The Use Of Activity Measures In Combination With Physiological Factors As Indicators Of Disease In Dairy Cattle

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ABSTRACT

Animal activity, in combination with physiological factors, can be used for early disease detection in dairy cattle. An initial study determined the impact of flunixin meglumine (FM), a non-steroidal anti-inflammatory drug, on activity measures, dry matter intake (DMI) and milk production during experimentally induced *Escherichia coli* mastitis. A total of 24 primiparous and multiparous lactating dairy cows were challenged with *E.coli* 727 in one quarter. Of the 24 *E.coli* challenged animals, 12 were administered FM at 0.002 µg/45.5kg of body weight at the onset of clinical mastitis signs. The remaining 12 cows were untreated. An additional 11 cows were infused with 1 mL of sterile phosphate-buffered saline, and served as the control group. Activity measures were collected through the use of the Afi PedometerPlus© and HOBO® data loggers. *E.coli* mastitis altered animal activity and improvement in DMI and milk production of animals given FM was observed, thus providing evidence for the use of an NSAID as supportive therapy for mastitis. Additionally, activity and blood metabolites concentrations were collected and analyzed from periparturient dairy cows at the Virginia Tech Dairy Center to determine the likelihood of disease occurrence. Primiparous and multiparous Holstein, Jersey, and Crossbred dairy cows were monitored for daily rest bouts, rest duration, and rest time throughout the pre and postpartum periods. Activity measurements were collected using Afi PedometerPlus© pedometers. It was concluded that evaluation of activity changes, and comparison of deviations from healthy herdmates, could allow producers to utilize activity monitoring to proactively manage dairy herd health.

Keywords: animal activity, periparturient disease, mastitis

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"Don't give up…don't ever give up."- Jimmy Valvano

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1.1 Introduction

Disease prevention and treatment is a constant focus in the management of a dairy herd. All medium and large sized dairy operations have reported at least one case of clinical mastitis, lameness, retained placenta, reproductive problems, or milk fever with an even higher percentage having at least one cow with a health problem (USDA, 2007). Of these diseases, those that are the most prevalent throughout the United State dairy industry are clinical mastitis occurring in 95%, lameness at 88% and reproductive problems at 84% of all size operations (USDA, 2007). The costs associated with these diseases can be anywhere from $200 for a case of clinical ketosis or mastitis, to more than $300 for an identified case of lameness (Kelton et al., 1998). The clinical state of these diseases is easily identifiable and therefore, it is easy to associate the cost to the overall dairy operation when they occur. However, each of these diseases begins with subclinical stages prior to the onset of clinical signs that are more difficult to identify. Therefore, the true cost to a dairy farm is largely unknown.

Historically, dairy producers have focused much of their health management efforts on the treatment of disease. However, more recently, dairy producers have adopted a more proactive health management strategy and in response, advanced technology tools for monitoring herd health have been developed (LeBlanc et al., 2006). A component of such on-farm monitoring systems is the tracking of animal activity, which can further enhance detection of subclinical and clinical diseases while also addressing cow comfort and animal welfare concerns associated with ill animals (Dawkins, 2003, von Keyserlingk et al., 2009).

The objectives of this review are to 1) provide background knowledge on the use of activity monitoring on dairy farms and the information these technologies provide regarding the health of dairy animals, 2) address how transition cow metabolic diseases and mastitis can be

better detected through the tracking of animal activity and behavior, 3) discuss the use of activity and physiological measurements to identify animals at risk for metabolic diseases and mastitis, and 4) discuss the evidence that diseases, specifically mastitis, cause discomfort in animals and, how the use of non-steroidal anti-inflammatory drugs (NSAIDs) could be used in the treatment of this discomfort.

1.2 Dairy Cattle Behavior

1.2.1 Video Classification of Behavior

The determination of dairy cattle behavior has been largely used to assess the well-being of animals in various environmental situations. Historically, time lapse video has been relied upon to determine the natural behaviors of animals in current production systems. In a study to examine time budgets, dairy cows housed in a stanchion barn were videoed continuously for 15 h of a day to determine the natural behaviors of animals in current production systems (Hedlund and Rolls, 1977). Of the observation period, cows spent 45% of their day lying, 26% eating, 22% ruminating (of which the majority was conducted while lying down) with the remaining percentages drinking and socializing. The majority of the resting period occurred from mid-day to late afternoon. Activity (eating, drinking and social activity) was determined to be highest during and after milking and feeding (Hedlund and Rolls, 1977). The ability of video observation has allowed researchers to examine normal cow behavior and has played a crucial part in understanding the importance of sound dairy cattle management.

1.2.2 Automatic Behavior Monitoring

While videography is considered the gold standard for behavioral studies, there are limitations with its capacity to accurately quantify dairy cattle behavior. It requires accurate observation and classification of behaviors by trained personnel, which is time consuming, labor

intensive, and may allow for a bias in results (Ledgerwood et al., 2010). Therefore, the development and use of automatic data loggers has become the most labor and time efficient method of monitoring animal activity. In using an automatic recording system, the subjectivity of observation becomes more objective and finite. Muller and Schrader (2003) validated a similar activity tracking mechanism known as the Actiwatch® activity monitoring system using video monitoring as the gold standard. The Actiwatch® is an accelerometer which quantifies the behavior through an intensity measure of animal movements. High and low activity was determined using dynamic thresholds based upon the daily recorded activity for each individual animal. These thresholds, from the accelerometer, were then correlated with the videoed behaviors. High activity was significantly correlated with locomotion at r = 0.75 (P<0.001) and low activity was significantly correlated to lying at r = 0.65. Additionally, an increased correlation between the activity loggers occurred when the system was attached to the same leg as opposed to opposite legs. The reported correlations indicated that these types automatic activity tracking systems could successfully distinguish between behavioral activity levels, but were not sensitive enough to determine exact behaviors (Muller, 2003). In a later study, a simplistic data logger was developed to distinguish standing from lying activity using voltage measurements. A 0.0 voltage was associated with standing while a 2.5 voltage indicated lying. A mercury tilt switch within the data logger determined what voltage was recorded. With a Kappa correlation of above 0.8, this simplistic automatic data logger was determined to be a good alternative to direct observation of cow behavior (O'DriScoll et al., 2008). Similar accelerometers were used to assess behaviors in beef calves (White et al., 2008). A 2-axis accelerometer consisting of an x and y-axis was utilized. Calves were video recorded in 2 h intervals and then subsequently compared to the posture classification from the accelerometer. In

this comparison to video, the accelerometer accurately predicted lying 96.4% of the time and standing 99.4% of the time (White et al., 2008).

An added level of sensitivity of such activity monitors occurs when a z-axis is added to the accelerometer. With the addition of a third axis the side the animal lies on can be distinguished. The additional dimension that is contained within the GP1 SENSR (SENSR, Elkader, IA) accelerometer added accuracy of classifying behaviors to 99.2% for lying and 98.0% for standing (Robert, 2009). Accuracy of quantifying walking activity averaged only 67.8% and therefore, this type of activity was still considered a shortcoming of this behavior monitor. The IceTag® (IceRobotics, Edinburg, UK) is another system that that has been used extensively to characterize behavior in dairy cattle (Munksgaard, 2006, Trenel et al., 2009). The device can record lying and standing time in addition to step time and step counts through the use of intensity measures as percentages of those specific activities. A lying period criterion was developed that modeled the deviations between the IceTag® and video to determine the sensitivity and specificity of the activity monitor (Trenel et al., 2009). While the IceTag® overestimated the moving activity (sensitivity + specificity = 1.39), accurate lying and standing measurements were able to be captured with higher level of sensitivity + specificity at 1.90. Additional accuracy was obtained when an action occurred and was recorded for at least 24.8 seconds (Trenel et al., 2009). Further evaluation of this system was performed and did show the IceTags® were able to quantify the number of steps with a correlation of r = 0.84 and bouts of walking r=0.95 ($P<0.0001$) when compared to video observation. Similar successful correlations to video for standing and lying were also observed (Munksgaard, 2006).

Because of these results, the IceTag® became the activity monitor of choice to validate subsequent systems including the PedometerPlus© pedometers (S.A.E. Afikim, Israel). The

PedometerPlus© are reported to record steps taken, lying time, and lying bouts for dairy cattle. When the IceTag® and the PedometerPlus© were placed on different legs, the correlation for steps taken was r=0.73 (Higginson, 2009). Upon further investigation of this relatively low correlation, it was discovered that dependent on the side the cow was laying on, movement of the outside leg while lying increased the activity value for that particular leg when compared to the other. Therefore, the researchers placed both devices on the same leg, and the step correlation increased to r=0.82, lying bout correlation increased to r=0.98 and lying time correlation increased to was r=0.90. The high correlations between the new PedometerPlus© system and previously validated IceTags® provide support for the use of the pedometers in commercial dairy production (Higginson, 2009).

