dairy monitoring technology variables for factors associated with the incidence of each disease compared to cows without the disease.[1-9]

Disease	Variable	Odds ratio	95% Confidence interval		*P*-value
Clinical metritis[1]	IQLT, h/d	0.46	0.44	0.48	< 0.01
Clinical ketosis[2]	IQSTEPS, steps/d	0.50	0.50	0.50	0.09
Subclinical ketosis[3]	THI	0.50	0.50	0.51	0.44
Hypocalcemia[4]	IQMI	0.50	0.50	0.50	0.07
	IQSTEPS	0.50	0.50	0.50	0.35
	DVMRT	0.70	0.37	0.90	0.23
	IQLT	0.44	0.37	0.51	0.09
	HRRUM	0.64	0.52	0.75	0.03
	PG	0.74	0.19	0.97	0.41

[1]Clinical metritis was defined as a cow with non-clear and thick uterine fluid, examined through rectal palpation and discharge expulsion

[2]Clinical ketosis was defined as a cow with any or all of the following symptoms: decreased feed intake, reduced milk production, lethargy, an empty-appearing abdomen, dehydration, abnormal licking, chewing incessantly on inanimate objects, incoordination, gait abnormalities, aggression, and bellowing.

[3]Subclinical ketosis was defined as cows with BHBA ≥1.2 mmol/L, obtained on days 3, 7, and 14 post-partum.

[4]Subclinical hypocalcemia was defined as a serum Ca level < 8.55 ng/dL, obtained on days 3, 7, and 14 post-partum. Clinical hypocalcemia represented a cow with mild excitement without recumbency, nervousness, anorexia, weakness, and rapid heart rate, a cow with sternal recumbency, depression, fine muscle tremors, rapid heart rate, cold ears, decreased gastrointestinal activity, and dilated pupils, or a cow with lateral recumbency progressing to loss of consciousness, severe bloat, profound gastrointestinal atony, rapid heart rate, and a pulse that was difficult to detect.

[5]IceQubes (IceRobotics Ltd., Edinburgh, Scotland) measured lying time (IQLT), motion index (IQMI), and number of steps (IQSTEPS) with a 3-axis accelerometer.

[6]The DVM Systems, LLC (Boulder, CO) bolus system monitored reticulorumen temperature using a passive RFID transponder (Phase IV Engineering, Inc., Boulder, CO) equipped with a temperature sensor queried by a panel reader placed in parlor entrances.

[7]HR Tags (SCR Engineers Ltd., Netanya, Israel) measured rumination time with a microphone and microprocessor.

[8]Hourly temperature and relative humidity were obtained from Kentucky Climate Data, calculated through the University of Kentucky College of Agriculture via a Campbell Scientific Inc. (Logan, UT) 23× data logger, located 5.63 kilometers from the farm.

[9]Parity group represented multiparous or primiparous cows.

Table 2.3. Sensitivity and specificity of rumination time, activity, reticulorumen temperature, lying time, and lying bouts on each disease using different alert thresholds for disease detection.[1-4]

Type of subclinical mastitis	Probability (alert threshold)	Sensitivity (%)	Specificity (%)	Accuracy
Clinical ketosis	0.009866	57.23	84.85	57.46
	0.013163	61.28	81.82	61.45
	0.021594	85.96	30.30	85.50
	0.026482	97.37	6.06	96.55
Subclinical ketosis	0.15682	98.14	5.77	93.56
	0.12586	85.75	37.02	83.34
	0.07178	58.89	88.94	60.38
	0.04685	55.30	90.87	57.07
Hypocalcemia	0.82433	97.45	7.95	55.10
	0.49557	93.88	17.05	57.53
	0.33468	89.80	19.32	56.45
	0.18152	84.69	22.16	55.10
Clinical metritis	0.01916	86.24	51.85	86.00
	0.01566	76.67	66.67	76.60
	0.01273	68.11	85.19	68.22
	0.01249	67.32	85.19	67.44

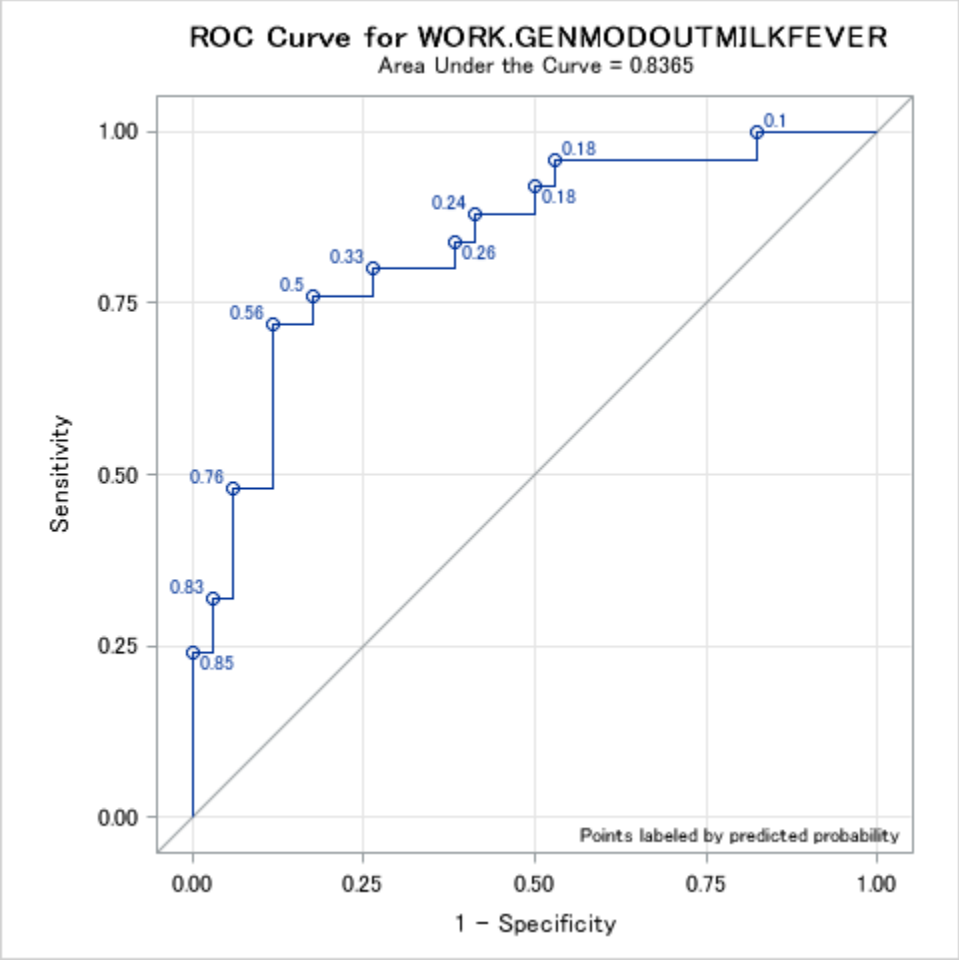
[1]Clinical ketosis was defined as a cow with any or all of the following symptoms: decreased feed intake, reduced milk production, lethargy, an empty-appearing abdomen, dehydration, abnormal licking, chewing incessantly on inanimate objects, incoordination, gait abnormalities, aggression, and bellowing.

[2]Subclinical ketosis was defined as cows with BHBA ≥1.2 mmol/L, obtained on days 3, 7, and 14 post-partum.

[3]Subclinical hypocalcemia was defined as a serum Ca level < 8.55 ng/dL, obtained on days 3, 7, and 14 post-partum. Clinical hypocalcemia represented a cow with mild excitement without recumbency, nervousness, anorexia, weakness, and rapid heart rate, a cow with sternal recumbency, depression, fine muscle tremors, rapid heart rate, cold ears, decreased gastrointestinal activity, and dilated pupils, or a cow with lateral recumbency progressing to loss of consciousness, severe bloat, profound gastrointestinal atony, rapid heart rate, and a pulse that was difficult to detect.

[4]Clinical metritis was defined as a cow with non-clear and thick uterine fluid, examined through rectal palpation and discharge expulsion.

Figure 2.1. ROC curve for the final GENMOD model evaluating the effects of reticulorumen temperature, lying time, rumination time, and parity group in cows with hypocalcemia versus cows without hypocalcemia.[1-4]



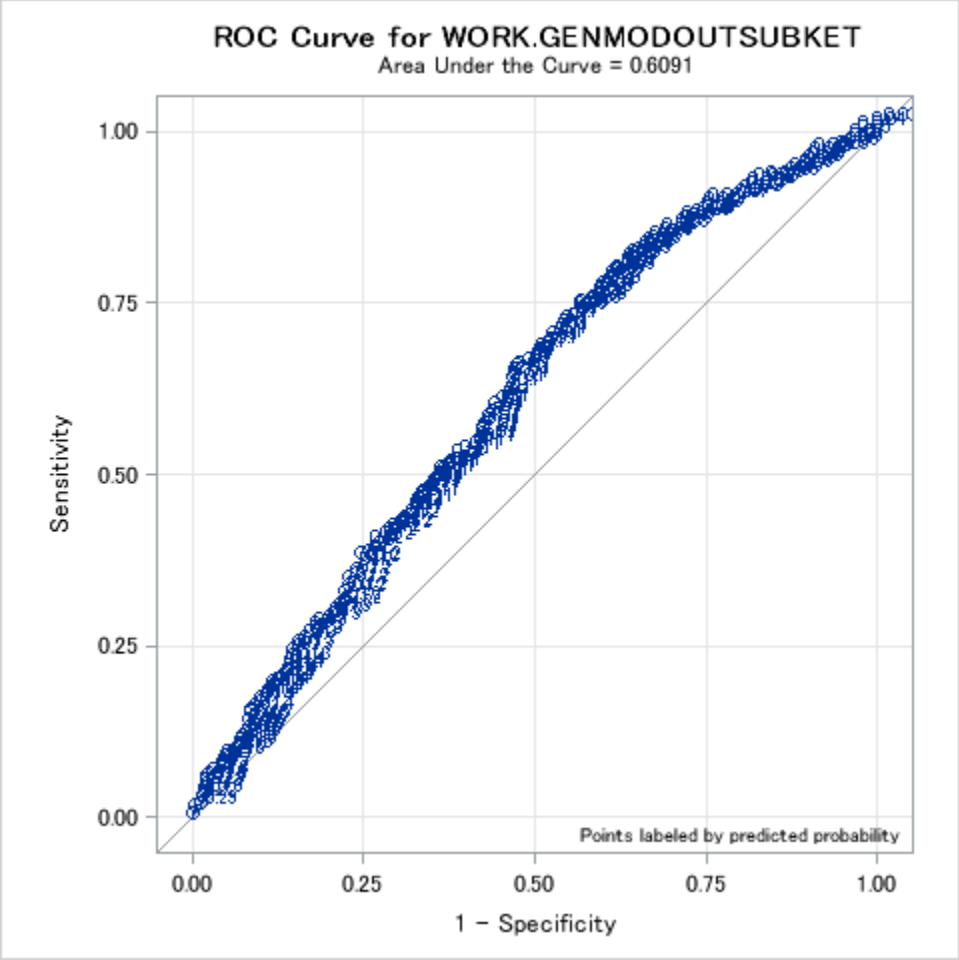
[1]The DVM Systems, LLC (Boulder, CO) bolus system monitored reticulorumen temperature using a passive RFID transponder (Phase IV Engineering, Inc., Boulder, CO) equipped with a temperature sensor queried by a panel reader placed in parlor entrances.

[2]IceQubes (IceRobotics Ltd., Edinburgh, Scotland) measured lying time with a 3-axis accelerometer.

[3]HR Tags (SCR Engineers Ltd., Netanya, Israel) measured rumination time with a microphone and microprocessor.

[4]Parity group represented multiparous or primiparous cows.

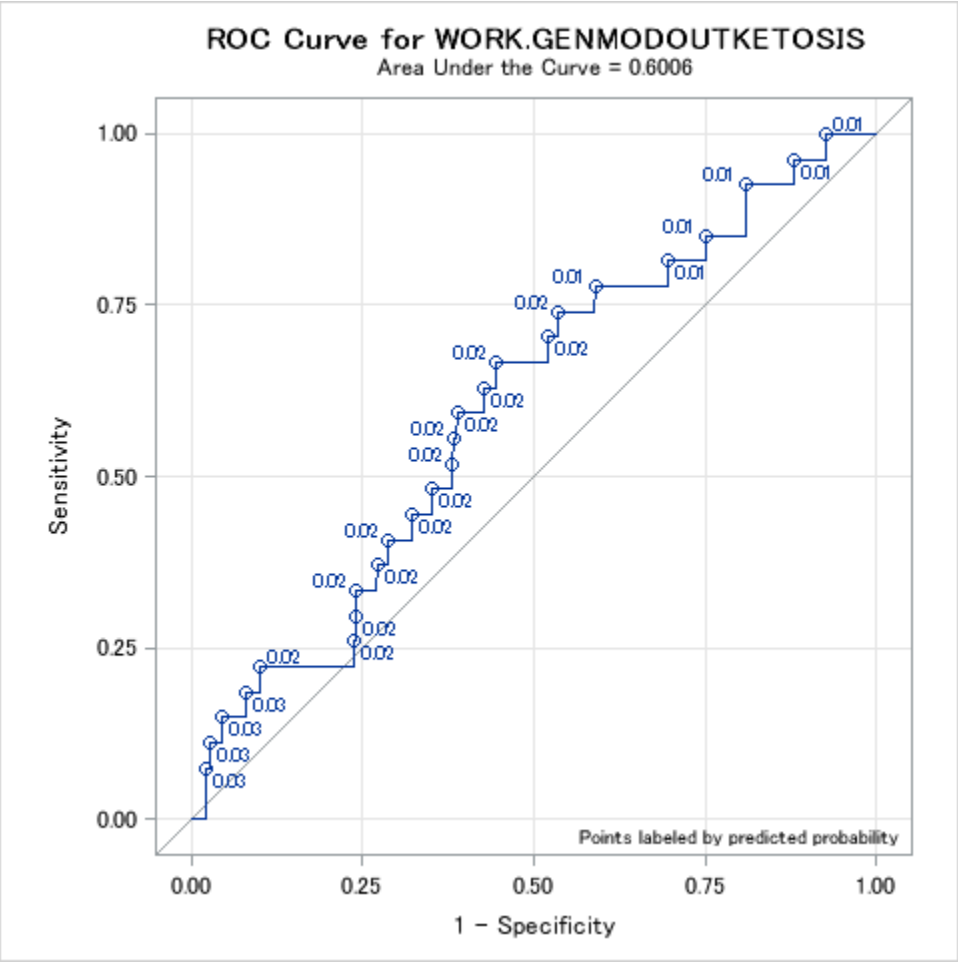
Figure 2.2. ROC curve for the final GENMOD model evaluating the effects of temperature humidity index, motion index, and number of steps in cows with subclinical ketosis versus cows without subclinical ketosis.



[1]Hourly temperature and relative humidity were obtained from Kentucky Climate Data, calculated through the University of Kentucky College of Agriculture via a Campbell Scientific Inc. (Logan, UT) 23× data logger, located 5.63 kilometers from the farm.

[2]IceQubes (IceRobotics Ltd., Edinburgh, Scotland) measured motion index and number of steps with a 3-axis accelerometer.

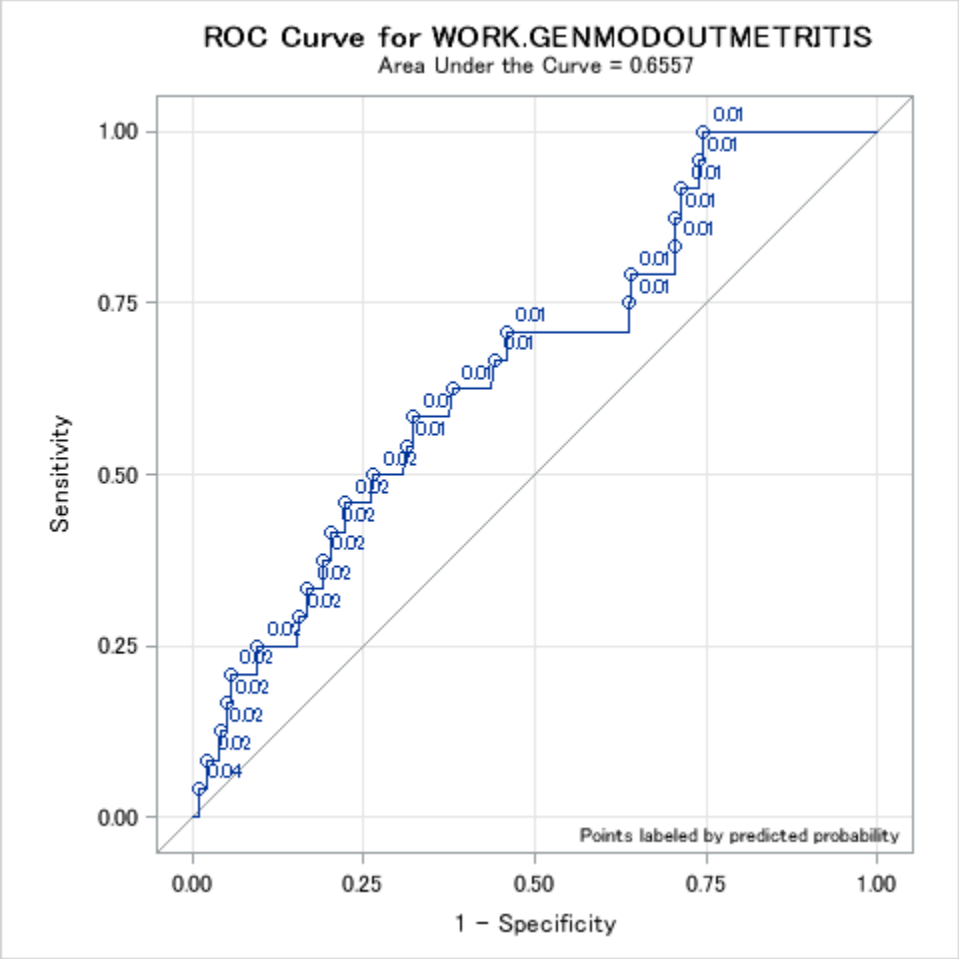
Figure 2.3. ROC curve for the final GENMOD model evaluating the effects of neck activity and number of steps in cows with clinical ketosis verus cows without clinical ketosis.



[1] HR Tags (SCR Engineers Ltd., Netanya, Israel) measured neck activity with a 3-axis accelerometer.

[2]IceQubes (IceRobotics Ltd., Edinburgh, Scotland) measured number of steps with a 3-axis accelerometer.

Figure 2.4. ROC curve for the final GENMOD model evaluating the effects of lying time in cows with clinical metritis versus cows without clinical metritis.[1-2]



[1]IceQubes (IceRobotics Ltd., Edinburgh, Scotland) measured lying time with a 3-axis accelerometer.

[2]Clinical metritis was defined as a cow with non-clear and thick uterine fluid, examined through rectal palpation and discharge expulsion.

Figure 2.5a. Three cows displaying different lying times around multiple diseases, or lack of, during the fresh period.[1-6]

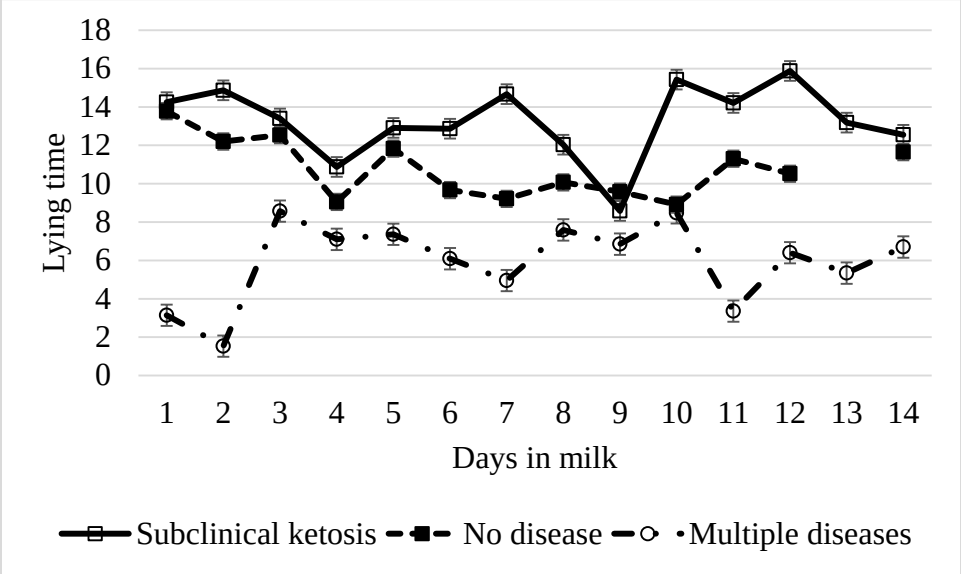


Figure 2.5b. Lying times of an example cow around multiple diseases during her fresh period.[1-6]

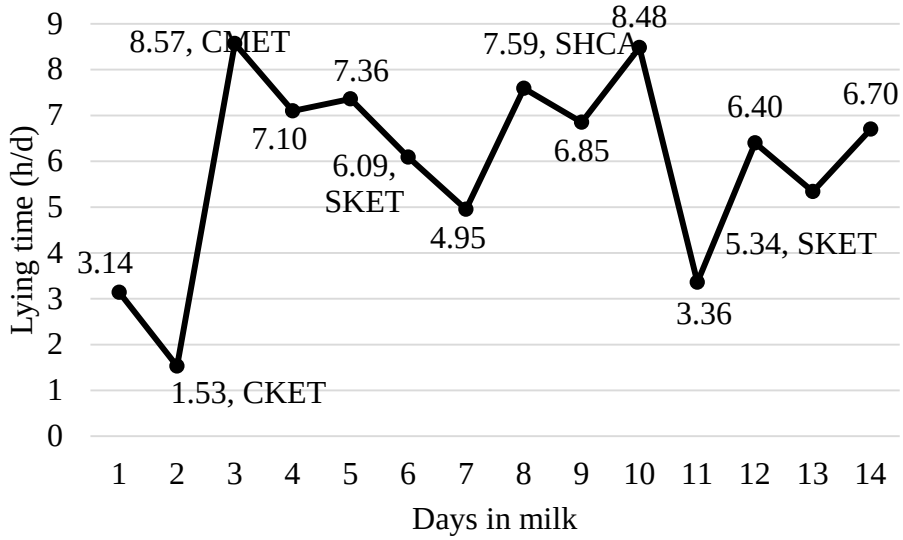


Figure 2.5c. Lying times of an example cow around subclinical ketosis events, meaured with BHBA on days 3, 7, and 14 DIM.[1-6]

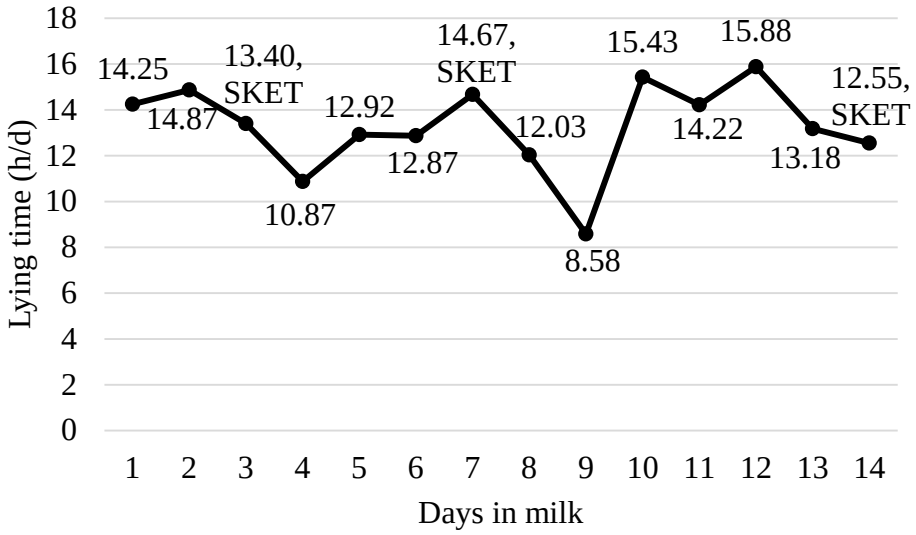
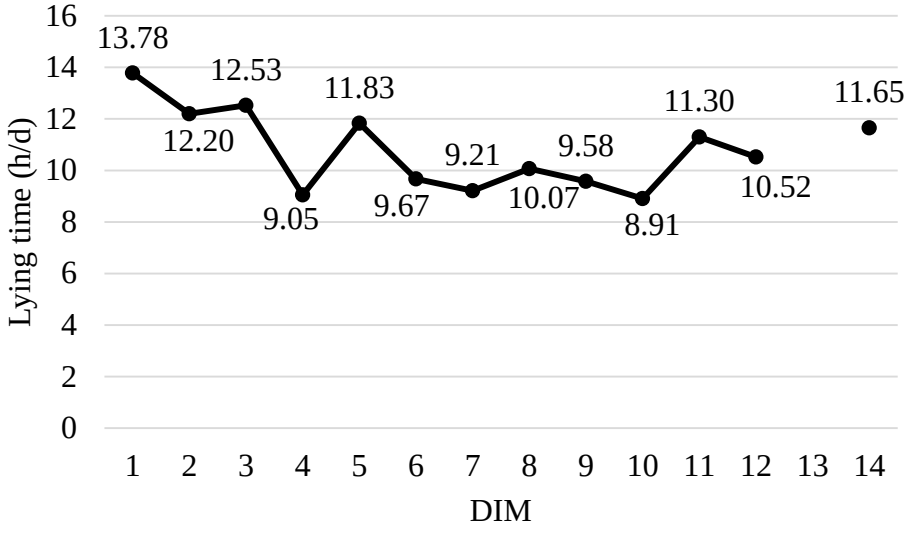


Figure 2.5d. Lying times of an example cow without subclinical ketosis or hypocalcemia, or clinical ketosis, hypocalcemia, or metritis throughout her fresh period.[1-6]



[1]Figure 2a is the overlay of Figures 2b, c, and d to show the differences of each different disease pattern in the example cows.

[2]IceQubes (IceRobotics Ltd., Edinburgh, Scotland) measured lying time with a 3-axis accelerometer. Predicted lying times from the MIXED procedure of SAS are displayed.

[3]Clinical ketosis (CKET) was defined as a cow with any or all of the following symptoms: decreased feed intake, reduced milk production, lethargy, an empty-appearing abdomen, dehydration, abnormal licking, chewing incessantly on inanimate objects, incoordination, gait abnormalities, aggression, and bellowing.

[4]Subclinical ketosis (SKET) was defined as cows with BHBA ≥1.2 mmol/L, obtained on days 3, 7, and 14 post-partum.

[5]Subclinical hypocalcemia (SHCA) was defined as a serum Ca level < 8.55 ng/dL, obtained on days 3, 7, and 14 post-partum. Clinical hypocalcemia (CHCA) represented a cow with mild excitement without recumbency, nervousness, anorexia, weakness, and rapid heart rate, a cow with sternal recumbency, depression, fine muscle tremors, rapid heart rate, cold ears, decreased gastrointestinal activity, and dilated pupils, or a cow with lateral recumbency progressing to loss of consciousness, severe bloat, profound gastrointestinal atony, rapid heart rate, and a pulse that was difficult to detect.

[6]Clinical metritis (CMET) was defined as a cow with non-clear and thick uterine fluid, examined through rectal palpation and discharge expulsion.

Chapter 3:

Evaluation of neck and leg activity, feeding time, lying time, rumination time, reticulorumen temperature, and milk yield, conductivity, lactose, protein, and fat percent to detect subclinical mastitis

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INTRODUCTION

Mastitis is an inflammatory reaction of udder tissue, usually caused by a bacterial infection in the mammary gland (Harmon, 1994, Sordillo et al., 1997, Oliver and Murinda, 2012). This disease alters udder secretory processes, lowers milk yield, and changes milk composition (Beck et al., 1992, Harmon, 1994). Mastitis has a detrimental effect on epithelial tissues and may destroy some secretory cells (Harmon and Heald, 1982, Beck et al., 1992). Additionally, mastitis may compromise animal welfare because of the resulting discomfort and pain (Medrano-Galarza et al., 2012, Fitzpatrick et al., 2013).

Dairy industry personnel generally accept that economic losses resulting from mastitis are sizable (Beck et al., 1992, Hogeveen et al., 2011). Dairy cattle economic efficiency is closely related to milk yield (Dohoo and Martin, 1984) and mastitis has a long lasting negative effect on milk yield (Rajala-Schultz et al., 1999a). Even after an infection is cured, milk yield remains depressed (Bar et al., 2008) and cows may be unable to reach their pre-mastitis milk yield (Rajala-Schultz et al., 1999a).

Subclinical mastitis constitutes an animal with an udder infection but no visible health changes. Because it cannot be detected by the human eye, cytological, biochemical and bacteriological milk tests are the only way to detect it (Bramley et al., 1996, Janzekovic et al., 2009). Subclinical mastitis is the most prevalent form of mastitis most herds experience, but many producers are unaware of the consequences of this disease because there are no outward signs. Subclinical mastitis causes the greatest overall loss to dairy producers because of decreased production as these cases may go undetected (Bramley et al., 1996).

Early intervention may set a cow up to produce more milk and remain healthier throughout her lactation (Aalseth, 2005). Proactive action may also decrease antibiotic use, which may decrease the chance of antibiotic residues in the bulk tank (Oliver and Murinda, 2012). Automated dairy cattle behavioral, physiological, and production monitoring systems, or precision dairy monitoring technologies, may be useful for early mastitis detection. Precision dairy monitoring technologies (**PDMT**) include sensors that monitor activity, body temperature, feeding behavior, location, lying behavior, milk parameters (yield, electrical conductivity, lactose, lactate dehydrogenase, blood, color, and SCC), and rumination time. Each of these parameters has mastitis detection potential because mastitis can affect dairy cattle behavior and physiology.

The primary objective of this study was to evaluate variation in neck and leg activity, feeding time, lying time, rumination time, reticulorumen temperature, and milk yield, lactose, protein, and fat percent around subclinical mastitis events. The secondary objective was to evaluate the sensitivity, specificity, and accuracy of alerts created from activity, feeding time, lying time, rumination time, milk yield and components, and body temperature in detecting subclinical mastitis.

MATERIALS AND METHODS

This study was conducted at the University of Kentucky Coldstream Research farm from May 8, 2015 to September 11, 2015. Every cow in the lactating herd remained on the study for its entirety or until they were dried off or left the herd. General cow demographic information was obtained from PCDart (Dairy Records Management Systems, Raleigh, NC) records.

Lactating cows were housed in two freestall barns with one barn of 54 dual chamber waterbeds (Advanced Comfort technology, Inc., Reedsburg, WI) and the other equipped with 54 rubber-filled mattresses, all covered with sawdust. Cow groups were balanced between barns by DIM and parity. Cows had access to fresh water from automatic fill Rubbermaid 150 gallon tanks. Cows had access to an exercise lot for about 1 h/d at 1000, weather permitting.

Cows had ad libitum access to water in each barn and shared a feedbunk between barns. Lactating cows were fed the same ration consisting of corn silage, alfalfa hay, concentrate mix, whole cottonseed, and alfalfa silage at 0600 and 1330 daily. Cows were milked 2X at 0430 and 1530. The milking routine included forestripping, pre-dipping with 0.5% iodine, drying teats with individual cloth towels, unit attachment, automatic takeoff, and post-dipping with 1% iodine. Cows received Tomorrow (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and Orbeseal (Zoetis Services LLC, Florham Park, NJ) in each quarter 45 to 60 days before their next calving due date and were housed in a pasture with access to a bedded pack barn during the dry period.

During analysis, cows were divided into two parity groups (**PG**), representing primiparous and multiparous cows. The herd manager was notified of cows with clinical mastitis and they were treated according to farm protocol, but remained in the study regardless of their treatment regimen. Data from cows with clinical mastitis were not included in the study the day of and 14 d after clinical detection to ensure no effects of clinical mastitis were still present. A cow was considered to have clinical mastitis if she had a visually abnormal milk secretion (e.g. clots, flakes, or watery milk) from one or more quarters as detected by the milkers at each milking. Milk clots were also detected

by examining the Ambic dairy cow mastitis detector (Coburn Company, Whitewater WI), connected to the milk hose, after each cow was milked. Cows < 22 DIM were removed from this study because this period is complicated by many other diseases that may affect the parameters of interest in this study (fresh cow disease detection using the same PDMT on the same herd is explained in Tsai et al., 2016 unpublished). Cows > 400 DIM were also removed because their behavior may not be representative of the rest of the herd. Any time a cow was removed from the freestall barn for more than milking or pasture time (e.g. judging contest held at the farm or hoof trimming), data from that day was removed for that cow. Data from cows were removed the day before, of, and after estrus, as detected by farm staff (estrus detection using similar PDMT is explained in Mayo (2015)).

Precision Dairy Technologies

A weather station (HOBO U23 Pro v2 External Temperature/Relative Humidity Data Logger - U23-002, Onset, Bourne, MA) was located inside each freestall barn that measured relative humidity and temperature every 15 minutes. Temperature humidity index (**THI**) was computed using Eq. 3.1.

THI = temperature (^{0}F) - [0.55 – (0.55 × relative humidity/100)] × [temperature (^{0}F) – 58.8] (NOAA, 1976) (**Eq. 3.1**).

Each cow in the herd was equipped with the following PDMT: AfiAct Pedometer Plus (afimilk, Kibbutz Afikim, Israel), which measured number of steps (**AFISTEP**), lying time (**AFILT**) and rest bouts (**AFILB**); DVM Bolus (DVM Systems, LLC, Greeley, CO), which measured reticulorumen temperature (**DVMRT**); CowScout (Gea Farm Technologies GmbH, Bönen, Germany), which measured leg activity (**GEAACT**);

HR Tag (SCR Engineers Ltd, Netanya, Israel), which measured rumination time (**HRRUM**) and neck activity (**HRACT**); IceQube (IceRobotics Ltd, Edinburgh, Scotland), which measured lying time (**IQLT**), standing time (**IQST**), lying bouts (**IQLB**), bout duration (**IQBD**), and total motion (**IQMOT**); SmartBow (MKW electronics GmbH, Jutogasse, Austria), which measured lying time (**SBLT**), standing time (**SBST**), inactive time (**SBINACT**), rumination time (**SBRUM**), high activity (**SBHACT**), and no activity (**SBNOACT**); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands), which measured head activity (**SENSACT**), no activity (**SENSNOACT**), feeding time (**SENSFT**), rumination time (**SENSRUM**); Track a Cow (ENGS, Hampshire, UK), which measured time at the feedbunk (**TACTFB**), number of feed bunk visits (**TACFV**), lying time (**TACLT**), number of steps (**TACSTEPS**).