Most recently, the ability of the Onset Pendant G data loggers (Onset Computer Corporation, Bourne, MA) to classify animal behavior in greater detail has been assessed (Ledgerwood et al., 2010). In addition to the lying and standing behavior, these data loggers, through recording of the *g* force on the x, y and z-axes can also determine the laterality of the animal using the degree of tilt of the axes. Cut off values for the *g* forces were determined through preliminary data collection and compared to the video to classify the occurrence of behaviors. Further data manipulation with Microsoft Excel macros allowed for the creation of a descriptive data file that included the specific behaviors that could then be compared to video observation. The length of sampling time determined the overall accuracy. Longer recording length of 300 s did show the strongest positive relationship ($R^2 \geq .99$) to the video observation. By using a shorter, 30-s recording interval and some data filtering to remove ambiguous, short-lasting behaviors, a 99% predictability, sensitivity, and specificity was realized. This further supports the use of Pendant G data loggers as another mechanism to quantify animal activity and

behavior (Ledgerwood et al., 2010). All of these validation studies have been conducted using a Holstein population or beef calves. As diversity of breed increases in dairy herds, it would be advantageous to assess the accuracy of current activity monitoring systems to accurately quantify activity in different breeds.

1.2.3 Use of Activity Monitoring to Determine Influencing Factors on Dairy Cattle Behavior

The normal behavior of dairy cows can be affected by a variety of factors, which can include the individual variation, management practices, environment, and physiologic state (Gomez and Cook, 2010, Overton et al., 2002). Without a large impact of these factors, cows' behavior when managed in loose housing, group settings has been shown to remain stable. (Muller and Schrader, 2005).

Management is arguably one of the most influential and volatile factors affecting animal behavior. Video taken of 205 dairy cows in a freestall environment in 16 Wisconsin farms was analyzed to assess the impact of management on cow behavior. Time lying in stalls, feeding, standing in the alley, standing in a stall and the length of milking time over a 24 h period were the primary behaviors tracked. It was found that overstocking of pens resulted in longer milking times (P=0.003) and therefore, reduced the time of the other behaviors observed. Additionally, the milking time consisting of the time spent away from the housing area, negatively influenced feeding and lying time and the time spent standing in the alleys (Gomez and Cook, 2010). Cows are also more apt to lay 2 h after milking compared to a longer time post-milking (Overton et al., 2002). Additionally, when certain behaviors are forcibly restricted, behavior is adversely impacted (Munksgaard et al., 2005). When free access to lying down, feeding, and social contact was incrementally decreased from 23 h to 15 h and 12 h, respectively. It was determined that cows will prioritize lying time and sacrifice the amount of time spent eating and socializing

when such time constraints were implemented (Munksgaard et al., 2005). Utilizing behavior studies such as these further enhance the overall understanding of the impact of management on cattle behavior.

Further, the type of housing greatly determines behaviors of dairy cattle, especially in regard to resting behavior. When cows are housed in an open, large pen, they will lay 40% more than when they are housed in tie stalls. Large pen housing also allows for more changes between lying and standing because of the ease of rising and laying back down (Haley et al., 2000). The tendency of cows to prioritize lying indicates that ideal resting time is critical to overall cow comfort and health.

Finally, the physiologic state of the animal, such as stage of lactation, reproductive/health status can affect the actions and behaviors expressed. Locomotor activity (feeding, drinking, walking, grooming and rumination activity) is apt to change between the dry period and immediately following calving in a grazing system (Piccione et al., 2011). Dry cows had 924.1 ± 23.9 min/d of total locomotor activity as compared to the same cows during their lactation, which only had 358.1 ± 33.7 min/d. Since there is no predefined restriction of time, such as milking or specific feeding times, in the dry period, the opportunity to express overall activity is possible during gestation. In a more conventional housing system, cows' resting behavior was monitored for a 3 d period at 40 d before calving and for a 3 d period at 60 d after calving (Dechamps et al., 1989). Animals that were housed in closed stalls prior to calving, had a decreased percentage of lying bouts lasting longer than 1 h at 21.3%, as compared to after calving where lying bouts lasting longer than 1 h occurred 28.8% of the time but the difference was not significant. Comparatively, standing bouts lasting less than 15 min were increased throughout the dry period but still no difference existed for the actual length of standing time.

The authors hypothesized that the differences in the activity was due to the assumed discomfort associated with fetus weight in the gestating animals which resulted in more frequent changes of position.

In addition to locomotion, feeding and drinking behavior have been assessed in cows within the transition period (Huzzey et al., 2005). It is accepted that cows gradually decrease their feed intake up to 3 weeks before calving and increase their intake post-calving. This has led to extensive research as to how this type of behavior later impacts energy balance and metabolic disease incidence within the transition period. Cow behavior was monitored from 10 d before calving to 10 d post-calving. Standing time remained relatively constant over the transition period where cows pre-calving spent 12.3 ± 0.3h/d as compared to 13.4 ± 0.3 h/d of standing time post-calving. However, there was a significant difference found on the actual day of calving where standing time increased to 14.39 ± 0.29 h/d. A similar trend was seen in the number of standing bouts where the difference pre and post-calving was not significant. However, during the calving period, bouts increased significantly to an average of 17.3 ± 1.1 bouts compared to 11.7 ± 1.1 pre-calving and 13.1 ± 1.1 post-calving. Along with these changes in standing behavior, it was shown that cows spent less time eating (average 61.7 ± 3.0 min) and displayed more drinking bouts (9.5 ± 0.4 bouts) post-calving, as compared with pre-calving. The changes in feeding and drinking behavior are likely due to the increased energy requirements of a lactating cow. Standing and lying behavior may indicate the restlessness associated with discomfort and adaptation to changes throughout the transition period (Huzzey et al., 2005).

Beyond the stage of lactation, reproductive and health status affects animal behavior. When cows are in estrus an increase in step activity was observed (Maatje et al., 1997, Roelofs et al., 2005). A cow's average number of steps taken was collected using pedometers over 10 d

prior to the onset of estrous. It was determined that when the number of steps taken was 2.5 standard deviations above the 10 d mean, such increase in activity could be used to predict time of ovulation to be 29.3 ± 3.9 h after the onset of increased activity (Roelofs et al., 2005). Also, when activity increased to greater than 100% in corresponding periods over 2 d, cows were classified as being in estrus, which was visually confirmed. From this increase in activity, cows could be serviced within the optimal time period for improved conception rates (Maatje et al., 1997). In addition, the quantification of lame cows can be determined through the use of activity measures (Ito et al., 2010, Mazrier et al., 2006). In 92% of confirmed clinical lameness cases, cows reduced their pedometer activity by greater than 15%. Over time, 55.3% of these lame cows showed a decline in activity 7-10 d prior to the actual clinical expression of lameness (Mazrier et al., 2006). Lameness also negatively impacts the lying behavior of dairy cattle. Cows that were severely lame (gait score of 4 or higher) laid down 1.6 ±0.1 h/d longer and had longer lying bouts (P<0.001). Also, cows that laid for greater than 14.5 h/d and those that had average lying bouts lasting longer than 90 min were 16.2 and 3.0 times more likely to be severely lame, (Ito et al., 2010). The association of physiologic state and behavior alteration provides support for the use of activity measurements to predict other common dairy diseases.

While each of these studies has provided valuable information as to the various factors influencing cattle activity, the opportunity to combine of physiological and behavioral measures to identify other disease onset has been limitedly researched. Video evidence for behavioral measures is helpful but as observed, the further accuracy of behavior quantification through automatic data loggers can provide additional insight to true time budgets of animals and how they are affected by external factors.

1.3 Disease Detection in Dairy Cattle

1.3.1 Use of Activity Monitoring to Detect Disease

In understanding the natural behaviors of cows and the multiple factors that can influence those behaviors, activity monitoring can also aid dairy producers in identifying those animals at risk for disease. A field study tracked animal activity through the use of a pedometry system in three Florida dairies from 1996 through 1999 to see if the activity of the animals could be predictive of disease (Edwards and Tozer, 2004). All health events and treatments were recorded by the same veterinarian on each of the dairies. A healthy cow was defined as having no metabolic or digestive disorder in the pre-breeding stage of lactation and a sick cow had at least one metabolic or digestive disorder in the same period of time. On average, sick cows walked an average of 8 to 14 steps/h less than the healthy cows. When the metabolic diseases were examined separately, ketosis was diagnosed 10.0 ± 8.2 DIM. Activity of ketotic animals was increased 8 to 9 d prior to the onset of disease. After that point, activity declined up to d 5 as compared to healthy cows. Ketotic cows additionally showed a 9 kg/d decrease in milk on d 0 compared to their healthy counterparts. Left displaced abomasums (LDA) was diagnosed around 14.0 ± 11.9 DIM and activity was greater than healthy cows on every day except d 2 relative to the healthy herdmates. In the first 5 d of the lactation, healthy cows had decreased milk yield as compared to cows with LDA but then, after d 7, LDA cows showed a significantly reduced milk yield. Overall, the activity of these sick animals increased 8 to 9 d prior to clinical diagnosis with a gradual decrease until the day of diagnosis. LDA cows actually showed increased activity from d -5 to d -2 but the difference was not statistically significant compared to the healthy cows. However, on d -1 and 0 for LDA animals and d 0 for ketotic animals, there was a spike in activity. In comparison, milk yield declined at 6 and 7 d prior to diagnosis for ketotic and LDA cows, respectively (Edwards and Tozer, 2004). The definitions of disease diagnosis and onset were not extremely specific in order to discern particular disease incidences from one another.