Activity (**AFISTEP, GEAACT, HRACT, SENSACT, SENSNOACT, IQMOT, SBINACT, SBHACT, SBNOTH**) and lying (**AFIRB, IQLT, IQST, IQLB, IQBD, TACLT, SBLT, SBST**) parameters were measured using 3-axis accelerometers. Rumination time was measured using a 3-axis accelerometer (**SENSRUM** and **SBRUM**) or a microphone and microprocessor (**HRRUM**). Feeding time was measured with a 3-axis accelerometer (**SENSFT**) and TACTFB and TACFV were measured using a cable that monitored when cows arrived and left the feed bunk.

All devices were assigned to cows and heifers at least 10 d before their predicted calving date. Leg and ear devices were placed on the same leg for each technology for every cow (Track a Cow on the right front, Cow Scout on the left front, Pedometer Plus on the right rear, and IceQube on the left rear leg; Smartbow on the right ear and CowManager SensoOr on the left ear). Ear tags were positioned using an ear tagger,

provided by each technology company to fit the respective device. Precision dairy monitoring technologies were removed from cows if they started to irritate the cow's skin or cause swelling and placed on the opposite leg if possible to prevent data loss. Once the area healed, the PDMT was re-applied to the original position. DVM boluses were inserted into the reticulorumen orally with a bolus gun.

The parlor was equipped with AfiLab (afimilk, S.A.E. AFIKIM, Kibbutz Afikim, Israel), which measured milk yield (**AFIYIELD**), fat (**AFIFAT**), protein (**AFIPROT**), lactose (**AFILACT**), conductivity (**AFICOND**), and milking order (**AFIORDER**). Cows were sorted into their respective groups using AfiSort (Afimilk, Kibbutz Afikim, Israel) after each milking and were manually checked daily to ensure correct sorting. During this check, tags were accounted for to ensure no tags were lost in the lot or pasture and these tags were recovered and replaced when loss occurred. All PDMT were monitored and replaced promptly when failure occurred, including dead batteries and broken tags. All computer clocks were set to synchronize with NIST Internet Time Service (NIST, Gaithersburg, MD, USA) automatically, and time was manually verified on all computers on a weekly basis.

Mastitis Sampling

Twice weekly (Monday and Friday) at the morning milking, composite milk samples were obtained for each cow in the herd (36 sampling periods). Composite milk samples were collected into clear, 90 mL polypropylene resin vials (Capitol Vial, Thermo Fisher Scientific, Hudson, NH). Samples were evaluated for SCC immediately following milking with a SomaCount FC (Bentley Instruments, Inc., Chaska, MN) and any cow with a SCC > 200,000 cells/mL was classified as having subclinical mastitis. Individual

quarter milk samples were obtained for bacteriological, milk leukocyte differential, and SCC evaluation from all four quarters of cows with subclinical mastitis at the afternoon milking on the same day. Cows without subclinical mastitis were not sampled at the afternoon milking.

For bacteriological culture, samples were obtained following the procedure described by Hogan et al. (1999). After forestripping and pre-dipping, teat ends were cleaned with cotton balls soaked in 70% ethyl alcohol. About 5 mL of milk from each quarter was stripped into an individual sterile polypropylene test tube (Falcon®, Corning Life Sciences, Corning, NY). Samples were frozen immediately after milking and delivered to a University of Kentucky laboratory for bacteriological analysis each week. In the lab, individual quarter milk samples were thawed and 0.1 mL of each quarter sample were aseptically obtained from each tube and plated onto one half of a Difco™ (BD Diagnostic Systems, Detroit, MI) Columbia blood esculin agar plate with 5% calf's blood, which was collected aseptically from calves at the University of Kentucky Coldstream Dairy. Plates were incubated at 37°C and bacterial growth was observed 48 h later. Bacteria on the primary culture medium were identified tentatively according to colony morphology and hemolytic characteristics. Contaminated and no growth plates were recorded and discarded. Isolates considered causative mastitis agents were placed in brain-heart-infusion broth and incubated at 37°C for 24 h. Ten μL of each broth was then heat-fixed to a microscope slide and Gram stained. Gram staining was conducted by drenching each slide in crystal violet for 1 min, Gram's iodine for 1 min, alcohol for 30 s, and safranin for 30 s. Between drenches, slides were rinsed and blotted with bibulous paper. Slides were examined under a microscope and isolates identified as Gram-

negative rods or streptococci were further evaluated by Vitek 2 Compact (bioMérieux, Durham, NC). Isolates identified presumptively as staphylococci were subsequently tested for coagulase activity (positive or negative) by the tube coagulase test using BBL™ coagulase rabbit plasma with ethylenediaminetetraacetic acid (BD Diagnostic Systems, Detroit, MI). Coagulase-positive staphylococci were considered *Staphylococcus aureus*. Samples with negative coagulase-status were considered coagulase negative staphylococci. Isolates identified as yeast or coryneforms were not confirmed beyond microscopic identification.

After milk samples were plated for bacteriological analysis, the samples were used to evaluate lactate dehydrogenase (**LDH**) with UdderCheck (Portacheck, Moorestown, NJ). An UdderCheck strip was dipped in each milk sample and results were recorded two minutes later by comparing the color of the test strip to the color chart on the vial.

During sampling, an additional 4 mL of milk was obtained from each quarter for milk leukocyte differential evaluation directly after the bacteriological samples were obtained. Samples were evaluated with the Q-Scout (Advanced Animal Diagnostics, Morrisville, NC) system directly after milking, according to manufacturer directions. Results of the milk leukocyte differential evaluation included total leukocyte, lymphocyte, macrophage, and neutrophil count.

Ninety mL of milk from each quarter was collected in a non-sterile polypropylene flip-top vial (Capitol Vial, Thermo Fisher Scientific, Hudson, New Hampshire) for SCC evaluation directly after the milk leukocyte differential sample was obtained. Samples

were preserved and refrigerated until individual quarter SCC was performed with a SomaCount FC (Bentley Instruments, Inc., Chaska, MN) within 2 d.

Data Editing and Analysis

Statistical analyses were conducted using SAS Version 9.3 (SAS Institute Inc., Cary, NC). Milk yields < or > 4 standard deviations from the previous week's average milk yield were removed, presumably caused by technology error. To account for decreased reticulorumen temperature caused by water bouts, DVMRT were removed if < 38.3ºC and if they were less than 4 standard deviations from the previous week's average temperature. Milk yield, IQLB, IQBD, IQST, IQMOT, IQLT, HRRUM, HRACT, SENNOACT, SENRUM, SENFT, SENACT, SENHACT, SBLT, SBST, SBINACT, SBHACT, SBNOTH, SBRUM, TACLT, TACTFB, and TACFV were each summed to create one value per variable per cow per day. Temperature humidity index, AFILACT, AFIPROT, AFIFAT, AFICOND, AND AFIORDER, GEAACT, and DVMRT were averaged to create one value per variable per cow per day. If any variable amounted to 0 for the day, that variable was set as missing for that cow day. Cow days were removed if < 90% of each day's data was recorded, but if a cow had > 90% of each day's data, that linear interpolation was used to include the missing 10% from that day. In cases where less than 24 hours of data were available, the percentage lying for that time period was used to calculate the percentage lying within 24 hours. The UNIVARIATE procedure was used on these variables and the 1^{st} and 99^{th} percentile of all variables were removed.

Pathogen groups were created to account for a small frequency of several individual pathogens. Pathogen groups included: Gram positive and Gram negative mixed cultures (**NPMIX**), Gram positive cultures (**GPOS**), and no growth or

contaminant cultures (**NOGROW**). The NPMIX and GPOS groups were only required to have one quarter with a Gram negative pathogen or a Gram positive pathogen, respectively, and the other quarter(s) could have been no growths or contaminants. All but one cow with a Gram negative pathogen isolated from at least one quarter also had a Gram positive pathogen isolated from at least one other quarter. The cow with a Gram negative pathogen isolated from one quarter and the other three quarters determined to be no growths was removed from the study since she did not fit in any of the groupings and may have responded differently to this infection than did the cows with NPMIX culture results. Cows with no subclinical mastitis (cows with < 200,000 SCC at the composite AM sampling) at the time of each sampling period were considered to be in the no subclinical mastitis group and were treated as the reference group for all analyses.

The previous day's data was used for each PDMT variable to account for the timing of data availability to producers (**d -1**). Baseline data for each cow each day was created by calculating a 7d rolling mean from day -2 to day -8 before each subclinical mastitis event (**baseline**). The percent change for each day was calculated using Eq. 3.2 and that data was used in all models.

Percent change = (d -1 – baseline) / baseline x 100 (**Eq. 3.2**).

Three separate models were analyzed for each individual PDF technology variable: 1) NPMIX versus no subclinical mastitis; 2) GPOS versus no subclinical mastitis; 3) NOGROW versus no subclinical mastitis. Models were analyzed with PROC GENMOD with binomial distributions with cow as repeated subject and subclinical mastitis status as the dependent variable (yes or no for each of the NPMIX, GPOS, or NOGROW subclinical mastitis models). The single variable generalized linear models

were used to screen for variables to include in the three multi-variable models and non-significant variables ($P \geq 0.10$) were not accounted for in any further analysis. Variables significant in each of these individual models were then included in multi-variable models for each of the three pathogen groups. If redundant variables were significant in screening models, each variable was tested against subclinical mastitis status using the CORR procedure. Only the variable with the greater Spearman correlation coefficient was included in the multi-variable model. Redundant variables included activity (AFISTEP, GEAACT, HRACT, SENSACT, SENSNOACT, IQMOT, SBINACT, SBHACT, and SBNOTH), lying behavior (AFIRB, IQLT, IQST, IQLB, IQBD, TACLT, SBLT, and SBST), rumination time (SENSRUM, SBRUM, and HRRUM), and feeding behavior (SENSFT, TACTFB and TACFV) parameters. Significance was set at $P \leq 0.05$ for the multi-variable models.

The LOGISTIC procedure of SAS was used to calculate ROC curves and determine probabilities of disease for each cow each day using the all of the variables included in each of the three multi-variable models. Probabilities that represented 80% sensitivity, 95% sensitivity, 80% specificity, and 95% specificity were then used to determine alert levels in GENMOD. All four probabilities were used for each of the NPMIX, GRAMPOS, and NOGROW final multi-variable models in order to determine alerts at each probability. If a probability in the dataset was greater than the probability associated with the respective sensitivity or specificity, an alert was created for that cow that day. Alerts were then used alongside subclinical mastitis determined by SCC in order to calculate sensitivity and specificity at the desired probabilities and to include these points on the ROC curves.

Correctly identified events were considered true positives (**TP**), non-alerted events were false negatives (**FN**), non-alerted non-events were true negatives (**TN**), and alerted non-events were false positives (**FP**) (Firk et al., 2002). Specificity is the probability that a negative sample is from a disease-negative cow. Sensitivity is the probability that a positive alert is a true indicator of a disease (Hamann and Zecconi, 1998, Sherlock et al., 2008, Hogeveen et al., 2010). Because sensitivity and specificity are interdependent, thresholds should be set to optimize both (Hogeveen et al., 2010). Accuracy can account for the prevalence of a disease whereas sensitivity and specificity cannot. Accuracy depends on how strongly and closely the measured parameters are associated with the event, how accurately the technology measures the parameters, and how well the manufacturer algorithm processes the data to create useful alerts (Dolecheck et al., 2015). Sensitivity, specificity, and accuracy for each final multi-variable model were determined using Eq. 3.3, 3.4, and 3.5 (Sherlock et al., 2008, Hogeveen et al., 2010).

Specificity = TN / (TN + FP) x 100 (**Eq. 3.3**).

Sensitivity = TP / (TP + FN) x 100 (**Eq. 3.4**).

Accuracy = [(TP + TN) / (TP + TN + FP + FN) X 100] (**Eq. 3.5**).

RESULTS AND DISCUSSION

In total, 354 subclinical mastitis cases were detected using SCC. Of the subclinical mastitis cases, 66, 148, and 140 cases were detected for NPMIX, GPOS, and NOGROW, respectively. Cow days without subclinical mastitis totaled 3,553 over the course of the study. Mean (± SD) DIM, milk yield, and parity for all cows over the study

period were 226.29 ± 142.29 days, 34.58 ± 9.97 kg/d, and 2.06 ± 1.15 lactations, respectively. Mean (± SD) milk lactose, fat, protein, and conductivity were 4.69 ± 0.26%, 3.40 ± 0.48%, 2.98 ± 0.24%, and 8.09 ± 0.85. Mean (± SD) SCC, leukocyte count, and LDH levels in cows with subclinical mastitis caused by NPMIX, GPOS, and NOGROW pathogens are displayed in Table 3.1.

Gram-negative pathogens release endotoxins, increasing the risk of death in cows with mastitis caused by this pathogen group (Hertl et al., 2011) and clinical mastitis caused by Gram negative pathogens is usually more severe than mastitis caused by Gram positive pathogens. Isolation of environmental pathogens from milk cultures is difficult and diagnosis of mastitis caused by environmental pathogens is difficult because of the short duration (Smith et al., 1985). Thus, some of the NOGROW may have been Gram negative pathogens and speculate that timing to detect these cases may have been too wide. Because samples were only taken twice weekly, it is plausible that we missed the window of detection for Gram negative pathogens. Unfortunately, studies evaluating behavioral, physiological, and production indicators around naturally-occurring mastitis studies are not available to attempt to alter the time windows post-hoc, which may have allowed for capture of the greatest variable changes.

Mean percent change (± SD) from d -1 to baseline for each PDMT variable evaluated are displayed in Table 3.2. The greatest percent change occurred for HRRUM (10.35%) for GRAMPOS. The least amount of change occurred for AFIPROT for both NOGROW and no subclinical mastitis (0.01%).

Multi-variable General Linear Models

The variables included in each general linear multi-variable model are listed in Table 3.3 alongside the odds ratios for having NPMIX, GPOS, and NOGROW subclinical mastitis based on the PDMT variables included in each model. The multi-variable NPMIX model included 8 cases of subclinical mastitis and 560 days of no subclinical mastitis. The multi-variable GRAMPOS model included 30 cases of subclinical mastitis and 769 days of no subclinical mastitis. The multi-variable NOGROW model included 38 cases of subclinical mastitis and 1091 days of no subclinical mastitis. Because each technology's data was missing at different points as a result of human error, tag error, or data cleaning, the amount of data available in each model varied. The only significant variable included in any of the models was AFILACT in the NPMIX model. The odds of a cow with a one percent decrease in AFILACT from the previous week's average having NPMIX subclinical mastitis was 58% greater than a cow without that decrease ($P = 0.05$).

The sensitivity, specificity, and accuracy of each model are displayed in Table 3.4. The best accuracy achieved in the NPMIX model was 98 %, obtained with 95% sensitivity and 5% specificity. The best accuracy achieved in the GPOS model was 95%, obtained with 5% sensitivity and 95% specificity. The best accuracy achieved in the NOGROW model was 84%, obtained with 8% sensitivity and 95% specificity. The sensitivities accompanying these accuracies were sufficient, but the specificities were low. The desired level of sensitivity, specificity, and accuracy depend on the needs of each individual producer intending to use the data for subclinical mastitis detection. For example, a producer may not want to decrease the chances of examining false positive

cows, in which case he or she could decide to lower the sensitivity threshold and increase the specificity.

The area under the curve for each of the multi-variable models were as follows: 0.42 for NPMIX (Figure 3.1), 0.37 for NOGROW (Figure 3.2), and 0.44 for GRAMPOS (Figure 3.3). The best NOGROW accuracy was not much higher than chance (0.67 versus 0.50), and thus this model was not ideal for detecting subclinical mastitis caused by pathogens that failed to grow on blood agar at the time they were sampled. The best area under the curve evaluated in this study was still only 0.44, implying that the best possible combination of variables was likely not achieved in any of the models. This may be a result of 1) the PDMTs used in this study were a few generations behind today's version and improvements may have already been made; 2) the variables evaluated not being the best predictors of subclinical mastitis; 3) the PDMTs may have been unsuccessful at monitoring or detecting changes within the variables of interest; 4) the effects of subclinical mastitis were not consistent or strong enough for the PDMTs to work well on detecting the disease; 5) patterns and fluctuations in the data making it difficult to discern anything meaningful; or 6) the analysis not being the best fit for the data.

Activity

Activity was a significant predictor of GRAMPOS (GEAAACT; $P < 0.01$), NPMIX (SENNOACT; $P < 0.01$), and NOGROW (SENNOACT; $P < 0.01$) subclinical mastitis. Activity was no longer significant when placed in the NPMIX, GPOS, and NOGROW multi-variable models ($P = 0.27$, $P = 0.22$, and $P = 0.51$, respectively). In the multi-variable GRAMPOS model, GEAACT was the only variable to remain significant

($P < 0.01$). Activity has been monitored to predict lameness (Van Hertem et al., 2013) and estrus (Aungier et al., 2012, Kamphuis et al., 2012, Neves et al., 2012), but to the knowledge of the authors, has not previously been examined for subclinical mastitis detection. While activity changes around the time of subclinical mastitis cases are not well understood, activity was clearly affected by subclinical mastitis with any of the causative pathogen groups studied in this paper.

Feeding Time

Feeding time (**SENFT**) was a significant predictor of NOGROW subclinical mastitis ($P = 0.09$), but was not significant in the multi-variable model ($P = 0.29$). Feeding time variables were not significant in the NPMIX and GPOS subclinical mastitis models and were excluded from those multi-variable models. AlZahal et al. (2011) observed that cows challenged with clinical mastitis caused by *Escherichia coli* consumed 23% less feed than control cows. Feeding time may differ from actual feed intake so the results of this study and that of AlZahal et al (2011) should be compared cautiously, but both variables are targeted at understanding feeding behavior. In Schirmann et al. (2012), a negative relationship was observed between average daily rumination time and feeding time ($r = -0.34$, $P = 0.03$), but no relation between daily rumination time and dry matter intake ($r = 0.11$; $P = 0.48$) was detected. Additionally, challenge studies are likely to elicit a stronger response than a naturally-occurring case of subclinical mastitis. Changes in feeding time may also be because of pain and not because of the actual disease, which may explain why cows with subclinical mastitis may not be as affected.

Lying Time

Lying time (SBLT) was a significant predictor of GRAMPOS subclinical mastitis ($P = 0.04$), but was no longer significant in the multi-variable GRAMPOS model ($P = 0.71$). Lying time (TACLT) and TACLB were significant predictors of NPMIX subclinical mastitis ($P = 0.06$ and $P < 0.01$, respectively), but were no longer significant in the multi-variable NPMIX model ($P = 0.71$ and $P = 0.90$ for TACLT and TACLB, respectively). All variables related to lying time, standing time, and number of bouts were not significant in the NOGROW subclinical mastitis model and were excluded from the multi-variable model.

Lying down is a high-priority behavior in dairy cows (Munksgaard et al., 2005). Lying times in this study averaged 8.78, 9.26, 9.53, and 12.95 h/d from AFILT, TACLT, IQLT, and SBLT, respectively. Smartbow lying time was determined from an ear tag, which may explain why the lying time was so much greater than the other lying times. However, none of the lying times obtained in this study represent the average lying times previously referenced between 10.5 and 11 h/d (Ito et al., 2009, Bewley et al., 2010). Cows in this study were housed in outdated freestall facilities and cow comfort was poor, which may have altered lying times.

In an *E. coli* lipopolysaccharide challenge study, cows spent 1.2 h/d less total time lying down on the day of challenge compared with baseline lying time. However, no differences were observed in the number of lying bouts or mean lying bout duration between baseline and the days post-challenge (Cyples et al., 2012). Although lying time, standing time, or lying buts were not significant predictors of subclinical mastitis in any of the multi-variable models, lying time variables were significant in both the NPMIX

and GPOS models, signifying that it may be a valid predictor of some subclinical mastitis cases.

Milking Order

Milking order was a significant predictor of NOGROW subclinical mastitis (P = 0.02), but not GPOS or NPMIX. In the NOGROW multi-variable model, AFIORDER was no longer significant (P = 0.53) and therefore was not the best predictor in the model after taking other variables into account. The authors hypothesized that cows may be slower to enter the milking parlor or may be less likely to show dominance in the herd when they are feeling ill. Although the pain in animals with subclinical mastitis has not been heavily evaluated in the literature, less pain may be associated with subclinical mastitis than some other dairy cow diseases and thus the cows may not show deviations in herd hierarchy or milking motivation as a result of subclinical mastitis as they potentially could for severe clinical mastitis or other diseases that may be present on a more systemic level. However, other variables likely also contribute to milking order, like herd hierarchy, entrance of new fresh cows, weather, and milker behavior when pushing cows into the parlor, making this variable difficult to completely account for.

Milk Yield and Components

Milk yield was not a significant predictor in any of the models evaluated. This result was not surprising because milk yield decreases typically occur after detection of clinical mastitis and this drop persists for the rest of the lactation. Finnish researchers observed that milk yield began to decline four weeks before clinical mastitis detection. Milk yield of cows with clinical mastitis dropped below that of the healthy cows in the first two weeks after diagnosis. However, the yield decrease of the cows with clinical

mastitis was not significantly different from the healthy cows (Rajala-Schultz et al., 1999b). French researchers developed a mastitis simulation model using data from three herds and determined that overall losses amounted to 8% of total projected production. The authors concluded that one-third of cows did not experience a significant milk yield decrease compared to control cows. However, the other two-thirds of study cows experienced a 144 kg milk loss between the week of mastitis occurrence and the five weeks following or experienced a 911 kg milk loss extended throughout their lactation (Lescourret and Coulon, 1994). Again, subclinical mastitis may create different reactions at different time windows related to milk yield.

Milk components, including lactose, protein, and fat were seemingly important variables for detecting subclinical mastitis. Lactose, AFIPROT, and AFICOND were significant predictors of GRAMPOS subclinical mastitis ($P < 0.01$, $P = 0.03$, and $P = 0.03$, respectively). However, AFIPROT and AFICOND were not significant in the GRAMPOS multi-variable model ($P = 0.66$ and $P = 0.61$, respectively). Lactose and AFIFAT were significant predictors of NOGROW subclinical mastitis ($P < 0.01$ and $P = 0.07$, respectively). Fat percent was no longer significant in the multi-variable NOGROW model ($P = 0.46$), but AFILACT remained significant in the multi-variable model ($P = 0.03$). Fat appears to increase during mastitis, but this change mostly occurs because the milk yield decrease is greater than the decrease in fat synthesis (Burriel, 1997) and thus the result that either fat or protein were significant in any individual model is slightly surprising. However, because this result does not imply causation, it is possible that cows with different fat and protein levels are more or less likely to have subclinical mastitis and not the other way around.

Lactose, AFIPROT, and AFICOND were significant predictors of NPMIX subclinical mastitis ($P < 0.01$, $P = 0.07$, and $P = 0.02$, respectively). Lactose remained a significant predictor of NPMIX subclinical mastitis in the final multi-variable model ($P < 0.01$). Lactose concentration decreases with mastitis, mainly because of the reduced synthesis capacity of damaged tissue (Burriel, 1997). In intramammary *Strep. uberis* and intravenous endotoxin-induced mastitis challenges, lactose significantly decreased for 3 milkings (Shuster et al., 1991) and on day 3 (Kester et al., 2014) post-challenge compared to controls. Although lactose concentration decreased with increasing mastitis severity, Berning and Shook (1992) explained that lactose was "not useful for mastitis detection" because it was least responsive to changes in bacterial status. Additionally, a variable detecting a change after human detection has already occurred is not likely as useful as a detection tool before human detection occurs. However, the results of this study imply that lactose may be a useful predictor of subclinical mastitis caused by NPMIX pathogens.

Rumination Time

Rumination time was a significant predictor of NPMIX ($P = 0.06$ for HRRUM) and NOGROW ($P = 0.03$ for SBRUM) subclinical mastitis. In the multi-variable model for NPMIX and NOGROW, HRRUM and SBRUM were no longer significant ($P = 0.82$, and $P = 0.08$, respectively).

Rumination time decreases with acute stress (Herskin et al., 2004) and disease (Welch, 1982, Hansen et al., 2003). The results of the NOGROW model relate to previous studies that detected a change in rumination time around the time of clinical mastitis. Decreased feed intake or increased feeding time may also negatively affect

rumination time (Schirmann et al., 2012). Several researchers have conducted *E. coli* mastitis challenge studies that demonstrated a rumination time decrease post-challenge (Siivonen et al., 2011, Fogsgaard et al., 2012, Fitzpatrick et al., 2013). In an *E. coli* challenge with 20 cows, rumination time decreased on the day of the challenge and gradually increased to pre-challenge levels during the following 2 d (Fogsgaard et al., 2012).

Reticulorumen Temperature

Reticulorumen temperature was only a significant predictor of GPOS subclinical mastitis ($P = 0.07$). However, in the multi-variable model, DVMRT was no longer a significant predictor of GPOS subclinical mastitis ($P = 0.90$). Fever, or a body temperature over a predefined threshold, is an indicator of disease (Leon, 2002, Burfeind et al., 2010). In an *E. coli* mastitis challenge, reticulorumen temperature peaked between 40.5 and 41.0°C and remained above 40.0°C for 2 h post-challenge (AlZahal et al., 2011). Siivonen et al. (2011) also conducted a mastitis challenge study and discovered that rectal temperatures started to increase 4 to 6 h post-infusion and remained above 39.2°C from 6 to 10 h post-infusion, returning to pre-challenge temperatures within 12 h post-infusion. Nevertheless, to the author's knowledge, this is the first evaluation of subclinical mastitis using temperature and the results of using DVMRT were sub-optimal. Subclinical mastitis likely manifests differently than clinical mastitis, particularly in a challenge study using *E. coli* which is known to affect the whole cow instead of just her mammary gland.

Variation

Examples of the variation by sampling period and AfiLab variables (AFILACT, AFIPROT, AFIFAT, AFICOND) are displayed in Figure 3.4. The cow in Figure 3.4a had one case of NOGROW subclinical mastitis early in the sampling period, but then did not register as subclinical mastitis case throughout the rest of the study period based on SCC testing. The cow in Figure 3.4b was chronically affected by *Staph. aureus*, as evidenced by being detected with subclinical mastitis based on SCC testing 9 different times throughout the study, some of which overlapped but some of which did not. The cows in Figure 3.4a and 3.4c still showed great variation in all of the variables shown on the graph, indicating that cow behavior is affected by many things and varies on a daily basis based on these factors, some of which are still not understood by researchers. These graphs depict the difficulty in detecting subclinical mastitis, particularly since great changes can be considered "normal" in a cow that does not have the disease. Visually, one could not tell the difference between the graphs without knowing which cow was represented in each.

CONCLUSIONS

Some of the variables evaluated in this paper may be useful in detecting subclinical mastitis caused by both NPMIX, GPOS, and NOGROW. The generalized linear models for NPMIX, GPOS, and NOGROW all included AFILACT, lying time from one or more of the technologies, and DIM. Not surprisingly, multiple variables together were better able to detect subclinical mastitis compared to using one variable alone. Unfortunately, none of the three models included variables from only one technology, which would have implied that a single technology was the best at detecting a particular type of subclinical mastitis. Instead, variables from multiple technologies

together were the best at predicting subclinical mastitis. Therefore, technology manufacturers should continue to seek ways to monitor multiple variables at once and to improve upon the variables they already monitor. However, the best area under the curve evaluated in this study was still only 0.44, implying that the best possible combination of variables was not achieved. Overall, using PDMT to predict subclinical mastitis is promising, but needs future work into evaluating the best variables and the best statistical methodology.

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Table 3.1. Mean (± SD) somatic cell count, leukocyte count, and lactate dehydrogenase levels in cows with subclinical mastitis caused by NPMIX, GPOS, and NOGROW pathogens.[1, 2, 3, 4]

Variable	Subclinical mastitis type		
	NPMIX	GPOS	NOGROW
Composite SCC, 1000 cells/mL	1044.68 ±1176.23	787.81 ±1108.75	887.18 ±1217.83
	(n = 38)	(n = 57)	(n = 86)
Individual quarter SCC, 1000 cells/mL	3820.61 ± 3934.41	2907.20 ± 2900.88	3938.95 ± 4752.92
	(n = 38)	(n = 56)	(n = 87)
Neutrophil count, 1000 cells/mL	2134.64 ± 2183.56	1382.41 ± 1511.56	1405.45 ± 1261.39
	(n = 36)	(n = 54)	(n = 78)
Lymphocyte count, 1000 cells/mL	631.67 ± 579.89	440.06 ± 415.97	473.40 ± 435.50
	(n = 36)	(n = 54)	(n = 78)
Macrophage count, 1000 cells/mL	293.33 ± 259.03	244.69 ± 284.78	291.19 ± 269.14
	(n = 36)	(n = 54)	(n = 78)
Total leukocyte count, 1000 cells/mL	3057.86 ± 2870.74	2041.56 ± 2097.28	2133.50 ± 1710.90
	(n = 36)	(n = 54)	(n = 78)

LDH, U/L	575 ± 263 (n = 4)	400 ± 216 (n = 7)	573 ± 297 (n = 11)

[1]Gram positive and Gram negative mixed cultures (NPMIX), Gram positive cultures (GPOS), and no growth or contaminant cultures (NOGROW). The NPMIX and GPOS groups were only required to have one quarter with a Gram negative pathogen or a Gram positive pathogen, respectively, and the other quarter(s) could have been no growths or contaminants.

[2]Lactate dehydrogenase (LDH) was determined with UdderCheck (Portacheck, Moorestown, NJ).

[3]Neutrophil, lymphocyte, macrophage, and total leukocyte count was determined by Q-Scout (Advanced Animal Diagnostics, Morrisville, NC) system.

[4]Milk samples were taken from individual quarters of each cow with subclinical mastitis (composite milk sample > 200,000 cells/mL) one milking after subclinical mastitis was detected and then averaged between quarters to have one value per cow per sample period.

Table 3.2. Mean ± SD percent change in behavioral, physiological, and production indicators monitored using precision dairy monitoring technologies the day before somatic cell count evaluation compared to a backward moving 5-d baseline for each cow.[1,2,3]

		Subclinical mastitis type			
Technology	Variable	GRAMPOS	NPMIX	NOGROW	No subclinical mastitis
Afi	Milk yield, % change	-0.97 ± 10.94	1.44 ± 9.75	-1.28 ± 9.46	-0.38 ± 7.72
	Milk lactose, % change	-0.60 ± 4.43	-0.57 ± 1.96	-0.47 ± 3.18	0.07 ±1.87
	Milk protein, % change	0.72 ± 4.41	0.20 ± 2.98	-0.01 ± 3.01	0.01 ± 2.63
	Milk fat, % change	-0.05 ± 5.61	-0.07 ± 6.92	1.05 ± 8.14	-0.04 ± 5.52
	Milking order, % change	1.21 ± 21.97	5.31 ± 22.75	4.00 ± 23.62	-0.22 ± 21.55
	Milk conductivity, % change	0.73 ± 4.92	1.22 ± 3.86	0.24 ± 3.73	-0.01 ± 3.34
	Steps, % change	0.45 ± 14.40	0.22 ± 18.18	0.30 ± 14.72	1.30 ± 13.60
	Lying time, % change	-1.72 ± 16.32	1.43 ± 20.49	-1.56 ± 18.70	-0.84 ± 16.27
	n, cow days	114	52	116	2537
IceQube	Lying bouts, % change	-3.39 ± 25.22	1.56 ± 19.02	-1.08 ± 22.82	0.03 ± 21.78
	Lying bout duration, % change	-2.93 ± 16.48	2.04 ± 15.19	-0.08 ± 15.65	-1.13 ± 14.04
	Standing time, % change	2.19 ± 10.29	-1.20 ± 10.38	0.35 ± 9.31	0.84 ± 8.98

	Lying time, % change	-2.99 ± 16.47	2.02 ± 15.59	-0.37 ± 18.19	-1.14 ± 14.02
	Total motion, % change	3.81 ± 21.47	3.25 ± 18.54	-0.09 ± 15.63	2.23 ± 18.47
	n, cow days	132	61	126	2790
GEA	Activity, % change	6.99 ± 22.09	3.27 ± 16.92	-0.29 ± 15.95	1.64 ± 16.31
	n, cow days	137	63	134	3269
DVM	Reticulorumen temperature, % change	0.11 ± 0.38	0.02 ± 0.43	0.03 ± 0.50	0.03 ± 0.50
	n, cow days	95	40	90	1746
SCR	Rumination time, % change	10.35 ± 20.93	-4.05 ± 8.62	-2.68 ± 9.82	0.41 ± 12.31
	Activity, % change	-5.21 ± 27.95	2.18 ± 11.27	-0.13 ± 11.57	-0.19 ± 12.31
	n, cow days	9	13	22	987
Sensoor	No activity, % change	0.03 ± 14.78	4.82 ± 15.91	3.20 ± 15.72	-0.84 ± 13.48
	Rumination time, % change	-1.34 ± 9.73	-1.67 ± 8.82	-1.33 ± 10.51	0.25 ± 9.53
	Eating time, % change	-0.56 ± 19.72	-1.54 ± 18.60	-2.69 ± 18.32	0.50 ± 16.50
	Activity, % change	6.82 ± 23.67	0.90 ± 21.97	2.15 ± 24.14	1.73 ± 20.79
	n, cow days	122	47	98	2313
Smartbow	Lying time, % change	1.74 ± 10.50	2.42 ± 10.23	2.04 ± 9.67	-0.16 ± 9.24

	Standing time, % change	1.67 ± 9.87	-2.24 ± 10.69	-1.61 ± 10.08	0.10 ± 9.22
	Inactive time, % change	-4.14 ± 18.55	4.74 ± 28.92	2.19 ± 21.17	-1.10 ± 19.35
	High active time, % change	7.59 ± 34.49	5.96 ± 46.07	1.43 ± 29.67	-2.49 ± 29.66
	Hours doing nothing, % change	-1.11 ± 6.58	0.30 ± 6.78	1.38 ± 7.32	-0.58 ± 6.31
	Rumination time, % change	1.94 ± 10.24	-0.38 ± 10.24	-2.28 ± 11.91	0.98 ± 9.86
	n, cow days	122	47	98	2313
Track-a-Cow	Lying time, % change	-1.13 ± 19.03	8.72 ± 21.26	1.43 ± 20.73	0.97 ± 22.62
	Feeding time, % change	-6.83 ± 32.52	-5.95 ± 27.68	-9.14 ± 31.50	-2.08 ± 27.59
	Feeding visits, % change	-0.05 ± 31.53	8.92 ± 29.86	-3.06 ± 30.73	-0.04 ± 26.17
	n, cow days	126	52	110	2507

[1]Gram positive and Gram negative mixed cultures (NPMIX), Gram positive cultures (GPOS), and no growth or contaminant cultures (NOGROW). The NPMIX and GPOS groups were only required to have one quarter with a Gram negative pathogen or a Gram positive pathogen, respectively, and the other quarter(s) could have been no growths or contaminants.