While the veterinarian was consistent, day and designation of clinical diagnosis can be extremely subjective leading to greater amounts of variability in diagnosis. Therefore, additional behavior measurements such as resting activity may further enhance proper identification of both clinical and subclinical disease states.

Similarly, cows that were at risk for metritis were identified through behavior monitoring (Huzzey et al., 2007). Cows were followed from 2 wk before calving until 3 wk after calving with feeding, drinking and social behavior quantified. Prepartum feeding time and DMI were the best identifiers of cows at risk for getting metritis. Metritis cows spent less time feeding and consumed less feed beginning 2 wk before clinical signs were expressed. For every 10 min decline in feeding time, odds of clinical disease increased 1.7 times and for every 1.0 kg drop in DMI, the odds ratio increased to 3.0. There was also a tendency for the sick animals to have less social interactions at the feed bunk. Once again, milk production of the metritis animals was also decreased by 8.3 ± 0.5 kg/d in severe cases through 21 d after calving (Huzzey et al., 2007). Feeding behavior may indeed be the most indicative of those cows at risk for metritis, yet regular monitoring is not commercially feasible in today's industry.

Dystocia is also a common ailment that may be better predicted through the tracking of behavior and feed intake (Proudfoot et al., 2009). Dystocia was defined as a birth that would classify as a calving ease of 3 or higher. Cows that experienced dystocia not only altered their eating and drinking habits prior to calving but also had more changes in position from standing to lying. Cows having greater than 30 standing bouts 24 h before calving were at a greater risk of dystocia. Further, cows that had a difficult calving consumed 12% and 24% less DM 48 and 24 h prior to calving, respectively. The daily feeding times of these animals also decreased up to 11 h before calving. It is likely that larger calves that cause dystocia take up more available space

within the animal, limiting rumen capacity and thus a decrease in intake occurs along with discomfort from a large, poorly positioned calf leading to more changes in position of the cow (Proudfoot et al., 2009). Through monitoring animal activity directly prior to calving, those animals predisposed to dystocia may be identified and aided earlier in the calving process.

Early metabolic disease diagnosis through the use of activity monitoring can be useful for disease detection for cows, especially within the transition period. However, behavior can also aid in detection of intramammary infections (IMI). Management plays a large role on the behavior and ultimate health status of an animal. A feeding management strategy was analyzed to determine the lying behavior of animals and how it may influence an animal contracting mastitis (DeVries et al., 2010). On average, cows will stand 78.6 min after they have been milked while housed in a tie-stall system. Cows that lied down after 60 minutes were 7.4 times more likely to acquire an IMI than those who lied between 40 and 60 min after milking. This is due to the increase in teat canal diameter that occurs as more milk begins to accumulate in the udder after 60 min of milking. While behavior can be altered by feeding strategy as in this study, it is not an effective way of preventing mastitis cases. However, those animals who can be identified with longer standing times post-milk may be ones that managers can selectively target for mastitis screening more regularly (DeVries et al., 2010).

The lying behavior of the cow may also indicate a mastitis infection. The laterality of cows that had mastitis was found to be significantly different than those without the infection (Kikkers et al., 2006). The animals that tended to lie more so on their left side had an increased chance of having mastitis in the right quarter even though the relationship was not significant. Significance in this relationship may have been observed if lying position had been visually recorded more often than just four times throughout the day (Kikkers et al., 2006). The use an

automatic data logger could provide measurements every minute to determine the true relationship of infected quarters and lying side.

Similar behavioral changes were also observed with cows that were experimentally challenged with lipopolysaccharide (LPS) mastitis where the animals' resting behavior changed (Hänninen et al., 2007). Cows rested for a longer period of time immediately after being challenged compared to when animals were not infected on d -1. Following that period of rest, the hourly rest time decreased. A similar change in behavior was also observed in the first 12 h after LPS infection, where cows infected spent less time lying in their stalls (40.7 ± 4.0%) as compared to the control animals (47.9 ± 3.4%). These infected animals also reduced the time spent eating (16.9 ± 0.8% versus 21.0 ± 1.2%) and cud chewing (35.8 ± 2.3% versus 39.8 ± 1.5%) (Zimov et al., 2011). An *E.coli* infection induced similar responses as cows stood idly longer on the day of the infection with associated decreases in DMI and feeding time (Fogsgaard, In Press). The experimental challenge model is extremely effective in understanding behavioral changes prior to and after a mastitis infection. However, naturally occurring cases of mastitis should also be considered as severity and infection pathogen varies within a herd and can cause differences in behavioral responses.

Traditionally, a drop in milk production and feed intake, along with increased somatic cell counts (SCC) are the first indicators to a dairy producer that a cow may be sick (Heuer et al., 1999, LeBlanc et al., 2006). While milk weight and composition are easily traceable in modern parlor systems, the availability to track individual feed intakes and specific behaviors are limited. However, it is evident that by monitoring activity and feeding behavior we have the ability to identify and attend to sick cows earlier than the traditional indicators allow, thus providing a feasible and an effective way to improve the health of a dairy herd. Therefore, identifying

behavioral patterns around the onset of specific diseases to create flags and critical thresholds to identify at risk animals would be valuable to dairy managers.

1.3.2 Physiological Factors to Detect Disease

Throughout the various stages of an animals' productive life, there are many physiological changes that occur. Significant metabolic demands are placed upon dairy cattle, specifically, in the periparturient period. By understanding the physiological impacts of such stress, the ability to intervene and prevent common disease in this stage of life may be possible.

During the periparturient period, the energy requirements of the animal increases, while DMI involuntarily decreases, thus leading to a state of negative energy balance (Sordillo et al., 2009). Within this state of negative energy balance, homeostasis is altered and requires the mobilization of fat from body storage in the form of non-esterified fatty acids (NEFA). These fatty acids affect the cellular functioning by being incorporated into membrane phospholipids while also altering gene expression and cellular signaling. The increase in blood NEFA concentrations as well as other metabolic predictors and milk component changes can be associated with an increased risk of mastitis, metritis, and various other metabolic diseases in dairy cattle (Sordillo et al., 2009).

Using metabolic indicators in blood NEFA, β-hydroxybutyrate (BHBA), glucose and calcium concentrations can aid in determining animals at risk for post-partum diseases and subsequent culling. A field study conducted on 16 farms took blood samples from animals within three weeks after calving and assessed the occurrences of displaced abomasums (DA), ketosis, and culling (Seifi et al., 2010). NEFA were analyzed from serum taken prior to calving and BHBA levels were determined from the sample taken within 8 DIM. The median time of

diagnosis for both DAs and ketosis was 10 and 11 DIM, respectively. When BHBA levels were greater than 1000 µmol/L, cows were 13.6 times more likely to be diagnosed with a DA (P=0.0008). Additionally, if calcium concentrations were below 2.3 mmol/L, cows were 5.1 times more likely to develop a DA. Older animals with an increased body condition score were at an increased risk to develop ketosis. Both blood BHBA and NEFA levels were associated with cows being diagnosed with ketosis. When BHBA levels were greater than 1200 µmol/L and post-partum NEFA levels were greater than 1.0 mmol/L, animals were 4.7 and 6.3 times, respectively, more likely of developing clinical ketosis. NEFA and calcium levels were also the largest predictors of cows that would be culled within the first 60 DIM (Seifi et al., 2010). The economic threat and higher culling rate, as well as numerous risk factors associated with animals that experience metabolic diseases, has dictated further research, primarily in the area of DA incidence. A wide spectrum of blood metabolite measurements were assessed in animals from one week pre-partum to one week post-partum (LeBlanc et al., 2005). DA diagnosis occurred at a median time of 10 DIM. Cows that experienced a DA had increased NEFA levels that elevated to increased levels at a quicker rate as compared to their non-diseased counterparts. BHBA levels also diverged from their healthy herdmates but began on the day of calving. For every increase of 1 mEq/L in NEFA concentrations before calving, cows were 4.2 times more likely to experience a DA. Thresholds of 1200 µmol/L for blood BHBA, 1.0 mEq/L for NEFA, and greater than 200 µmol/L milk BHBA were more strongly associated with DA incidence. Furthermore, when compared to healthy counterparts, NEFA (1.36 mmol/L) and BHBA (1.56 mmol/L) in cows with DAs were greater than the healthy controls (0.34 and 0.90 mmol/L, respectively) (Stengarde et al., 2010). Therefore, these levels in addition to the identification of the additional risk factors can be used as determining animals that are more likely to have a DA

(LeBlanc et al., 2005). Each of these studies supports the use of blood parameters for the aid of identifying animals at risk for metabolic diseases. However, there is limited practicality of taking weekly blood samples consistently from both dry and lactating dairy cows and the availability of diagnostic tools to quickly identify diseased animals. Therefore, the use of already available management tools could allow for more real-time, labor-friendly disease detection aids.