[2]Each cow in the herd was equipped with the following PDMT: AfiAct Pedometer Plus (afimilk, Kibbutz Afikim, Israel), which measured number of steps (AFISTEP), lying time (AFILT) and rest bouts (AFILB); DVM Bolus (DVM Systems, LLC, Greeley, CO), which measured reticulorumen temperature (DVMRT); CowScout (Gea Farm Technologies GmbH, Bönen, Germany), which measured leg activity (GEAACT); HR Tag (SCR Engineers Ltd, Netanya, Israel), which measured rumination time (HRRUM) and neck activity (HRACT); IceQube (IceRobotics Ltd, Edinburgh, Scotland), which measured lying time (IQLT), standing time (IQST), lying bouts (IQLB), bout duration (IQBD), and total motion (IQMOT); SmartBow (MKW electronics GmbH, Jutogasse, Austria), which measured lying time (SBLT), standing time (SBST), inactive time (SBINACT), rumination time (SBRUM), high activity (SBHACT), and no activity (SBNOACT); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands), which measured head activity (SENSACT), no activity (SENSNOACT), feeding time (SENSFT), rumination time (SENSRUM); Track a Cow (ENGS, Hampshire, UK), which measured time at the feedbunk (TACTFB), number of feed bunk visits (TACFV), lying time (TACLT), number of steps (TACSTEPS). The parlor was equipped with AfiLab (afimilk, S.A.E. AFIKIM, Kibbutz Afikim, Israel), which measured milk yield

(AFIYIELD), fat (AFIFAT), protein (AFIPROT), lactose (AFILACT), conductivity (AFICOND), and milking order (AFIORDER).

[3]A 1 d daily lagged variable was created for each variable to account for the timing of data availability to producers. Baseline data for each cow each day was created by calculating a 5d rolling mean from day -2 to day -6 before each subclinical mastitis event. The percent change was calculated by taking the difference between the daily lagged variable and the baseline data divided by the baseline data multiplied by 100.

Table 3.3. Odds ratios of cows having subclinical mastitis based on precision dairy monitoring technology variables for factors associated with the incidence of subclinical mastitis compared to cows without subclinical mastitis.[1,2,3]

Subclinical mastitis type	Variable	Odds ratio	95% Confidence interval		*P*-value
NPMIX	AFILACT, %	1.04	1.34	1.50	< 0.01
GPOS	GEAACT, %	1.03	1.38	1.49	< 0.01
NOGROW	AFILACT, %	1.02	1.32	1.41	< 0.01
	PG				0.03

[1]Gram positive and Gram negative mixed cultures (NPMIX), Gram positive cultures (GPOS), and no growth or contaminant cultures (NOGROW). The NPMIX and GPOS groups were only required to have one quarter with a Gram negative pathogen or a Gram positive pathogen, respectively, and the other quarter(s) could have been no growths or contaminants.

[2]Each cow in the herd was equipped with the following PDMT: AfiAct Pedometer Plus (afimilk, Kibbutz Afikim, Israel), which measured number of steps (AFISTEP), lying time (AFILT) and rest bouts (AFILB); DVM Bolus (DVM Systems, LLC, Greeley, CO), which measured reticulorumen temperature (DVMRT); CowScout (Gea Farm Technologies GmbH, Bönen, Germany), which measured leg activity (GEAACT); HR Tag (SCR Engineers Ltd, Netanya, Israel), which measured rumination time (HRRUM) and neck activity (HRACT); IceQube (IceRobotics Ltd, Edinburgh, Scotland), which

measured lying time (IQLT), standing time (IQST), lying bouts (IQLB), bout duration (IQBD), and total motion (IQMOT); SmartBow (MKW electronics GmbH, Jutogasse, Austria), which measured lying time (SBLT), standing time (SBST), inactive time (SBINACT), rumination time (SBRUM), high activity (SBHACT), and no activity (SBNOACT); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands), which measured head activity (SENSACT), no activity (SENSNOACT), feeding time (SENSFT), rumination time (SENSRUM); Track a Cow (ENGS, Hampshire, UK), which measured time at the feedbunk (TACTFB), number of feed bunk visits (TACFV), lying time (TACLT), number of steps (TACSTEPS). The parlor was equipped with AfiLab (afimilk, S.A.E. AFIKIM, Kibbutz Afikim, Israel), which measured milk yield (AFIYIELD), fat (AFIFAT), protein (AFIPROT), lactose (AFILACT), conductivity (AFICOND), and milking order (AFIORDER).

[3]A 1 d daily lagged variable was created for each variable to account for the timing of data availability to producers. Baseline data for each cow each day was created by calculating a 5d rolling mean from day -2 to day -6 before each subclinical mastitis event. The percent change was calculated by taking the difference between the daily lagged variable and the baseline data divided by the baseline data multiplied by 100.

Table 3.4. Sensitivity and specificity of rumination time, activity, reticulorumen temperature, lying time, and lying bouts on each disease using different alert thresholds for disease detection.[1]

Type of subclinical mastitis	Probability (alert threshold)	Sensitivity (%)	Specificity (%)	Accuracy
NPMIX	0.00998	95	5	98
	0.01014	80	25	87
	0.01048	5	95	89
	0.01031	31	80	89
GRAMPOS	0.02703	95	5	60
	0.03037	80	25	64
	0.04588	5	95	95
	0.03687	30	80	82
NOGROW	0.01433	95	13	52
	0.01527	80	40	56
	0.03215	8	95	84
	0.02877	30	80	68

[1]Gram positive and Gram negative mixed cultures (NPMIX), Gram positive cultures (GPOS), and no growth or contaminant cultures (NOGROW). The NPMIX and GPOS groups were only required to have one quarter with a Gram negative pathogen or a Gram positive pathogen, respectively, and the other quarter(s) could have been no growths or contaminants.

Figure 3.1. ROC curve for the final GENMOD model evaluating the effects of activity, DIM, lying time and number of bouts, rumination time, and milk lactose, protein, and conductivity percent in cows with subclinical mastitis caused by NPMIX Gram negative and Gram positive pathogens versus cows without subclinical mastitis.

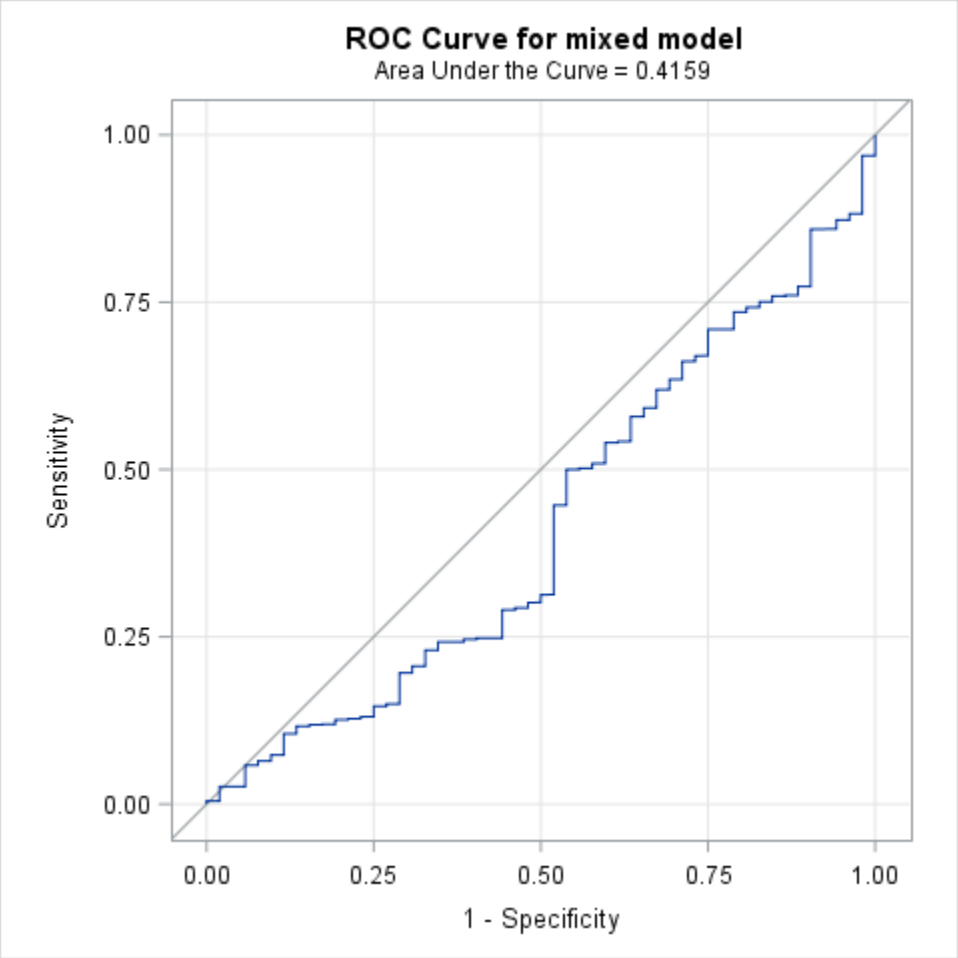


Figure 3.2. ROC curve for the final GENMOD model evaluating the effects of activity, feedig time, rumination time, DIM, parity group, milking order, and milk lactose and fat percent in cows with subclinical mastitis with no growth cultured versus cows without subclinical mastitis.

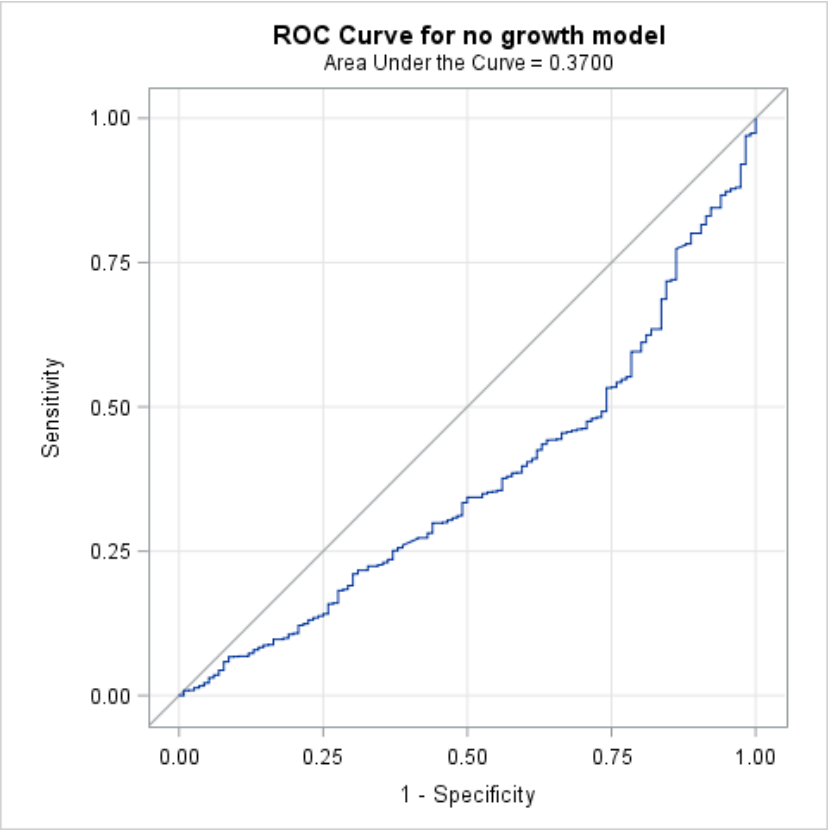


Figure 3.3. ROC curve for the final GENMOD model evaluating the effects of activity, lying time, reticulorumen temperature, number of steps, DIM, and milk lactose, protein, and conductivity percent in cows with subclinical mastitis caused by Gram positive pathogens versus cows without subclinical mastitis.

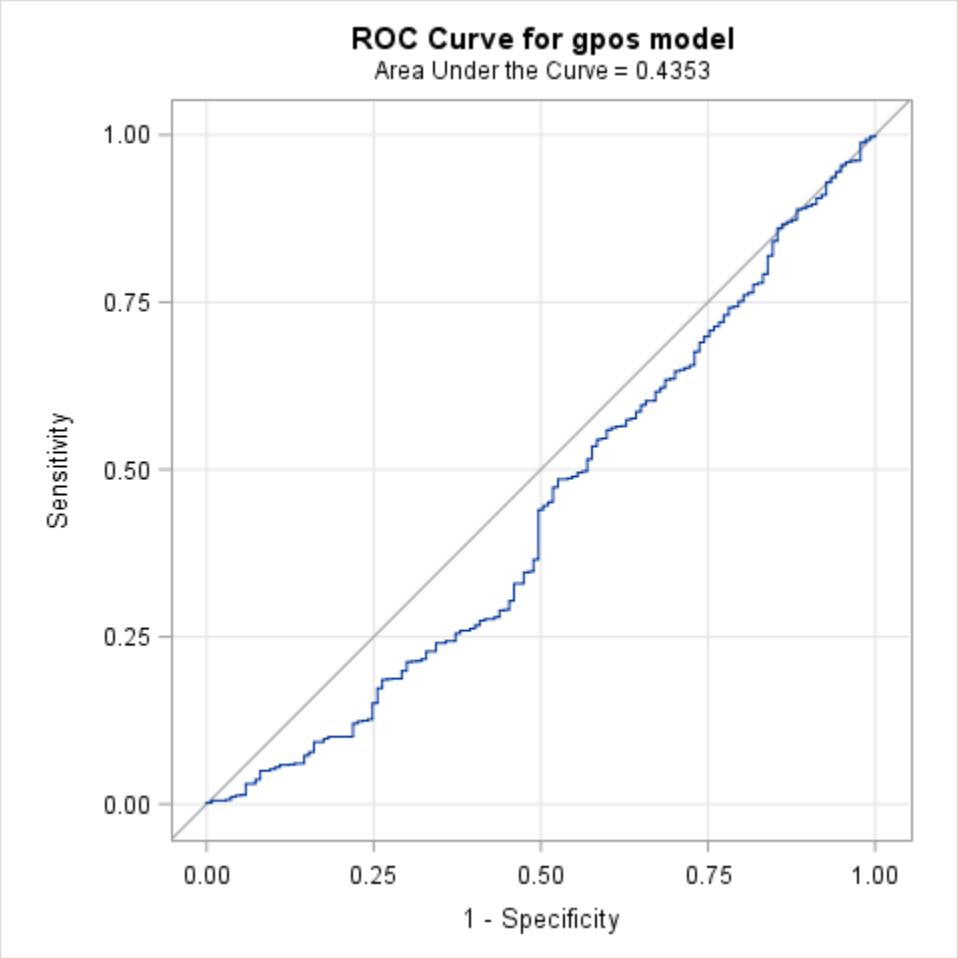


Figure 3.4a. Example cow displaying AfiLab variable percent changes around the time of a NOGROW subclinical mastitis case.[1,2,3]

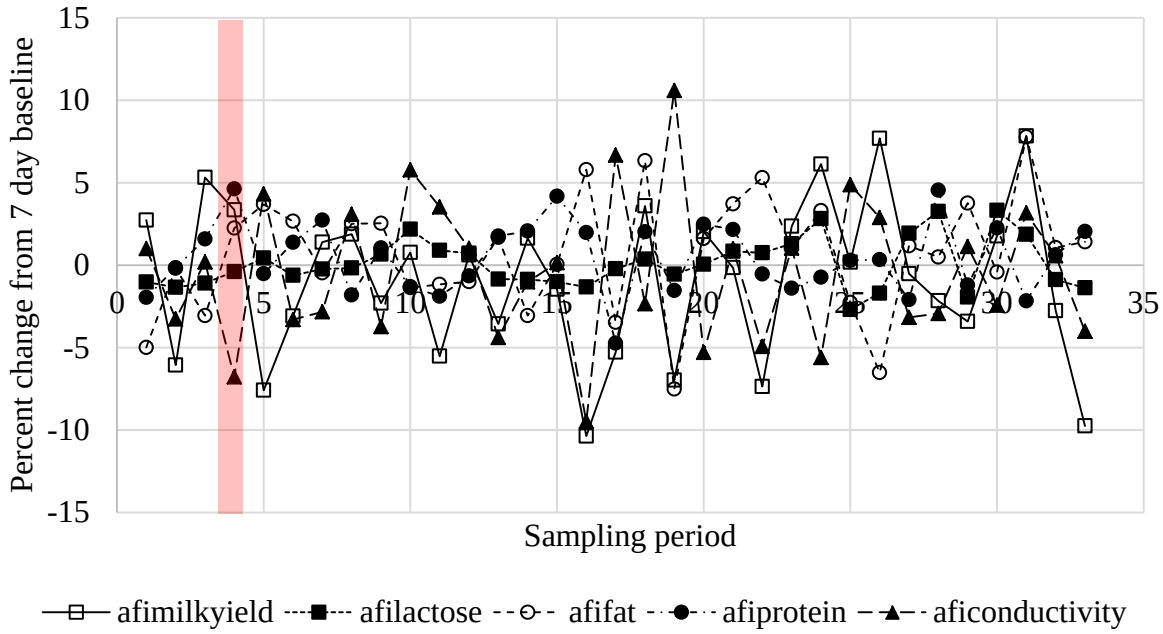


Figure 3.4b. Example cow displaying AfiLab variable percent changes around the time of nine GRAMPOS subclinical mastitis cases detected by SCC.[1,2,3]

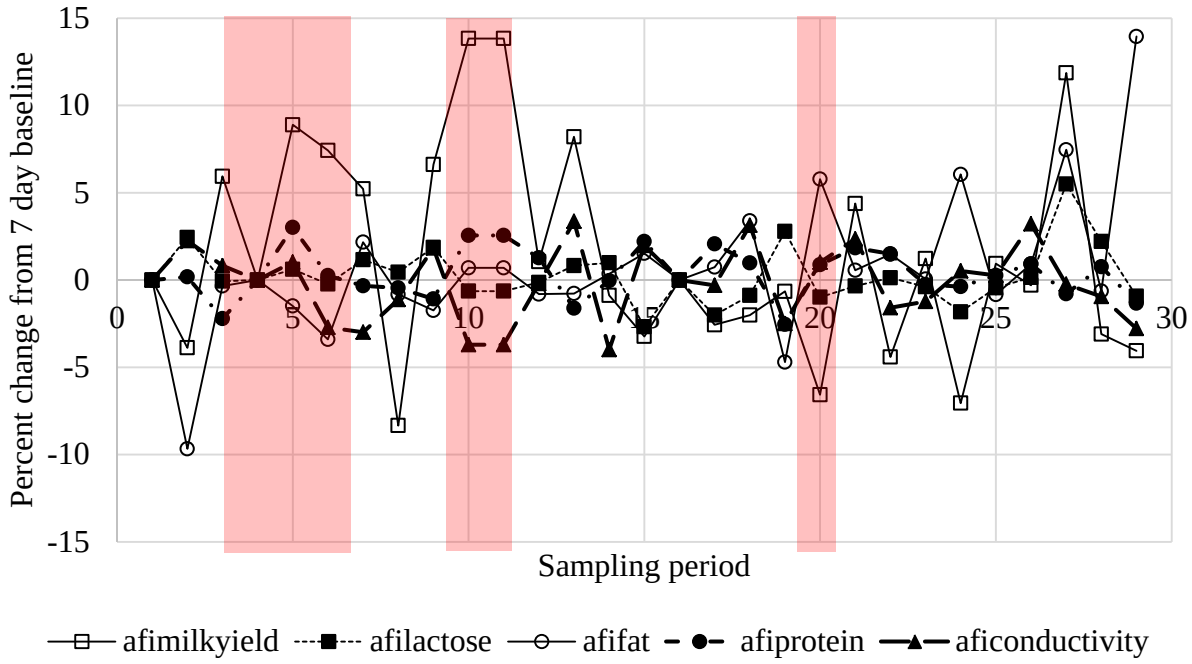
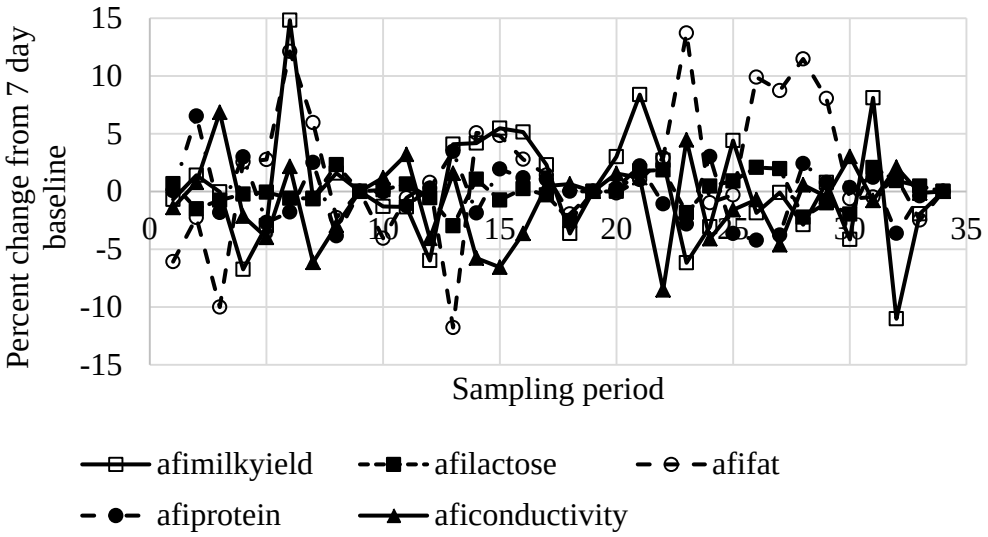


Figure 3.4c. Example cow displaying AfiLab variable percent changes throughout the study period when no subclinical mastitis cases were detected by SCC.[1,2,3]



[1]The red bar represents the sampling period(s) where subclinical mastitis caused by pathogens that did not grow on the culture media was detected by SCC testing.

[2]Milk yield, lactose, fat, and protein collected from AfiLab (afimilk, Kibbutz Afikim, Israel).

[3]A 1 d daily lagged variable was created for each variable to account for the timing of data availability to producers. Baseline data for each cow each day was created by calculating a 5d rolling mean from day -2 to day -6 before each subclinical mastitis event. The percent change was calculated by taking the difference between the daily lagged variable and the baseline data divided by the baseline data multiplied by 100.

Chapter 4:

Evaluation of Precision Dairy Monitoring Technologies to Detect Clinical Mastitis

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INTRODUCTION

Mastitis is an inflammatory reaction of udder tissue, usually caused by a bacterial infection in the mammary gland (Harmon, 1994, Sordillo et al., 1997, Oliver and Murinda, 2012). This disease alters udder secretory processes, lowers milk yield, and changes milk composition (Beck et al., 1992, Harmon, 1994). Mastitis destroys some to all secretory cells and epithelial tissue (Harmon and Heald, 1982, Beck et al., 1992). Mastitis may also compromise animal well-being because of the resulting discomfort and pain (Medrano-Galarza et al., 2012, Fitzpatrick et al., 2013).

Dairy industry personnel generally accept that economic losses resulting from mastitis are sizable (Beck et al., 1992, Hogeveen et al., 2011). Dairy cattle economic efficiency is closely related to milk yield (Dohoo and Martin, 1984). Mastitis has a long lasting negative effect on milk yield (Rajala-Schultz et al., 1999). Even after an infection is cured, milk yield remains depressed (Bar et al., 2008) and cows may be unable to reach their pre-mastitis milk yield (Rajala-Schultz et al., 1999).

Today's non-automated clinical mastitis detection approach involves observing inflammation through visualization and palpation of the udder or presence of abnormal milk. Producers can also monitor declines in milk yield because they can indicate a health problem (Lukas et al., 2009, Leslie and Petersson-Wolfe, 2012). However, these changes are not immediate, making early intervention difficult. This type of intervention may set a cow up to produce more milk and remain healthier throughout her lactation (Aalseth, 2005). Early diagnosis of clinical mastitis may reduce production losses, enhance recovery prospects (Milner et al., 1996), and improve animal welfare (AlZahal et al., 2009), making it very important to the dairy industry (Viguier et al., 2009). Proactive

action may also decrease antibiotic use, which may decrease the chance of antibiotic residues in the bulk tank (Oliver and Murinda, 2012).

Automated dairy cattle behavioral, physiological, and production monitoring systems, or precision dairy monitoring technologies (**PDMT**), may be useful for early mastitis detection. Precision dairy monitoring technologies include sensors that monitor activity, body temperature, feeding time, location, milk parameters (yield, electrical conductivity, lactose, lactate dehydrogenase, blood, color, and SCC), and rumination time. Each of these parameters has mastitis detection potential because mastitis can affect dairy cattle behavior and physiology.

The primary objective of this study was to evaluate variation in neck and leg activity, feeding time, lying time, rumination time, reticulorumen temperature, and milk yield, conductivity, lactose, protein, and fat percent around clinical mastitis events. The secondary objective was to evaluate the sensitivity, specificity, and accuracy of alerts created from neck and leg activity, feeding time, lying time, rumination time, reticulorumen temperature, and milk yield, conductivity, lactose, protein, and fat percent in detecting clinical mastitis.

MATERIALS AND METHODS

This study was conducted at the University of Kentucky Coldstream Research farm from September 9, 2014 to September 8, 2015. Every cow in the lactating herd remained on the study for its entirety or until they left the herd. General cow demographic information was obtained from PCDart (Dairy Records Management Systems, Raleigh, NC) records. During analysis, cows were divided into two parity groups (**PG**), representing primiparous and multiparous cows.

Lactating cows were housed in two freestall barns with one barn of 54 dual chamber waterbeds (Advanced Comfort technology, Inc., Reedsburg, WI) and the other equipped with 54 rubber-filled mattresses, all covered with sawdust. Cow groups were balanced between pens by DIM and parity. Cows had ad-libitum access to fresh water from automatic fill Rubbermaid 150 gallon tanks. Cows had access to an exercise lot for about 1 h/d at 1000, weather permitting.

Lactating cows were fed the same ration consisting of corn silage, alfalfa hay, concentrate mix, whole cottonseed, and alfalfa silage at 0600 and 1330 daily. Cows were milked 2X at 0430 and 1530. The milking routine included forestripping, pre-dipping, drying teats with individual cloth towels, unit attachment, automatic takeoff, and post-dipping with 1% iodine. Pre-dip was 0.5% iodine except from February 21, 2015 to March 21, 2015 when Oxycide (GEA, Naperville, IL) was used for another research study.

Cows < 21 DIM were removed from this study because this period is complicated by many other diseases that may affect the parameters of interest in this study (fresh cow disease detection using the same PDMT on the same herd is explained in Tsai et al., 2016 unpublished). Any time a cow was removed from the freestall barn for more than milking or pasture time (e.g. judging contest held at the farm or hoof trimming), data from that day was removed for that cow. Data from cows were removed the day before, of, and after estrus, as detected by farm staff (estrus detection using similar PDMT is explained in Mayo (2015)).

Precision Dairy Technologies

A weather station (HOBO U23 Pro v2 External Temperature/Relative Humidity Data Logger - U23-002, Onset, Bourne, MA) was located inside each freestall barn that measured relative humidity and temperature every 15 minutes. Temperature humidity index (**THI**) was computed using Eq. 4.1.

THI = temperature (^{0}F) - [0.55 – (0.55 × relative humidity/100)] × [temperature (^{0}F) – 58.8] (NOAA, 1976) (**Eq. 4.1**).

Each cow in the herd was equipped with the following PDMT: AfiAct Pedometer Plus (afimilk, Kibbutz Afikim, Israel), which measured number of steps (**AFISTEP**), lying time (**AFILT**) and rest bouts (**AFILB**); DVM Bolus (DVM Systems, LLC, Greeley, CO), which measured reticulorumen temperature (**DVMRT**); CowScout (Gea Farm Technologies GmbH, Bönen, Germany), which measured leg activity (**GEAACT**); HR Tag (SCR Engineers Ltd, Netanya, Israel), which measured rumination time (**HRRUM**) and neck activity (**HRACT**); IceQube (IceRobotics Ltd, Edinburgh, Scotland), which measured lying time (**IQLT**), standing time (**IQST**), lying bouts (**IQLB**), bout duration (**IQBD**), and total motion (**IQMOT**); SmartBow (MKW electronics GmbH, Jutogasse, Austria), which measured cow location (**SBLOC**), lying time (**SBLT**), standing time (**SBST**), inactive time (**SBINACT**), rumination time (**SBRUM**), high activity (**SBHACT**), and hours doing nothing (**SBNOTH**); CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands), which measured head activity (**SENSACT**), no activity (**SENSNOACT**), feeding time (**SENSFT**), rumination time (**SENSRUM**); Track a Cow (ENGS, Hampshire, UK), which measured time at the feedbunk (**TACTFB**), number of feed bunk visits (**TACFV**), lying time (**TACLT**), number of steps (**TACSTEPS**).

Activity (**AFISTEP, GEAACT, HRACT, SENSACT, SENSNOACT, IQMOT, SBINACT, SBHACT, SBNOTH**) and lying (**AFIRB, IQLT, IQST, IQLB, IQBD, TACLT, SBLT, SBST**) parameters were measured using 3-axis accelerometers. Rumination time was measured using a 3-axis accelerometer (**SENSRUM** and **SBRUM**) or a microphone and microprocessor (**HRRUM**). Feeding time was measured with a 3-axis accelerometer (**SENSFT**) or a cable that monitored when cows arrived and left the feed bunk (**TACTFB** and **TACFV**).

All devices were assigned to cows and heifers at least 10 d before their predicted calving date. Leg and ear devices were placed on the same leg for each technology for every cow (Track a Cow on the right front, Cow Scout on the left front, Pedometer Plus on the right rear, and IceQube on the left rear leg; Smartbow on the right and CowManager SensoOr on the left ear). Ear tags were positioned using an ear tagger, provided by each technology company to fit the respective device. Precision dairy monitoring technologies were removed from cows if they started to irritate the cow's skin or cause swelling and placed on the opposite leg if possible to prevent data loss. Once the area healed, the PDMT was re-applied to the original position. DVM boluses were inserted into the reticulorumen orally with a bolus gun.

The parlor was equipped with AfiLab (afimilk, S.A.E. AFIKIM, Kibbutz Afikim, Israel), which measured milk yield (**AFIYIELD**), fat (**AFIFAT**), protein (**AFIPROT**), lactose (**AFILACT**), conductivity (**AFICOND**), and milking order (**AFIORDER**). Cows were sorted into their respective pens using AfiSort (Afimilk, Kibbutz Afikim, Israel) after each milking and were manually checked daily to ensure correct sorting. During this check, tags were accounted for to ensure no tags were lost in the lot or

pasture and these tags were recovered and replaced when loss occurred. All PDMT were monitored and replaced promptly when failure occurred, including dead batteries and broken tags. Data that was not already missing from these time periods were deleted. All computer clocks were set to synchronize with NIST Internet Time Service (NIST, Gaithersburg, MD, USA) automatically, and time was manually verified on all computers on a weekly basis.

Mastitis Sampling

Clinical mastitis (**CM**) was identified and recorded by the milkers at each milking using visual and tactile assessment of milk (flakes, clots, or serous milk) and the udder (red, hard, swollen) before unit attachment. Milk clots were also detected by examining the Ambic dairy cow mastitis detector (Coburn Company, Whitewater WI), connected to the milk hose, after each cow was milked.

Individual quarter milk samples were obtained for bacteriological, milk leukocyte differential, lactate dehydrogenase (**LDH**), and SCC evaluation from the affected quarter(s) at the first notice of clinical mastitis signs, before the milking unit was attached. If clots were noticed after milking through the Ambic system, individual quarter milk samples were obtained at the following milking. The herd manager was notified of cows with CM and they were treated as he deemed necessary.

A new case of CM was defined as the first recorded case of CM for each quarter per cow per lactation or if the case was following > 14 days of normal milking from the quarter that was diagnosed as having CM (Schukken et al. 1990; Barkema et al. 1997; Hertl et al. 2010). Cows were only sampled during periods of new CM cases and were

not re-sampled in the 14 d window. Data from cows with CM removed from the study for 14 d after clinical detection to ensure no effects of CM were still present.

For bacteriological culture, approximately 5 mL of milk was collected aseptically from the infected quarter(s) of a cow diagnosed with clinical mastitis. Samples were obtained following the procedure described by Hogan et al. (1999). After forestripping and pre-dipping, teat ends were cleaned with cotton balls soaked in 70% ethyl alcohol. About 5 mL of milk from each quarter was stripped into an individual sterile polypropylene test tube (Falcon®, Corning Life Sciences, Corning, NY). Samples were frozen immediately after milking and delivered to a University of Kentucky laboratory for bacteriological analysis. In the lab, individual quarter milk samples were thawed and 0.1 mL of each quarter sample were aseptically obtained from each tube and plated onto one half of a Difco™ (BD Diagnostic Systems, Detroit, MI) Columbia blood esculin agar plate with 5% calf's blood, which was collected aseptically from calves at the University of Kentucky Coldstream Dairy. Duplicates of each individual quarter milk sample were plated to verify results. Plates were incubated at 37°C and bacterial growth was observed 48 h later. Bacteria on the primary culture medium were identified tentatively according to colony morphology and hemolytic characteristics. Isolates considered causative mastitis agents were placed in brain-heart-infusion broth and incubated at 37°C for 24 h. Ten μL of each broth was then heat-fixed to a microscope slide and Gram stained. Gram staining was conducted by drenching each slide in crystal violet for 1 min, Gram's iodine for 1 min, alcohol for 30 s, and safranin for 30 s. Between drenches, slides were rinsed and blotted with bibulous paper. Slides were examined under a microscope and isolates identified as Gram-negative rods and streptococci were further evaluated by Vitek 2

Compact (bioMérieux, Durham, NC). Isolates identified presumptively as staphylococci were subsequently tested for coagulase activity (positive or negative) by the tube coagulase test using BBL™ coagulase rabbit plasma with ethylenediaminetetraacetic acid (BD Diagnostic Systems, Detroit, MI). Coagulase-positive staphylococci were considered *Staphylococcus aureus*. Samples with negative coagulase-status were considered coagulase negative staphylococci. Isolates identified as yeast or coryneform were not confirmed beyond microscopic identification.

Four mL of milk was obtained from each quarter for milk leukocyte differential evaluation directly after the bacteriological samples were obtained. Samples were evaluated with the Q-Scout (Advanced Animal Diagnostics, Research Triangle Park, NC) system directly after milking, according to manufacturer directions.

After milk samples were plated for bacteriological analysis, the samples were used to evaluate LDH with UdderCheck (Portacheck, Moorestown, NJ). An UdderCheck strip was dipped in each milk sample and results were recorded two minutes later by comparing the color of the test strip to the color chart on the vial. Lactate dehydrogenase levels were categorized by the manufacturer as follows: low was < 100 U/L, moderate represented 100 to 200 U/L, high represented 200 to 500 U/L, and very high represented > 500 U/L.