Milk component analysis can also be used to identify cows at risk of disease. As discussed previously, when cows are in negative energy balance, lipid mobilization occurs to compensate for the lack of sufficient of energy (Toni et al., 2011). As such, there is an impact on milk fat percentage (Heuer et al., 1999). Under normal circumstances, the fat:protein ratio should be in the range of 1.0 to 1.5 (Toni et al., 2011). When the fat:protein ratio is elevated at the first DHI test date, it has been shown that the animal is indeed energy deficient and this puts the cow at an increased risk for ketosis, DAs, lameness, and mastitis. As the fat:protein ratio increased above 1.5, ketosis was 3.2-fold more likely to occur as compared to cows with a fat:protein ratio greater than 1.5 were 1.7 times more likely to have mastitis and 1.5 times more likely to become lame. A similar trend was seen in cows with a DA, where those animals with a ratio greater than 1.5 were 5.3 times more likely to get the disease as compared to their healthy counterparts (Heuer et al., 1999). Further analysis was conducted to determine if taking a milk sample seven days into lactation, as compared to the first test day, may provide more valuable health status information in post-partum animals (Toni et al., 2011). In first lactation cows, as the fat:protein ratio increased, the incidence of LDA, metritis, and endometritis increased and were greater than the incidence in their multiparous herdmates. While there was no clear trend associated with mastitis incidence, a fat:protein ratio of greater than 2.0 was a significant risk factor for retained placenta and metritis, while a fat:protein ratio greater than 2.5 also caused a significant risk for a

DA (Toni et al., 2011). Overall, when the fat:protein ratio is elevated, it presents a greater the risk of the spectrum of peripartum diseases. In addition to fat:protein ratios, the analysis of blood parameters with these studies would have provided more overall support of the lipid mobilization that is occurring in cows during the transition period.

Physiological changes in animals can also be seen in cows afflicted with other diseases, including mastitis. The physiological alteration that occurs during metabolic diseases, such as ketosis, can also cause an increase in clinical mastitis cases due to the impairment of udder defense mechanisms (Suriyasathaporn et al., 2000). When ketone bodies are mobilized in the state of negative energy balance post-partum, leukocytes have a decreased phagocytic capacity and there is decreased cytokine production in the presence of a bacterial infection in ketotic animals. When polymorphonuclear leukocytes (PMNs) are impaired, there is an increased severity of mastitis. Increased BHBA levels also inhibit neutrophil phagocytosis and killing capacity, which occurs when cows are in a ketotic state. When the immune function of animals is compromised due to the alteration in blood metabolites caused by a metabolic disease, the ability for the cow to self-clear a mastitis bacterial infection is reduced (Suriyasathaporn et al., 2000).

Beyond the association between metabolic disease and mastitis, mastitis itself causes both mammary specific and systemic physiological changes. Coliform mastitis caused by gram-negative environmental pathogens, such as *Escherichia coli,* enter the mammary gland through the teat canal, multiply and cause damage but are also rapidly eliminated by the host (Bradley, 2002). Such bacteria colonize the mammary gland and multiply without attaching to the epithelial cells, and survive by using lactose as a carbohydrate source in anaerobic conditions (Hogan and Smith, 2003). When a coliform infection is present, neutrophils are recruited for the

phagocytosis and killing of the bacteria. The virulence of such infections is dictated by how susceptible the specific pathogen is to the phagocytosis. Cell surface components presented by *E.coli* may allow for such infections to have a longer duration in the mammary gland. When the bacteria are effectively killed, endotoxin is released from the lysing of the bacterial cell wall and initiates the inflammatory response. Toll-like receptor 4 (TLR4) on the neutrophils is responsible for LPS recognition and induces inflammatory cytokine production along with the upregulation of anti-microbial genes during infection (De Schepper et al., 2008, Werling and Jungi, 2003). Along with cytokine production, the lipid-A portion of LPS is bound by lipopolysaccharide-binding protein (LBP). The LPS-LBP complex is then recognized by CD14 and stimulates mitogen-associated protein (MAP) kinases and the transcription factor, NF-κB (De Schepper et al., 2008, Miyake, 2004). NF-κB activation occurs not only during a time of infection but also in regular mammary gland development throughout the various stages of lactation (Connelly et al., 2010). At this point in the infection, clinical signs are typically expressed through anorexia, fever, dehydration and diarrhea along with a loss in milk production. *E.coli* infections do not typically last longer than ten days with 85% of the infections causing clinical cases (Hogan and Smith, 2003). However, it is not recommended to treat these infections with antibiotics due to the shortness of duration, self-cure rate, and possible induced sepsis if the cells have not yet been phagocytized upon treatment (Hogan and Smith, 2003).

E.coli infections infect at least 25% of cows annually (Hogan and Smith, 2003). Therefore, experimentally induced mastitis research has allowed for valuable additional information to be gained about this type of infection. When inoculated with *E.coli* 727, cows will show an increased rectal temperature at approximately 14 h post-infection with a return to normal by 24 h (Todhunter et al., 1991). Along with a spike in temperature, $\log_{10}$cfu peaks

between 9 h and 14 h, begins to decline by 24 h. When this decline begins, clinical signs are then displayed. As mentioned previously, a 36% loss in milk production has been shown, but animals are able to return to pre-challenge levels between d 4 and d 5. This type of infection also alters the DMI of the infected animals with a reduction in intake of approximately 4% with recovery between d 1 and d 2 (Todhunter et al., 1991). The amount of bovine serum albumin, which indicates when vascular leakage is occurring in the gland, was increased in animals post-challenge and was indicative of the mammary inflammation related to the infection (Hogan et al., 1995). Milk appearance also remained at an elevated clinical score (4) for a longer duration in the animals challenged with *E.coli* without treatment as compared to those administered a vaccine (Hogan et al., 1995). The physiological changes associated with *E.coli* mastitis are well understood, however, no behavioral changes were assessed in these studies.

The changes in behavior associated with mastitis have been recently researched to determine ways to alleviate the perceived reduced well-being of infected animals (Leslie, 2010). An experimentally induced endotoxin infection caused similar physiological effects as the *E.coli* 727 strain with an increase in SCC, rectal temperature and serum cortisol (Zimov et al., 2011). To further support the adverse effects of mastitis on the overall welfare of the cow, cud chewing, eating, and reduction in lying time was reduced in the infected animals. While it has been acknowledged that DMI is the most sensitive measure of behavioral changes associated with this type of infection (Fogsgaard, In Press), monitoring lying behavior during experimentally induced mastitis provides yet another avenue for proactively identifying mastitic animals. Within the first 24 h of induced *E.coli* mastitis, the length of time cows spent standing idle was significantly increased. These animals indeed respond to the infection with the expected clinical symptoms

but also through the quantification of behavioral changes a better understanding of the discomfort the animal is experiencing can be developed.

1.4 Sickness and Discomfort in Dairy Cattle

Animals that experience sickness show changes in their behavior due to their inability to perform normal activities (Aubert, 1999). Sickness behavior is comprised of the metabolic and physiologic changes required to address the infection causing the sickness. The behavior traditionally associated with a sick animal is a decrease in activity and an increase in rest time so that energy can be conserved for a full immune response to take place (Aubert, 1999). This same deviation in normal behavior has also been used as an indicator of animals experiencing pain. While pain can only be quantified subjectively, it has been defined as an unpleasant sensory and emotional experience associated with the actual or potential tissue damages (Anil et al., 2005). It aligns with sickness behavior in the way that it has been quantified to cause discomfort, impaired physiologic function, and a negative impact on immunity. While humans can formally express their pain, animals too have are believed to share the same sensory and emotional capacity to express experienced pain. However, the physiological measures of pain have not been adequately developed on the farm level (Anil et al., 2005).

1.4.1 Treatment of Discomfort

Responses to discomfort vary from animal to animal and in the same animal with various events. Therefore, it is increasingly difficult to treat effectively. NSAIDs have been used to alleviate inflammation and potentially the perceived discomfort that occurs in response to an infection. In general NSAIDs are anti-inflammatory, analgesic, anti-hyperalgesic, and/or anti-pyretic and are able to address each of these physiological responses by inhibiting one or both of the cyclooxygenase (COX) pathways thus blocking the production of prostaglandin, which is the

primary contributor to sensations of discomfort. By inhibiting COX-1 and COX-2, both the maintenance of homeostasis from the COX-1 pathway and proinflammatory enzymes from the COX-2 pathway are affected. NSAID administration alters various cellular processes that block the NF-κB signaling pathway. This occurs because of inhibition of IκB kinase which prevents the translocation of NF-κB to the nucleus of the epithelial cell and thus preventing the upregulation of target genes (Gupta and Dubois, 2001, Ulrich et al., 2006).

The dehorning of calves is generally accepted to be an uncomfortable experience and therefore, extensive research has been conducted in an attempt to reduce this discomfort through the use of NSAIDs (Heinrich et al., 2010). Calves were administered meloxicam, an NSAID with a half-life of up to 26 h that preferentially inhibits the COX-2 pathway which specifically addresses pain and inflammation resulting from tissue injury. The use of video and accelerometers showed that the NSAID treated calves showed less ear flicks and head shaking, were less active up to 5 h after dehorning and were overall less sensitive to the pain as quantified by an algometer. Further, the treated animals ate more on d 1 after dehorning than on d 0 (Heinrich et al., 2010).

Lameness is another dairy cattle ailment that is assumed to cause a distinguishable amount of discomfort as seen by the change in locomotion gait scores (Chapinal et al., 2010). Therefore, the administration of ketoprofen, an NSAID, was given to lame cows to determine if the pain could be reduced. The gait scores and distribution of weight on the rear legs was analyzed, which showed that ketoprofen allowed for a more uniform distribution of weight on the rear legs which also was the best predictor of lameness. The untreated counterparts stood with more asymmetrical weight distribution and shifted weight more often (Chapinal et al., 2010). The apparent alleviation of the pain associated through the use of NSAIDs has, in turn,

stimulated further research in using such products to address pain and discomfort with other common dairy diseases.

1.4.2 Discomfort Associated with Mastitis

In surveys conducted, veterinarians and farmers alike believe that mastitis is painful (Huxley, 2007). On a scale from one to ten, with ten being the worst pain imaginable, veterinarians scored a grade three case of mastitis a seven with producers scoring the pain associated with this grade of mastitis an eight (Huxley, 2007). In analyzing both behavioral and physiological changes that occur during mastitis, dairy cows exhibit clear signs that indicate that there is a considerable amount of discomfort associated with a mastitis infection.

Cows with mastitis have an increased sensitivity to pain (Fitzpatrick, 1998). Naturally occurring mastitis cases were analyzed to determine the severity of the case and the impact on the welfare of the animal (Kemp et al., 2008). In moderate cases of mastitis, the animals experienced increased rectal temperatures (38.9^{o} C), heart rate (78.5 bpm) and respiratory rates (24.1 breaths/min) above that of non-infected animals, which, as mentioned previously, are associated with both sickness and pain expression. Additionally, the width between the hocks of the control animals at 225 mm was less than the mastitic animals which indicated less swelling of the udder and thus less discomfort. When pressure was applied to both legs at random, the pain threshold on the unaffected side was greater than the affected side. This was a possible indication that the discomfort associated with mastitis hinders those animals' desire to shift weight to the side that is affected. (Kemp et al., 2008). Similar results were observed when animals that were exposed to leg stimulation on the infected side of the udder showed an increased sensitivity to pain when compared to those animals without mastitis (Fitzpatrick,

1998). In both studies, it may have enhanced the actual quantification of pain thresholds if the pressure was applied directly to the mammary system of the cow as opposed to just the leg.

When stimulation was actually applied to the mammary gland of cows experimentally infected with *E.coli* mastitis, the proportion of cows that stepped (indicating discomfort from the infection) was significantly increased on d 1 of the infection (Rasmussen et al., 2011). SCC also increased and was positively correlated to the prevalence of kicking (P=0.06, r=0.87). The increase in rectal temperature on d 1 was also positively correlated with the length of time it took for the animal to move her leg where the more elevated the body temperature the longer it took the animal to move her leg (P=0.02, r=0.84). The latency associated with the leg movement further support the type of behavior seen in the previous study where responsiveness to stimulation declines with such an infection due to the pain associated with the inflammation by measuring a secondary response in the movement of the leg (Rasmussen et al., 2011).

Fogsgaard (In Press) and Zimov (2011) both showed that eating behaviors decreased significantly in infected animals and a sharp decline in lying time with increased idle standing time was observed. As outlined previously, the alteration from the normal behaviors provides evidential support that infected animals are indeed in a state of discomfort. Therefore, with the acknowledgment of this poor state of well-being, NSAIDs would be an appropriate therapy for mastitis cases (Leslie, 2010).

1.4.3 NSAID therapy for Discomfort associated with Mastitis

In addition to antibiotics, NSAIDs, such as flunixin meglumine (FM), have been researched for the therapeutic usage during mastitis (Fitzpatrick, 1998). When FM was administered intravenously to animals with naturally occurring cases of mild and moderate

mastitis, a large decline in the responsiveness to applied pressure to the leg on the same side of the mastitic quarter occurred. However, at the next sample period, pain responsiveness had returned to the control levels. There was no significant difference in SCC between the treatment groups. Due to the brevity of apparent pain alleviation, the researchers had suggested the potential for additional doses of FM for prolonged positive effects (Fitzpatrick, 1998).

Zimov et al. (2011) examined the effects of FM during endotoxin induced clinical mastitis. Milk measurements and behavioral activity was monitored in the study animals. FM was given 4 h post-infection and was able to reduce the rectal temperatures of infected animals by the 6 h time point. The frequency of rumen sounds were numerically increased in challenge animals but DMI was not affected by the infection or FM treatment. The lack of DMI difference was likely due to the feeding management or the actual length of the infection time during the study but cows did show an increased eating time 9-12 h after its administration along with showing an increase in cud chewing compared to the non-treated control group. While infected cows spent less time lying in the first 12 h after infection, FM had no effect on the lying behavior (Zimov et al., 2011). This study was effective in showing the impact of FM administration against non-treated controls. However, the FM treatment was given at a predetermined time point instead of when clinical mastitis was displayed. In dairy operations, treatment is typically given upon clinical diagnosis which may have altered these behavioral responses.

The efficacy of FM was directly compared to another NSAID, isoflupredone acetate, when cows were experimentally induced with LPS mastitis (Wagner and Apley, 2004). The treatment groups consisted of the two NSAID groups and a saline control. NSAIDs were administered upon the cows showing clinical signs of mastitis. The NSAID treated individuals did experience decreased heart rates with no significant differences in milk production as

compared to the control animals. The largest drop in milk production was observed on d 1 post-infection in all treatment groups but all returned to baseline production by d 10. The rectal temperatures of FM treated cows were decreased below the isoflupredone acetate and the control cows while also having an increased occurrence of rumen compared to the other two treatment groups (Wagner and Apley, 2004).

FM and the steroidal drug dexamethasone, as treatments for LPS induced mastitis cases, have also been researched (Anderson and Hunt, 1989). Cows were administered FM at 2 h and 10 h post-challenge in comparison to dexamethasone which was given once just at 2 h post-challenge. Each of these treatments was compared to a saline control group. Changes in milk appearance began at 2 h post-infusion and a rise in rectal temperature was observed in all three treatment groups. FM treated cows had intermediary body temperatures that were increased above the control group but not as low as the dexamethasone treated. All treatment groups displayed depressed milk production by an average 74% of the baseline production levels. However, dexamethasone also caused a greater decrease in milk production as compared to FM and the control groups with no significant differences seen between FM and the control. It was once again suggested that an increased dosage of FM would possibly allow for a more pronounced therapeutic effect. Administering either an NSAID or a steroid had both benefits and detriments. FM did alter milk production but did not have as much benefit on rectal temperature. In comparison, dexamethasone decreased temperature more effectively but at the cost of milk production. (Anderson and Hunt, 1989). From the results of these studies, further analysis of body temperature, milk production, in combination with behavior should be analyzed using FM as an NSAID treatment against saline controls to determine the overall impact of this particular therapy.