Data Editing and Analysis

Statistical analyses were conducted using SAS Version 9.3 (SAS Institute Inc., Cary, NC). Milk yields < or > 4 standard deviations from the previous week's average milk yield were removed, presumably caused by technology error. To account for decreased reticulorumen temperature caused by water bouts, DVMRT were removed if <

38.3ºC and if they were less than 4 standard deviations from the previous week's average temperature. Milk yield, IQLB, IQBD, IQST, IQMOT, IQLT, HRRUM, HRACT, SENNOACT, SENRUM, SENFT, SENACT, SBLT, SBST, SBINACT, SBHACT, SBNOTH, SBRUM, TACLT, TACTFB, and TACFV were each summed to create one value per variable per cow per day. Temperature humidity index, AFILACT, AFIPROT, AFIFAT, AFICOND, AND AFIORDER, GEAACT, and DVMRT were averaged to create one value per variable per cow per day. Daily data equal to 0 or missing was deleted for variables within GEA, IceQube (except lying bouts), Smartbow, Track-a-Cow, and CowManager SensOor. Cow days were removed if < 90% of each day's data was recorded, but if a cow had > 90% of each day's data, that linear interpolation was used to include the missing 10% from that day. In cases where less than 24 hours of data were available, the percentage lying for that time period was used to calculate the percentage lying within 24 hours. The UNIVARIATE procedure was used on these variables and the 1st and 99th percentile of all variables were removed. The previous day's data for each PDMT variable was used in all models to account for the timing of data availability to producers, who do not receive alerts in real time.

The results of this study were analyzed as a matched case control. Each day a cow had CM, two cows without CM were chosen as matches. Cows were matched based on date, PG, housing group, DIM, and milk yield. In order to prevent confounding data with AFIMY, which was evaluated as a predictor of CM, the DHIA test day milk yield closest to the date of CM occurrence for each case was used for matching purposes.

A separate model was analyzed for each individual PDMT variable using PROC GENMOD with binomial distributions with cow as repeated subject, CM status as the

dependent variable, and a strata statement to account for the matched cow groups. The one-variable generalized linear models were used to screen for variables to include in a multivariable model and non-significant variables ($P \geq 0.10$) were not accounted for in any further analysis. Variables significant in the one-variable models were then included in the multivariable model and significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Over the study period, 233 CM cases were visually detected. Of these cases, 31.37% were detected in the left front quarter, 29.41% were detected in the right front quarter, 26.80% were detected in the right rear quarter, and 12.42% were detected in the left rear quarter. Most samples (60.78%) were contaminated, 23.53% were no growths, 5.23% were caused by *Staphylococcus aureus*, 4.58% were caused by coagulase-negative staphylococci, 1.96% were caused by *Escherichia coli*, 2.62% were caused by yeast and Corynebacteria, and 1.31% were caused by *Klebsiella* spp. Mean ± SD LDH was 3.89 ± 2.57 U/L. The mean ± SD behavioral, physiological, and production indicators monitored using precision dairy monitoring technologies on the day before clinical mastitis detection are displayed in Table 4.1.

In the one-variable generalized linear models, AFIMY, AFIPROT, AFIFAT, AFICOND, and DVMRT were significant predictors of CM status ($P = 0.03$, $P = 0.06$, $P = 0.01$, $P < 0.01$, and $P = 0.05$, respectively). When these variables were included in a multivariable generalized linear model, AFIFAT and AFICOND remained significant ($P < 0.01$; Table 4.2). Reticulorumen temperature, AFIMY, AFIPROT were not significant in the multivariable model ($P = 0.12$, $P = 0.20$, $P = 0.72$, respectively). Cows with a 1% decrease in AFIFAT were 0.23 more likely to experience CM. Cows with a 1 mS/cm

greater AFICOND were 0.29 times more likely to experience CM. Fat is generally understood to increase during mastitis, but this change mostly occurs because the milk yield decrease is greater than the decrease in fat synthesis (Burriel, 1997) and thus the result that either fat or protein were significant is slightly surprising. However, because this result does not imply causation, it is possible that cows with different fat and protein levels are more or less likely to have CM and not the other way around.

Sensitivity and specificity of AFIMY, AFIPROT, AFIFAT, AFICOND, and DVMRT on CM detection using different alert thresholds are displayed in Table 4.3. The best accuracy obtained using the probability thresholds evaluated was 67.94%, which encompassed 99.13% sensitivity and 8.84% specificity. Although the sensitivity is suitable, it only implies that 99 of 100 cows with changes in the variables were detected. In this scenario, only 8 of 100 cows with alerts actually had CM.

The ROC curve for the multivariable GENMOD model evaluating the effects of AFIMY, AFIPROT, AFIFAT, AFICOND, and DVMRT in cows with CM versus cows without CM displayed in Figure 4.1. The area under the curve for using these variables to detect CM was only 0.71, implying that this combination is not ideal and adding other variables may help increase the effectiveness of this model.

Although challenge studies have produced significant responses in similar variables studied here, naturally occurring mastitis likely manifests differently than LPS-induced mastitis. In naturally occurring mastitis studies, different pathogens cause the infections and the exact timing of infection is unknown in naturally occurring mastitis studies, making them difficult to predict and detect. To the author's knowledge, this is the first evaluation of naturally-occurring CM using reticulorumen temperature.

CONCLUSIONS

Some of the variables evaluated in this paper may be useful in detecting clinical mastitis. Of the 5 variables significant in the one-variable models, 4 came from AfiLab (AFIMY, AFIFAT, AFIPROT, and AFICOND). Reticulorumen temperature was the other significant variable included in the multivariable model. Although DVMRT and milk temperature are not likely the same, this result may imply that milk temperature may be something to evaluate alongside the other Afi variables used in this study. Technology manufacturers should continue to seek ways to monitor multiple variables at once and to improve upon the variables they already monitor. However, the area under the curve for the multivariable model evaluated in this study was only 0.71 and the best accuracy obtained was 67.94%, implying that the best possible combination of variables was not achieved to detect CM. Overall, using PDMT to predict clinical mastitis is promising, but needs future work into evaluating the best variables and the best statistical methodology.

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Table 4.1. Mean ± SD in behavioral, physiological, and production indicators monitored using precision dairy monitoring technologies on the day before clinical mastitis detection.[1,2,3]

Technology	Variable	Clinical mastitis	No clinical mastitis
Afi	Milk yield, kg/d	31.86 ± 10.77	34.51 ± 10.05
	Milk lactose, %	4.70 ± 0.26	4.72 ± 0.25
	Milk protein, %	3.10 ± 0.24	3.16 ± 0.21
	Milk fat, %	4.08 ± 0.77	3.87 ± 0.60
	Milking order, parlor entry number	56.87 ± 20.96	53.93 ± 25.31
	Milk conductivity, mS/cm	7.80 ± 0.88	7.50 ± 0.65
	Steps/d	3428 ± 914.41	3433.19 ± 887.52
	Lying time, h/d	10.49 ± 2.63	9.96 ± 2.67
	n, cow days	125	213
IceQube	Lying bouts, bouts/d	18.31 ± 6.65	17.58 ± 7.09
	Lying bout duration, min/bout	12.11 ± 2.27	10.54 ± 2.41
	Standing time, h/d	13.46 ± 2.58	13.45 ± 2.43
	Lying time, h/d	12.05 ± 2.02	12.05 ± 2.02
	Total motion, motion units	4071.01 ± 1516.75	3935.45 ± 1508.41
	n (cow days)	145	262
GEA	Activity, activity units	1306.82 ± 436.84	1394.31 ± 430.59
	n, cow days	11	26
DVM	Reticulorumen temperature, °C	39.40 ± 0.92	39.23 ± 0.71

	n, cow days	84	164
SCR	Rumination time, h/d	7.87 ± 1.40	7.73 ± 1.29
	Activity, activity units	386.79 ± 109.88	391.39 ± 108.78
	n, cow days	106	206
Sensoor	No activity, %	8.46 ± 2.78	481.05 ± 136.30
	Rumination time, h/d	9.49 ± 1.92	581.86 ± 112.29
	Eating time, h/d	3.68 ± 1.92	3.98 ± 1.62
	Activity, activity units	74.86 ± 27.32	71.60 ± 25.90
	n, cow days	65	109
Smartbow	Lying time, h/d	7.65 ± 5.62	6.87 ± 5.82
	Standing time, h/d	11.89 ± 2.27	11.95 ± 2.02
	Inactive time, h/d	5.85 ± 1.86	5.66 ± 1.90
	High active time, h/d	3.09 ± 1.90	3.51 ± 1.85
	Rumination time, h/d	8.85 ± 1.42	8.70 ± 1.34
	n, cow days	94	166
Track-a-Cow	Lying time, h/d	9.83 ± 4.13	9.52 ± 3.39
	Feeding time, h/d	2.98 ± 1.32	3.20 ± 1.19
	Feeding visits, h/d	7.58 ± 2.76	7.69 ± 2.62
	n, cow days	111	173

[1]Each cow in the herd was equipped with the following: AfiAct Pedometer Plus (afimilk, Kibbutz Afikim, Israel), DVM Bolus (DVM Systems, LLC, Greeley, CO), CowScout (Gea Farm Technologies GmbH, Bönen, Germany), HR Tag (SCR Engineers Ltd,

Netanya, Israel), IceQube (IceRobotics Ltd, Edinburgh, Scotland), SmartBow (MKW electronics GmbH, Jutogasse, Austria), CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands), and Track a Cow (ENGS, Hampshire, UK).

[2]The previous day's data was used for each variable to account for the timing of data availability to producers.

[3] Each cow in the herd was equipped with the following precision dairy monitoring technologies: AfiAct Pedometer Plus (afimilk, Kibbutz Afikim, Israel), which measured number of steps, lying time, and rest bouts; DVM Bolus (DVM Systems, LLC, Greeley, CO), which measured reticulorumen temperature; CowScout (Gea Farm Technologies GmbH, Bönen, Germany), which measured leg activity; HR Tag (SCR Engineers Ltd, Netanya, Israel), which measured rumination time and neck activity; IceQube (IceRobotics Ltd, Edinburgh, Scotland), which measured lying time, standing time, lying bouts, bout duration, and total motion; SmartBow (MKW electronics GmbH, Jutogasse, Austria), which measured cow location, lying time, standing time, inactive time, rumination time, and high activity; CowManager SensoOr (Agis Automatisering, Harmelen, Netherlands), which measured head activity, no activity, high activity, feeding time, rumination time; Track a Cow (ENGS, Hampshire, UK), which measured time at the feedbunk, number of feed bunk visits, lying time, number of steps.

Table 4.2. Odds ratios of cows having clinical mastitis based on precision dairy monitoring technology variables for factors associated with the incidence of sclinical mastitis compared to cows without clinical mastitis.[1,2,3]

Variable	Odds ratio	95% Confidence interval		*P*-value
DVMTEMP	0.39	0.26	0.53	0.12
AFIMY	0.50	0.49	0.50	0.20
AFIPROT	0.58	0.20	0.88	0.72
AFIFAT	0.23	0.13	0.37	< 0.01
AFICOND	0.29	0.18	0.44	< 0.01

[1] The previous day's data was used for each variable to account for the timing of data availability to producers.

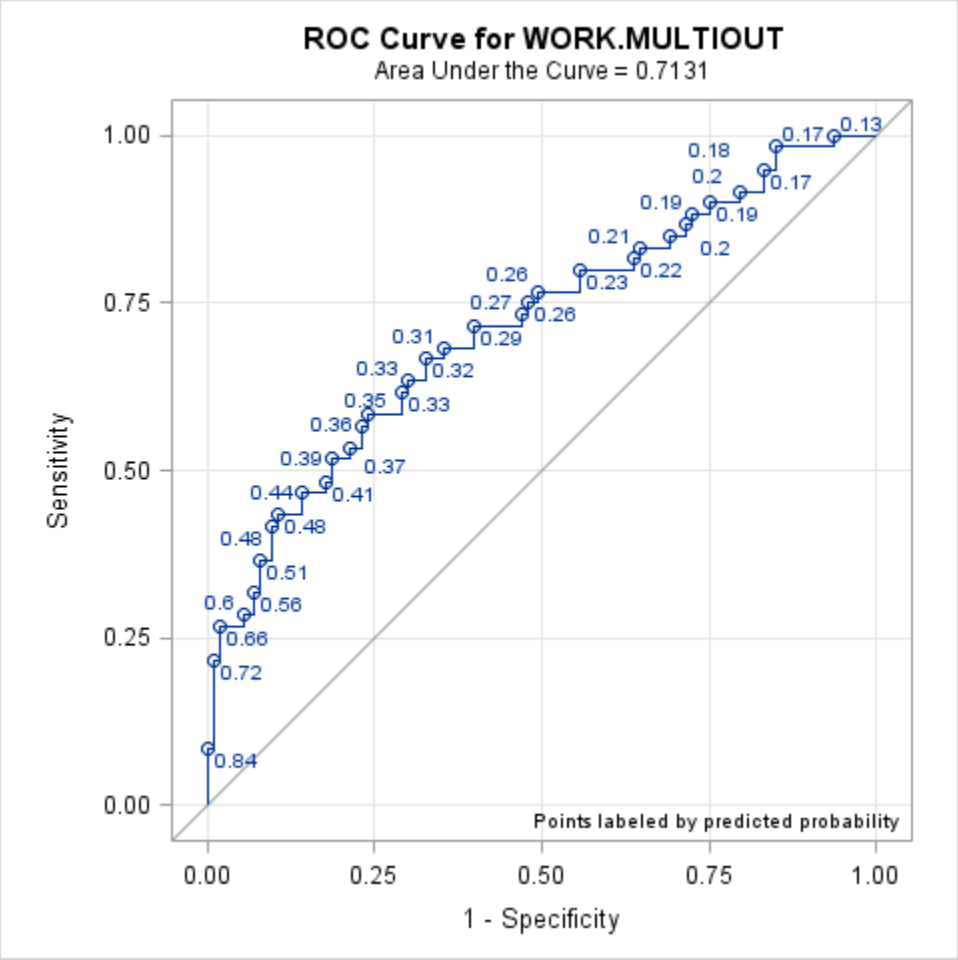
[2]The parlor was equipped with AfiLab (afimilk, S.A.E. AFIKIM, Kibbutz Afikim, Israel), which measured milk fat (AFIFAT), protein (AFIPROT), and conductivity (AFICOND).

[3]Each cow was equipped with a DVM Bolus (DVM Systems, LLC, Greeley, CO), which measured reticulorumen temperature.

Table 4.3. Sensitivity and specificity of rumination time, activity, reticulorumen temperature, lying time, and lying bouts on clinical mastitis using different alert thresholds to detect clinical mastitis.[1]

Probability (alert threshold)	Sensitivity (%)	Specificity (%)	Accuracy
0.60124	99.13	8.84	67.94
0.38033	76.09	27.73	59.73
0.23156	79.88	26.52	61.45
0.16944	72.59	30.39	58.02

Figure 4.1. ROC curve for the multivariable GENMOD model evaluating the effects of reticulorumen temperature, milk yield, milk protein percent, milk fat percent, and milk conductivity in cows with clinical mastitis versus cows without clinical mastitis.



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multiparous and primiparous dairy cows differently. Journal of Dairy Science 94(5):2408-2412.

Curriculum Vitae

EDUCATION:

UNIVERSITY OF KENTUCKY, Lexington, KY

Masters of Science in Animal Sciences: Dairy Systems Management, 3.6 GPA, 2013

Thesis: Management and technology solutions for improving milk quality

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Bachelor of Science in Animal Science and Bachelor of Science in Biology, 3.5 GPA, 2009

EXPERIENCE:

Graduate Extension Assistant	University of Kentucky, Lexington, Kentucky	2/14 to Present
Graduate Research Assistant	University of Kentucky, Lexington, Kentucky	6/10 to 2/14
Dairy Farm Intern	Wise Acres Dairy, Findlay, OH	1/09 to 12/09
Student Assistant	University of Findlay, Findlay, OH	1/07 to 12/09

SUMMARY OF ACCOMPLISHMENTS

PUBLICATIONS:

* Last name changed from Sterrett to Stone in June 2014.

§ From undergraduate mentorship.

† From thesis research.

Manuscripts Published in Refereed Journals (7)

1. Wadsworth, B.A., **A. E. Stone**, J. D. Clark, D. L. Ray, and J. M. Bewley. 2016. Stall cleanliness and stall temperature of two different freestall bases. J. Dairy Sci. 98:4206–4210.
2. Shelley, A.N., D. Lau, **A.E. Stone**, and J.M. Bewley. 2016. Measuring feed volume and weight with machine vision. J. Dairy Sci. 99:386–391.
3. Dolecheck, K. A., W. J. Silvia, G. Heersche Jr, Y. M. Chang, D. L. Ray, **A. E. Stone**, B. A. Wadsworth, and J. M. Bewley. 2015. Behavioral and physiological changes around estrus events identified using multiple automated monitoring technologies. J. Dairy Sci. 98:8723-8731.
4. Wadsworth, B.A., **A.E. Stone**, J.D. Clark, D.L. Ray, J.M. Bewley. Stall cleanliness and stall temperature of two different freestall bases. 2015. J. Dairy Sci. 98 (6) 4206-4210.
5. ƛ Lowe, J.L., **A.E. Stone**, K.A. Akers, J.D. Clark, and J.M. Bewley. 2015. Effect of alley floor scraping frequency on Escherichia coli, Klebsiella species, environmental Streptococcus species, and coliform counts. The Professional Animal Scientist 31 (3) 284-289.
6. † **Sterrett, A.E.*** and J.M. Bewley. 2013. Characterization of management practices used by low somatic cell count Kentucky dairy herds. The Professional Animal Scientist 29 (4) 359-366.
7. † **Sterrett, A.E.***, C.L. Wood, K.J. McQuerry, and J.M. Bewley. 2013. Changes in teat end hyperkeratosis after installation of an individual quarter pulsation milking system. J. Dairy Sci. 96 (6) 4041-4046.