FM is not the only NSAID that has been evaluated for mastitis therapy. Ketoprofen is another NSAID utilized by the dairy industry (Fitzpatrick, 1998). Ketoprofen inhibits both of the COX pathways. Therefore, the effectiveness of ketoprofen in both experimentally induced and naturally occurring mastitis cases has been analyzed. Three treatment groups were studied where two groups of experimental animals were inoculated with LPS and compared to an untreated and served as the control group. The two groups of experimental animals were given ketoprofen either orally or intramuscularly 2 h after LPS mastitis was induced (Banting et al., 2008). Untreated control animals showed an increase in rectal temperature to an average of 40.5^{o}C with differences between the groups seen at 6, 8 and 10 h post-challenge. By 2 h post-challenge, respiratory rates were increased but the treated groups started to decline by 6 h and were normal after 24 h. Rumen contractions were reduced by 50 percent in the 2 h post-challenge but within 6 h, ketoprofen treated animals began to recover with full recovery by 24 h whereas the control group did not recover until d 7. As the udder of the animals was palpated, a visual analogue scale assessed the pain experienced. Ketoprofen allowed for a more rapid decline in pain scores as compared to the untreated control. Further, milk thromboxane β_2 levels, an indicator of the general inflammatory status of an animal, were reduced at 6 h post-challenge as compared to 12 h post-challenge in the control animals. A field trial showed similar benefits by using ketoprofen (Shpigel et al., 1994). Upon initial diagnosis of clinical mastitis, the animals were given antimicrobials in combination with ketoprofen. A secondary portion of the study included ketoprofen treated versus a placebo treated control group. The animals treated with ketoprofen had an average 93.5% recovery rate as compared to the average recovery rate from the control groups of 78.4%. Recovery was only based upon production parameters however, the combination of Banting et al. (2008) findings and the field study, Shipgel et al. (1994), has

shown ketoprofen to also be an effective mastitis therapeutic NSAID in addressing a portion of parameters associated with discomfort.

Carprofen, a prostaglandin synthetase inhibitor that also preferentially inhibits the COX-2 pathway, has also been explored to address *E.coli* infected animals (Vangroenweghe et al., 2005). Carprofen has a half-life of 30.7 h as compared to the 8.1 h of FM and 0.5 h of ketoprofen, leading to the assumption that the benefits could be greater with the longer duration of efficacy. The increase in rectal temperature within infected animals, as seen in previous challenge studies, was once again observed in this study. However, upon carprofen treatment, temperatures decreased by 3 h post-treatment. Heart rates were reduced in the carprofen treated group and remained reduced throughout the trial period and rumen motility improved by 3 h post-treatment administration as compared to the saline treated control group. However, immunological measures IL-8, C5a and sCD14 were not significantly affected by the treatment itself (Vangroenweghe et al., 2005). The improvements in temperature and motility support carprofen as another feasible option in addressing the adverse effects of mastitis.

Another COX-2 specific inhibitor, meloxicam, has been shown to be beneficial in naturally occurring mastitis cases. When mastitis cases were determined by the producer, a technician administered the meloxicam intravenously along with an antibiotic given intramuscularly (McDougall et al., 2009). SCC was decreased in the treated animals (550 ± 48 SCC/mL) as compared to the cows given just the vehicle for meloxicam who served as the control group (711 ± 62 SCC/mL) and while milk yield decreased in the infected animals, there was no difference between the two groups of animals. The animals treated with meloxicam had a cull rate of 16.4% as compared to 28.2% in animals not treated with meloxicam (McDougall et

al., 2009). The advantage of keeping animals in the herd along with maintaining reduced SCC once again provides evidence that NSAID therapy for mastitis can be extremely beneficial.

Sodium salicylate showed similar benefits to meloxicam when administered prior to an endotoxin experimentally induced mastitis case (Morkoc et al., 1993). SCC increased as the gland showed signs of inflammation and peaked at 10 h post-infection. Cows given sodium salicylate displayed numerically decreased SCC as compared to the saline control, but no statistical differences were found. There was no difference seen in the inflammation that either group experienced yet prostaglandin levels were reduced in the milk. Peak rectal temperature was decreased and returned to baseline levels 12 h before the untreated control animals. Appetite and milk production both declined as seen previously, yet the control animals returned to baseline milk production levels earlier than the treated group with no effect of sodium salicylate observed (Morkoc et al., 1993).

Despite which specific NSAID is used, it is clear that the benefits on temperature, rumen function, SCC, milk production, behavior, and pain sensitivity in animals during mastitis constitute this therapeutic treatment throughout the dairy industry. As the health and well-being of dairy cattle continues to be scrutinized by consumer groups, it is essential that the alleviation of any perceived pain or discomfort associated with one of the most common dairy diseases, mastitis, be addressed.

1.5 Research Objectives

The benefit of early disease detection and possible prevention in a dairy herd extends beyond the economic benefit. Ensuring improved herd health, animal well-being can be enhanced and overall longevity of dairy cattle can be achieved. Mastitis, as one of the most

costly and common diseases in the dairy industry has known detrimental effects to the dairy cow. However, the extent to which the disease effects behavior and quantification of the possible discomfort experienced during such an infection has not been addressed. Therefore, the first objective of the research was to determine if FM (an NSAID) administration had an impact on activity measures, feed intake, and milk production during experimentally induced *E.coli* mastitis.

Further, the transition period is a critical time in a dairy animal's life where immunosuppression causes a host of subsequent diseases post-partum. While physiological measures and clinical symptoms have accurately quantified the onset of such diseases, behavior of dairy cows to predict such diseases has been limitedly utilized. Therefore, the second objective of the research was to further use animal activity along with blood metabolite measurements pre and postpartum to determine if postpartum diseases could be predicted prior to the expression of clinical signs.

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Chapter 2: The effects of flunixin meglumine administration on activity measures, feed intake, and milk production during experimentally induced *Escherichia coli* mastitis

ABSTRACT

The use of flunixin meglumine (FM), a non-steroidal anti-inflammatory drug, during experimentally induced *Escherichia coli* mastitis was evaluated. A total of 24 primiparous and multiparous lactating dairy cows were challenged with 1 x 10^2 cfu of *E.coli* 727 in one quarter. Of the 24 *E.coli* challenged animals, 12 were administered FM (ECF, 0.002 µg/45.5kg of body weight) at the onset of clinical mastitis signs. The remaining 12 cows were untreated (EC). An additional 11 cows were infused with 1 mL of sterile phosphate-buffered saline and served as the control (CTL) group. Activity measures, dry matter intake (DMI), and milk production were collected on all animals. Activity measurements were collected using both Afi PedometerPlus© pedometers and HOBO® data loggers. Activity was summarized by day (PedometerPlus©) and in three hour time periods (HOBO®). An examination of animal activity indicated that EC and ECF cows stood more and laid less as compared to the CTL animals in the first 6 h after FM administration. When DMI was analyzed, CTL and ECF had greater DMI than the EC animals on d 1 post-challenge. However, by d 2 post-challenge, DMI for ECF and EC cows was significantly lower than the CTL. The ECF cows had greater milk yield than EC animals by d 3 and by d 4 post-challenge and there was no significant difference in yield between the ECF and CTL animals. Additionally, when primiparous EC animals were compared to their multiparous counterparts, the multiparous animals had a reduced milk yield post-challenge. This difference was not observed in the ECF animals. Therefore, it can be concluded that *E.coli* mastitis does alter animal activity and may have a negative impact on animal well-being. However, the improvement in DMI and milk production for ECF animals provides evidence for using an NSAID as supportive therapy in alleviating the adverse effects associated with *E.coli* mastitis.

Key Words: *Escherichia coli*, mastitis, animal activity, NSAID

INTRODUCTION

Mastitis is defined as an inflammation of the mammary gland, which is typically caused by a bacterial infection. It is one of the most costly diseases affecting dairy cattle costing a dairy producer upwards of $200 per clinical case (Smith and Hogan, 2001). A common cause of clinical environmental mastitis in dairy cows, *E.coli* causes a host immune response, which alters milk composition causing the clinical expression of mastitis (Hogan and Smith, 2003). This type of infection not only causes alterations in the milk but also in the physiological functioning of the animal.

Cows experience increased SCC, rectal temperature, serum cortisol levels and decreased rumen motility during experimentally induced *E. coli* mastitis (Hogan et al., 1999, Hogan et al., 1995). However, few reports have examined the physiological and behavioral changes that mastitis inflicts on animals. One study showed reduced lying behavior, eating time, and cud chewing following lipopolysaccharide (LPS) infusion (Zimov et al., 2011). Additionally, time spent idly standing increased and self-grooming decreased when animals were infected with *E.coli* (Fogsgaard, In Press). It is hypothesized that such behavioral changes associated with mastitis may be attributed to the discomfort associated with the infection (Fogsgaard, In Press, Kemp et al., 2008, Leslie, 2010).

While effective management and treatment protocols for mastitis have been successfully implemented, therapies to address the behavioral and physiological changes have not been well documented. The NSAIDs have been well-proven to reduce inflammation, pain, pain sensitivity, and fever (Leslie, 2010). Therefore, the use of an NSAID to counteract these negative impacts of mastitis may be an appropriate treatment to recommend in a standard operating procedure. Cows

that were experimentally induced with endotoxin or *E.coli* followed by the administration of an NSAID showed decreased rectal temperatures (Anderson and Hunt, 1989, Banting et al., 2008, Vangroenweghe et al., 2005, Wagner and Apley, 2004) and restored rumen motility (Banting et al., 2008, Vangroenweghe et al., 2005) as compared to those not treated. The use of an NSAID during naturally occurring mastitis has shown limited but positive effects. In one study, animals treated with an NSAID displayed reduced SCC and increased survivability post-infection (McDougall et al., 2009). However, the ability to identify behavioral changes associated with mastitis following the administration of flunixin meglumine (**FM**), using a pedometry system, has not been previously reported.

Therefore, the objectives of the current trial were to assess the effects of FM treatment on activity measures, feed intake, milk production, $\log_{10}$cfu, and SCS during experimentally induced *E.coli* mastitis.

MATERIALS AND METHODS

Animals

Thirty-five primiparous (n = 20) and multiparous (n = 15) lactating dairy cows (29 Holstein, 6 Jersey) housed at the Virginia Tech Dairy Center (Blacksburg, Virginia) were used in the study. Cows were housed in a freestall barn containing sawdust bedded stalls with rubber mattresses. Stalls were bedded with sawdust twice weekly. A week-long adaptation period was utilized for socialization and adjustment to eating from Calan© door feeders. Animals were 35 to 170 DIM (median=117 DIM) and were milked twice daily at 12-h intervals in a double-eight milking parlor.

Quarter milk samples were aseptically collected at -7, -5, and -3 d relative to intramammary challenge to determine pre-existing infection status. Samples were evaluated for

microbiological status and SCC to determine quarter-level infection status. Procedures regarding aseptic milk sampling and microbiological culture were followed as described previously (Hogan et al., 1999) with SCC being determined at the DHIA laboratory (United Federation DHIA; Blacksburg,VA). Mammary quarters with a pre-existing IMI (two out of three positive samples) were not considered for challenge. Animals were challenged in groups of six per week with cows randomly assigned to one of three treatment groups; *E.coli* challenged (EC, n=12), *E.coli* challenged followed by FM treatment (Intervet/Schering-Plough Animal Health; The Netherlands; ECF, n=12) or saline control (CTL, n=11).

Intramammary Challenge

The challenge bacterium, *E. coli* 727 was obtained from The Ohio State University and stored at -80ºC until needed (Hogan et al., 1995). Preparation of the challenge inoculum followed a previously published protocol (Hogan 1995). In brief, prior to challenge, the bacteria were streaked for isolation on blood agar. Upon isolation, the bacterium was cultured in trypticase soy broth at 37ºC for 24 h. The bacterial culture was then centrifuged at 6000 rpm at 4°C for 20 minutes to achieve a pellet of the bacterium. The pellet was diluted in phosphate buffered saline (**PBS**) to achieve a final concentration of 1.0×10^2 cfu/mL. Colony-forming units of the challenge inoculum were determined by delivering two 1 mL replicates and two 100 µl replicates of the final dilution on to the surface of a plate containing liquid MacConkey agar.

Prior to infusion, challenge teats were cleaned with cotton swabs soaked in 70% ethanol. An inoculum of 1 mL with approximately 1.0×10^2 cfu/mL of the bacterial suspension was infused into one randomly selected mammary quarter of the EC and ECF cows using a sterile 1 mL syringe fitted with a sterile teat cannula (Jorgensen Laboratories, Inc., Loveland, Colorado) immediately following the morning milking. The CTL cows were infused with 1 mL of sterile

PBS using the same procedure as described above. Teats were post-dipped with a teat disinfectant immediately after inoculation.

Activity

Each of the animals had an Afi PedometerPlus© affixed to a rear leg fetlock, which collected activity data on a daily basis. Cow activity was collected between milking sessions and stored in the memory of the pedometer. The activity information collected was then transmitted at each milking through a reader box to the AfiFarm© (S.A.E. Afikim, Israel) Herd Management software program. Activity variables collected by the Afi PedometerPlus© included steps taken, resting bouts, and resting time between milking sessions for each and summed for daily measurements of these activities.

The HOBO© data loggers (HOBO Pendant G Data Logger, Onset; Pocasset, MA) were attached to the same rear leg as the Afi PedometerPlus©. This device measured acceleration and angle displacement on an X, Y, and Z axis. Through these measurements, activity was classified. Cow activity variables associated with the acceleration and angle displacement included lying bouts and average lying times for both left and right sides in addition to total lying and standing time and average daily steps. The data loggers were set to start recording acceleration and angle displacement data upon the time of challenge with data collected every minute throughout the study. At the final sample point, the HOBO© data loggers were removed and the collection of data ceased. To assess daily activity, the minute data were summarized into 3 h time periods and then further summed together to obtain daily activity. The purpose of Afi PedometerPlus© data collection was to validate the daily resting time when compared to HOBO© data loggers (considered the ‘gold’ standard).

Dry Matter Intake (DMI)

Cows were fed approximately 45.5 kg of a TMR daily at 1230 h using a Data Ranger© (American Calan Inc.; Northwood, NH). On the following day, the refusals were removed via vacuum by the Data Ranger© and weighed with the Data Ranger© scale prior to the 1230 h feeding. A daily representative sample of the TMR was collected to determine the DM of the feed to achieve a DMI daily on each animal as described previously (Cyriac et al., 2008).

Data Collection Post-challenge

Aseptic quarter milk samples were collected from all quarters and rectal temperatures were recorded from all study cows at h 0, 3, 6, 9, 15, 18, 21, 24, 36, and d 2, 3, 4, 5 and 6 post-challenge. Clinical status of all quarters was recorded on the 5-point scale as previously described Hogan et al. (1995): 1 = normal milk and normal quarter, 2 = normal quarter but milk was questionable, 3 = normal quarter but abnormal milk, 4 = a swollen quarter and abnormal milk, and 5 = swollen quarter, abnormal milk, and systemic signs of infection. A challenge quarter was considered clinically mastitic if the milk appearance score was ≥ 3.

Each sample was subsequently analyzed for microbiological, SCC, and $\log_{10}$cfu determination. Detection of *E.coli* 727 in challenge quarters was by duplicate in 1.0 mL pour plates using MacConkey agar for EC and ECF animals. The total $\log_{10}$cfu counts were determined by pour plates or serial dilutions plated on MacConkey agar. Following incubation (37°C, 18 h), the counts were determined. The final bacterial numbers were recorded as $\log_{10}$cfu/mL. An infection was considered to be present if there was ≥1 cfu/mL of *E.coli* 727 in two of three consecutive samples. Somatic cell counts were recorded as SCC $\log_{10}$/mL. Milk production was collected using the AfiMilk© (S.A.E. Afikim, Israel) system.

Flunixin Meglumine Injection

At the onset of clinical signs (milk appearance score ≥ 3), ECF animals received a single dose of FM. As prescribed by the Virginia Tech Veterinary staff and the label recommendations, the FM was intravenously administered to the ECF treatment group at 0.002 µg/45.5 kg BW through the jugular vein. Weight was determined on the day of challenge using scales at the milking parlor exit. Following administration of flunixin meglumine, the ECF treatment group received the same management as the other treatment groups.

Data Management and Statistical Analyses

All data was stored in a database (Microsoft Access 2000 and Microsoft Excel 2007 for Windows©; Microsoft Corporation, Redmond, Washington) until analysis. The HOBO© data logger data required the utilization of macros for the data to be descriptively analyzed as previously described by Ledgerwood et al. (2010). HOBO© data logger information recorded in minutes and were later summarized into 3 h time periods to coincide with milk sample collection. To accurately compare all three treatment groups, in the lying time, standing time and lying bouts models, periods prior to flunixin meglumine administration and after the specified label effectiveness were truncated.

The analyses of animal activity, DMI, and milk production were performed using the PROC GLIMMIX procedure in SAS (version 9.2, SAS Institute Inc., Cary, NC). The variables offered into the lying time and standing time and average daily steps activity models included treatment, lactation number, period or day, DIM, SCS, temperature and covariates (at period -1) with associated interactions. Within the lying bouts model, treatment, lactation number, period, DIM and baseline covariates were offered. The lying side preference model was offered treatment, lactation number, period and the side of infection. The variables offered to the DMI model included treatment, lactation number, day, DIM, daily total standing with associated

interactions. The variables offered into the milk production model included treatment, lactation number, day, DIM, day of challenge DIM, covariates and associated interactions. The variables offered into the log_{10}cfu and SCS models included treatment, lactation number, hour, and DIM with associated interactions. The relationship between total activity data collected from the HOBO© data loggers and Afi PedometerPlus© was analyzed using the PROC GLM procedure to assess the correlation of the Afi PedometerPlus© from the HOBO© data loggers.

All variables entered into the model were removed manually by backwards elimination based upon significance of the p-value, from highest to lowest until all variables were significant ($P \leq 0.05$). For each model, least square means and standard errors were determined for the significant variables. Slices were also used to determine significant days, periods, or hours within treatment interactions. Tukey's adjusted *P*-values were calculated for all class variables.

RESULTS

The average number of bacteria infused at the time of challenge was 69 cfu/mL (range 59-74 cfu/mL). No significant differences were found in the challenge inoculums by week. Intramammary challenge with *E.coli* 727 resulted in 100% (24/24) of quarters becoming infected. Two animals did not have activity data recorded beyond d 1 post-challenge as the HOBO© data logger was set to record in seconds instead of minutes thus filling the memory capacity of the logger. These two animals were removed from the data analyses involving activity parameters.