Abstracts and Conference Papers (23)

1. **A.E. Stone**, C. L. Blakely, K.A. Bochantin, P. D. Krawczel, M.A. Myers, D. T. Nolan, C. S. Petersson-Wolfe, G.M. Pighetti, S. H. Ward, and J.M. Bewley. Housing and demographic effects on somatic cell score in southeast United States dairies. 2016. Abstract 17111. American Dairy Science Association Annual Meeting. Salt Lake City, Utah.
2. Wadsworth, B.A., **A.E. Stone**, J.D. Clark, and J.M. Bewley. 2016. Identification of lameness using lying time, rumination time, neck activity, reticulorumen

temperature, and milk yield. Abstract 17786. American Dairy Science Association Annual Meeting. Salt Lake City, Utah.

3. Wadsworth, B.A., Mayo, L.M., Tsai, I.C., **Stone, A.E.**, Ray, D.R., Clark, J.D., Bewley, J.M. 2016. Behavioral comparisons among lame versus sound cattle using precision technologies. Precision dairy farming conference. Leeuwarden, The Netherlands.

4. Wadsworth, B.A., L.M. Mayo, N.I. Tsai, **A.E. Stone**, D.L. Ray, J.D. Clark, and J.M. Bewley. 2015. Comparison of lying times, milk yield, rumination times, and eating times of lame versus sound cattle using Precision Technologies. Abstract 61. 10th International Conference on Lameness in Ruminants. Valdivia, Chile.

5. **Stone, A.E.**, T.B. Mark, and J.M. Bewley. 2015. A Decision Support Tool for Escherichia coli Bacterin Mastitis Vaccine Use in Dairy Cows. Abstract 64014. American Dairy Science Association Annual Meeting. Orlando, FL.

6. Wadsworth, B.A., L.M. Mayo, N.I. Tsai, **A.E. Stone**, D.L. Ray, J.D. Clark, and J.M. Bewley. 2015. Comparison of lying times of lame versus sound dairy cattle using a leg-based accelerometer. Abstract 63378. American Dairy Science Association Annual Meeting. Orlando, FL.

7. ƛ Bochantin, K.A., **A.E. Stone**, and J.M. Bewley. 2015. Effect of milking procedures and mastitis detection methods on somatic cell count in the southeastern United States. Abstract 64382. American Dairy Science Association Annual Meeting. Orlando, FL.

8. ƛ Myers, M.A., **A.E. Stone**, and J.M. Bewley. 2015. Effect of management practices and housing type on somatic cell count in the southeastern United States. Abstract 64354. American Dairy Science Association Annual Meeting. Orlando, FL.

9. **Stone, A.E**., B.A. Wadsworth, C.A. Becker, and J.M. Bewley. 2015. Breed, production, and temperature humidity index influences on reticulorumen temperature, lying time, neck activity, and rumination behavior. Article in Review, 7th European Conference on Precision Livestock Farming, Milan, Italy.

10. Borchers M., Chang Y., **Stone A.**, Wadsworth B., and Bewley J.. 2015. Predicting impending calving using automatic activity and rumination measures in dairy cattle. Article in Review, 7th European Conference on Precision Livestock Farming, Milan, Italy.

11. **Sterrett, A.E.***, B.A. Wadsworth, J.D. Clark, and J.M. Bewley. 2014. Milk yield, reticulorumen temperature, rumination time, and neck activity changes around mastitis. National Mastitis Council Regional Meeting. Ghent, Belgium.

12. **Sterrett, A.E.***, B.A. Wadsworth, R.J. Harmon, L.M. Arnold, J.D. Clark, E.P. Aalseth, D. L. Ray, J.M. Bewley. 2014. Detection of subclinical milk fever and

ketosis in fresh dairy cows using rumination time, lying time, reticulorumen temperature, and neck activity. Abstract 7966. American Dairy Science Association Annual Meeting. Kansas City, MO.

13. Dolecheck, K.A., W.J. Silvia, G. Heersche Jr., **A.E. Sterrett***, B.A. Wadsworth, and J.M. Bewley. 2014. Changes in behavioral and physiological parameters around estrus in partially synchronized cows. Abstract 1491. American Dairy Science Association Annual Meeting. Kansas City, Missouri.

14. ƛ Lowe, J.L., K. A. Akers, **A. E. Sterrett***, J. D. Clark, and J. M. Bewley Case study: Effect of alley floor scraping frequency on environmental mastitis-causing pathogen counts., Abstract 29. American Dairy Science Association Annual Meeting. Kansas City, MO.

15. **Sterrett, A.E.***, B.A. Wadsworth, J.D. Clark, and J.M. Bewley. 2013. Influence of breed, milk yield, and temperature humidity index on dairy cow reticulorumen temperature, lying time, and rumination behavior. Abstract 522. American Dairy Science Association Annual Meeting. Indianapolis, IN

16. Wadsworth , B.A., A.E. **Sterrett***, C.L. Wood, K.J. McQuerry, J.D. Clark, D.L. Ray, and J.M. Bewley. 2013. Characterization of lying time, milk yield, and rumination time with different freestall bases. Abstract 278. American Dairy Science Association Annual Meeting. Indianapolis, IN.

17. Wadsworth, B.A., **A.E. Sterrett***, J.D. Clark, D.L. Ray, and J.M. Bewley. 2013. Changes in lying behavior and milk yield associated with changing freestall dimensions and bases. Abstract TH199.American Dairy Science Association Annual Meeting. Indianapolis, IN.

18. Wadsworth, B.A., **A.E. Sterrett***, D.L. Ray, J.D. Clark, and J.M. Bewley. 2012. Changes in lying time and milk yield associated with changing freestall dimensions and bases. Page 41 in Proceedings of Dairy Cattle Welfare Symposium. Guelph, Ontario, Canada.

19. **Sterrett, A.E.***, C.L. Wood, K.J. McQuerry, and J.M. Bewley. 2012. Potential utility of a parlor-based individual quarter milking system. Page 23 in Proceedings of the 38th of the International Committee for Animal Recording (ICAR) Session. Cork, Ireland.

20. **Sterrett, A.E.*** and J.M. Bewley. 2012. Changes in teat end condition following installation of an individual quarter pulsation system. National Mastitis Council 51st Annual Meeting. St. Pete's Beach, Florida.

21. **Sterrett, A.E.***, C.L. Wood, K.J. McQuerry, and J.M. Bewley. 2012. Potential utility of a parlor-based individual quarter milking system. Abstract 534. American Dairy Science Association Annual Meeting. Phoenix, Arizona.

22. **Sterrett, A.E.***, K.N. Brock, B.I. Kiser, J.D. Clark, D.L. Ray, and J.M. Bewley. 2012. Detection of clinical and subclinical mastitis using reticulorumen temperatures. Abstract M110. American Dairy Science Association Annual Meeting. Phoenix, Arizona.

23. **Sterrett, A.E.*** and J.M. Bewley. 2011. Characterization of management practices utilized by low somatic cell count Kentucky dairy herds. Abstract M160 . American Dairy Science Association Annual Meeting. New Orleans, Louisiana.

Peer-Reviewed Extension Publications (2)

1. Wadsworth, B.A., **A.E. Stone**, L.M. Mayo, N.I. Tsai, and J.M. Bewley. 2015. Methods of precisely managing precision dairy farming technologies on-farm. University of Kentucky College of Agriculture Extension Factsheet.

2. **Sterrett, A.E.***, D. Amaral-Phillips, L.M. Arnold, and J.M. Bewley. 2014. A Fresh Cow Health Monitoring System. University of Kentucky College of Agriculture Extension Factsheet ASC-218.

Peer-Reviewed Newsletter Publications (6)

1. Stone, A.E. and J.M. Bewley. 2015. Maintaining Animal Health in Organic Dairy Herds. Kentucky Dairy Notes (November).

2. Wadsworth, B.A., **A.E. Stone**, L.M. Mayo, N. Tsai, and J.M. Bewley. 2015. Methods of precisely managing precision dairy farming technologies on-farm. Kentucky Dairy Notes (May).

3. **Sterrett, A.E.*** and J.M. Bewley. 2014. Using an On-Farm Culturing System to Identify Mastitis-Causing Pathogens. Kentucky Dairy Notes (May).

4. **Sterrett, A.E.*** and J.M. Bewley. 2013. The Future of Disease Detection: Should you Check the Cow or Check the Computer? Kentucky Dairy Notes (October).

5. Bewley, J.M., **A.E. Sterrett***, R.A. Black, D. Liang, N. Rosario, S.M. Smith, A. Thompson, A.N. Waldeck, and A.J. McAllister. 2012. Lessons learned from visiting Dutch and Danish dairies. Kentucky Dairy Notes (June).

6. **Sterrett, A.E.*** and J.M. Bewley. 2011. What can you learn from Kentucky dairy producers with low SCC? Kentucky Dairy Notes (June).

National Industry Magazine Publications (6)

1. **Stone, A.E.** 2016. Milk quality observations from 300 farm visits. The Progressive Dairyman (April).
2. Wadsworth, B.A., **A.E. Stone**, L.M. Mayo, N. Tsai, and J.M. Bewley. 2016. Methods of managing precision dairy farming technologies. The Progressive Dairyman Canada (February).
3. Dairy Herd Management (article on published research). 2015. 3-D cameras coming to dairy feedbunks? Dairy Herd Management (December).
4. Wadsworth, B.A., **A.E. Stone**, L.M. Mayo, N. Tsai, and J.M. Bewley. 2015. Methods of managing precision dairy farming technologies. The Progressive Dairyman (October).
5. Cooley, W. (Interview with **A.E. Sterrett***). 2014. New fact sheet for fresh cows available. The Progressive Dairyman (May).
6. **Sterrett, A.E.** 2012. Precision Dairy Showcase presents the future of dairy farming. The Progressive Dairyman (December).

PRESENTATIONS:

Invited Presentations (6)

1. 3D Cameras and Other Precision Technologies. Professional Dairy Producers of Wisconsin Meeting. March 18, 2015. Madison, WI.
2. 3D Cameras: Measuring Feed Intake and Body Condition. Precision Agriculture Summit. January 20, 2014. Jamestown, North Dakota.
3. Breed Comparisons of Lying, Temperature, and Rumination. Precision Agriculture Summit. January 20, 2014. Jamestown, North Dakota.
4. Automated Mastitis Detection for Dairy Farms. MILK 2020. November 14, 2013. New Brunswick, Canada.
5. Automated Mastitis Detection for Dairy Farms. 2013 Atlantic Bovine Practitioners Association 14th Annual Conference. November 15, 2013. New Brunswick, Canada.

6. Precision Dairy Farming. American Dairy Science Association Graduate Student Division Dairy Tales. July 16, 2012. Phoenix, Arizona.

Extension Presentations (16)

1. **Introduction to On-Farm Culturing.** December 18, 2014. Todd County, KY.
2. **Southeast Quality Milk Initiative Research Update.** Kentucky Milk Quality Conference. August 27, 2014. Cadiz, KY.
3. **Health Care Management- Diagnostics for sick cows and heifers.** March 13, 2014. University of Kentucky FarmStart Shortcourse. Lexington, KY.
4. **Improving and Maintaining Milk Quality.** March 12, 2014. University of Kentucky FarmStart Shortcourse. Lexington, KY.
5. **Introduction to On-Farm Culturing.** February 13, 2014. Taylor County, KY.
6. **Precision Agriculture in Dairy Housing: The 'Udder' Side of Precision Dairy Monitoring**. Penn State Technology Tuesday webinar series. October 8, 2013. Webinar.
7. **University of Kentucky Dairy Research Update.** Kentucky Milk Quality Conference. August 28, 2013. Cadiz, KY.
8. **Breed Differences Using Precision Dairy Technologies.** University of Kentucky Dairy Research Showcase. June 19, 2013. Lexington, KY.
9. **Evaluation of an Automated 3D Feed Intake Monitoring System.** University of Kentucky Dairy Research Showcase. June 19, 2013. Lexington, KY.
10. **Using Precision Dairy Technologies to Predict Mastitis Events.** University of Kentucky Dairy Research Showcase. June 19, 2013. Lexington, KY.
11. **Effects of Individual Quarter Pulsation on Teat End Health.** University of Kentucky Precision Showcase. December 3, 2012. Lexington, KY.
12. **Precision Dairy Technologies to Predict Health Events.** University of Kentucky Precision Showcase. December 3, 2012. Lexington, KY.
13. **New technologies for monitoring dairy cattle.** March 28, 2012. Daisy Dairy Group (Texas). Lexington, KY.
14. University of Kentucky Dairy Research Update. Kentucky Dairy Development Council Young Dairy Producers Field Day. January 28, 2012. Lexington, KY.

15. **Can technologies reduce dairy farmer and dairy cow stress?** Kentucky Dairy Development Council Coverall Meetings. January 31 and February 10 2012.
16. **Results of survey of low somatic cell count Kentucky dairy herds.** Burkmann Feeds Consultant Training. March 1, 2011. Glasgow, KY.

TEACHING:

Courses

1. **ASC 564: Milk Secretion** (Primary Instructor: Spring 2016, Teaching Assistant: Spring 2015). This course is designed to guide students in learning the anatomy of a cow's udder the physiology and endocrinology of mammary development, lactogenesis, and maintenance of lactation, and the impact, disease process, and control of mastitis.
2. **ASC 323 Advanced Dairy Cattle Evaluation** (Co-Instructor: Fall 2012). This course allows students to apply the skills learned in Dairy Cattle Evaluation at several dairy farms and national college judging competitions.
3. **ASC 321 Dairy Cattle Evaluation** (Co-Instructor: Spring 2012). This course is designed to assist students in evaluating and judging dairy cattle.
4. **ASC 205: Livestock, People, and Their Interactions** (Teaching Assistant: Fall 2011). This course is designed to assist students in evaluating career opportunities in animal sciences.
5. **ASC 382: Livestock Production Principles** (Teaching Assistant: Fall 2010). This course is a broad survey of animal agricultural management covering cattle, horses, poultry, swine, sheep, and goats. Emphasis is placed on the practical application of scientific disciplines including anatomy, physiology, nutrition, reproduction, and genetics.

Student Organizations

Dairy Club Junior Advisor (approximately 30 members), 2015-2016. Assist in advising student organization for all activities including participation in Southern and National American Dairy Science Association Student Affiliate Division meetings, industry field trips, leadership development, and fundraising activities.

SCHOLARSHIPS AND AWARDS:

1. Young Dairy Leaders Institute Participant, 2015-2016
2. Gamma Sigma Delta Outstanding PhD Student, 2014
3. Farm Animal Integrated Research Graduate Student Recorder, 2012
4. National Mastitis Council Scholars Award Scholarship Recipient, 2012
5. University of Kentucky Animal & Food Sciences Graduate Association Poster Symposium Award Recipient, 2011 & 2012
6. National Milk Producers Federation National Dairy Leadership Scholarship Recipient, 2011
7. Student Employee of the Year, 2009
8. Symposium for Scholarship and Creativity Award Recipient, 2008

RECOGNITION AND PROFESSIONAL SERVICE:

International and National

1. National Mastitis Council Member, 2012-present
2. Gamma Sigma Delta Member, 2011-present
3. American Dairy Science Association Member, 2010-present
4. American Dairy Science Association Graduate Student Division President, 2013-2014
5. American Dairy Science Association Graduate Student Division Vice President, 2012-2013

6. American Dairy Science Association Graduate Division Communications Committee Member, 2011-2012

Youth Development Activities

1. Proper Milking Routine and Mastitis Management. Kentucky 4H Teen Conference. June 9, 2015. Lexington, KY.
2. Proper Milking Routine and Explanation of Precision Dairy Technologies. Dare to Dairy. October 25, 2014.
3. Dairy Cow Anatomy, including Udder, Bones and Teeth. Tri-County Dairy Meeting. July 16, 2013.
4. Proper Milking Routine, Explanation of Precision Dairy Technologies, and Udder Anatomy. Dare to Dairy. October 20, 2012. Lexington, KY.
5. Evaluating dairy cattle health and nutrition. Tri-County Dairy Meeting. June 29, 2012.
6. Cow Health. Kentucky 4H Teen Conference. June 13, 2012. Lexington, KY.
7. Dairy Cow Anatomy. Tri-County Dairy Meeting. July 19, 2011.
8. Proper Milking Routine and Explanation of Precision Dairy Technologies. Kentucky 4H Teen Conference. June 16, 2011.

Departmental and College Committees and Activities

1. University of Kentucky Dairy Judging Coach, 2012
2. University of Kentucky Animal & Food Sciences Graduate Association Symposium Chair, 2011-2012
3. University of Kentucky Animal & Food Sciences Graduate Association President, 2010-2011
4. University of Kentucky Animal & Food Sciences Graduate Association Co-Founder, 2010

UNDERGRADUATE STUDENT MENTORSHIP:

Undergraduate Research Assistants - Managed Own Project (4)	
Student	**Completion**
Mickayla Myers	May 2016 (Expected)
Kerri Bochantin	August 2015
Gustavo Mazon	July 2015
Jessica Lowe (now in MS program at University of Washington)	December 2014