Animal Activity

Least square means and standard errors for lying and standing time are shown by treatment group in Table 2.1. Treatment group had a significant impact on lying (Figure 2.1) and standing time (Figure 2.2). In periods 0 and 1, relative to FM injection, EC (64.8 ± 12.7 min and

86.4 ± 12.7 min, respectively) and ECF (56.7 ± 13.3 min and 64.4 ± 13.3 min, respectively) animals spent less time lying per period than the CTL (108.2 ± 13.9 min and 119.9 ± 13.9 min, respectively). Similarly, in periods 0 and 1, relative to FM injection, EC (115.2 ± 12.7 min and 93.6 ± 12.7 min, respectively) and ECF (123.3 ± 13.3 min and 115.6 ± 13.3 min, respectively) spent more time standing per period than the CTL (71.8 ± 13.9 min and 60.1 ± 13.9 min respectively). No significant differences were seen after period 2 relative to flunixin meglumine injection.

The steps taken per hour was influenced by day relative to challenge ($P<0.05$) but was not affected by treatment group (Figure 2.3). Additionally, treatment group did not impact the lying side preference. As DIM increased, the number of lying bouts decreased. Furthermore, primiparous cows expressed 1.9 ± 0.1 lying bouts per period as compared to the multiparous animals which showed an average of 1.6 ± 0.1 lying bouts ($P<0.001$). Treatment did not influence average lying bouts per period following FM administration. However, a numerical difference was observed for the CTL and EC groups (1.9±0.8 and 1.7 ± 0.7 per period, respectively, $P=0.08$).

Predictability of Activity Monitors

The sum of total daily lying time variable collected by the HOBO© data loggers was compared to the Afi PedometerPlus© daily rest time, which is the equivalent variable. Daily rest time from the PedometerPlus© was highly correlated to the sum of total daily lying time from the HOBO© data loggers (R=0.97, $P<0.001$, Figure 2.4).

Milk Production

The treatment group by time interaction in the milk production model showed a significant impact on d 1, 2, 4 and 6 (Figure 2.5) between the three treatments. On d 1 and 2

post-challenge, the CTL group displayed greater milk yield (32.2 ± 2.3 kg and 31.1 ± 2.3 kg, respectively) than the EC (16.0 ± 2.2 kg and 18.4 ± 2.2 kg, respectively) and ECF (17.7 ± 2.2 kg and 20.3 ± 2.2 kg, respectively) groups. On d 3 post-challenge, a significant difference was observed between all three groups with CTL yielding 33.9 ± 2.3 kg, EC yielding 17.0±2.2 kg, and ECF yielding 26.2 ± 2.2 kg (P<0.05). However, by d 3, ECF cows had significantly higher yield (26.2 ± 2.2 kg) than the EC cows (17.0 ± 2.2 kg). By d 4 and 6 post challenge, ECF cows reached similar level of milk yield (28.74 ± 2.2 kg and 27.58 ± 2.2 kg, respectively) as that of the CTL cows (34.8 ± 2.3 kg and 33.5 ± 2.3 kg, respectively). The yield of the EC cows on d 4 and 6 (23.4 ± 2.2 kg and 21.5 ± 2.2 kg, respectively, P<0.05) was significantly less than CTL. On d 30, 60, and 90 post-challenge there was no significant difference between treatment groups.

Additionally, a significant effect of parity was seen on milk yield. On d 2, 3, 4 and 6 post-challenge, EC multiparous (26.6 ± 2.8 kg, 25.6 ± 2.8 kg, 30.7 ± 2.8, and 27.7 ± 2.8 kg, respectively) cows produced significantly less milk than the EC primiparous cows (10.3 ± 3.3 kg, 8.4 ± 3.3 kg, 16.0 ± 3.3 kg, and 15.2 ± 3.3 kg, respectively, P<0.05, Figure 2.6). However, this parity difference was not observed for the ECF or CTL cows.

Dry Matter Intake

On d 1 post-challenge, DM consumption was not different between the ECF (18.9 ± 1.4 kg) and the CTL cows (21.1 ± 1.4 kg). ECF and CTL animals consumed a greater amount of DM as compared to the EC animals (14.0 ± 1.4 kg). On d 2 post-challenge, the CTL cows consumed more DM (20.0 ± 1.4 kg) than both the EC and ECF animals (14.7 ± 1.4 kg and 16.6 ± 1.4 kg, respectively, P<0.05, Figure 2.7). After d 2, DMI was not significantly different between the three treatment groups.

CFU and SCS

There was no significant difference between the average $\log_{10}$cfu of the EC (2.8 ± 0.2 cfu) and ECF (2.6±0.2 cfu) animals throughout the entire study period. The interaction between time and lactation number was significant (P<0.0001, Figure 2.8), where multiparious animals displayed greater $\log_{10}$cfu counts from 15 h through 96 h post-challenge, as compared to the primiparous population.

There was a significant difference between the average SCS of the CTL animals (2.3±0.4) and the EC and ECF animals (6.6±0.4 and 6.4±0.4, respectively, P<0.0001). The interaction of treatment by time for SCS was significant where CTL cows had lower SCS (P<0.0001) than both the EC and ECF animals from 9 h until the end of the trial period. No difference was seen between EC and ECF cows for SCS (P>0.05). While there was a significant interaction between time and parity (P=0.0004), the pairwise comparison showed no difference between the parities at each time point (Figure 2.9). Additionally, as DIM increased, the SCS decreased (estimate = -0.01 ± 0.01, P = 0.04).

DISCUSSION

A cow's behavior can be influenced by a variety of factors including environment, management, and physiologic status (Overton et al., 2002). Within a day, it has been estimated that a healthy cow will spend 45% of her daily budget lying down, 55% standing and 26% eating with 18 to 20 resting bouts (Hedlund and Rolls, 1977). Gomez and Cook (2010) also determined that healthy cows spend 4.3 ± 1.1 h/d feeding, 2.5 ± 1.5 h/d standing in the alley, and 2.7 ± 2.1 h/d standing in their stall. This leaves approximately 11.9 ± 2.4 h/d for resting behavior which also includes 12.9 ± 6.6 lying bouts. However, if a cow's physiologic state is compromised by a disease, such as lameness or mastitis, an alteration in behavior has been shown (Gomez and Cook, 2010, Mazrier et al., 2006).

Classic sickness behavior has been described as loss of appetite, increased body temperature, depression, pain, lethargy, and an overall alteration in an animals' normal behavior. The change in normal behavior that has been observed in animals with clinical mastitis suggests that these animals may also have reduced well-being (Leslie, 2010). A decline in lying behavior was shown when cows were challenged with LPS. Infected animals spent 40.7 ± 4.0% of the day lying in the stall compared to 47.9 ± 3.4% for control animals (Zimov et al., 2011). In the present study, cows infected with *E.coli* reduced lying time to 57.4 ± 5.4 minutes and 58.3 ± 5.6 minutes per 3 h period (EC and ECF treatments, respectively) as compared to 78.0 ± 5.9 minutes per period for the CTL animals as determined by the HOBO© data loggers as previously validated by Ledgerwood et al., (2010). Similarly, lying time of cows infected with LPS was shortened from 2 hr through 13 hr post-infection as compared to the day prior to infection (Hänninen et al., 2007). The deviations from normal lying behavior may be indicative of the discomfort associated with the mastitis infection. The behavior expressed was possibly dictated by pain experienced as opposed to the natural sickness behavior of increased rest. When daily steps were analyzed using the Afi PedometerPlus© as previously validated by Higginson (2009) in the current study, there was no significant difference seen between the treatment groups. To our knowledge, Hanninen (2007) is the only other study to analyze daily steps, and also observed no difference in the number of steps taken. During the sampling periods, all cows in the pen were unintentionally disrupted, causing similar walking behavior during those time periods. This is likely the reason for the lack of alteration in this behavior. This lack of behavioral change between FM treated and non-treated animals that were infected with LPS was also observed in Zimov et al, (2011) where lying times did not significantly differ.

Furthermore, Kikkers et al. (2006) determined that cows, which developed naturally occurring mastitis, increased their left side lying behavior with an infection on the right side as compared to animals that were not infected. While this was related to an increased probability that the cow was infected in her right side, it was not a significant relationship, which coincides with the present study's findings. In the current project, there was no relationship between the percent of time the cow spent lying on the uninfected side relative to the challenged quarter. This may indicate that cows do have a preference as to which side they lay upon, regardless of infection status.

The *E.coli* infection also resulted in changes in DMI and milk production. The ECF cows consumed the same amount of dry matter as the CTL cows on d 1 post-challenge, and both were significantly greater than the EC cows. By d 2, however, consumption by the ECF animals was similar to the EC cows, and the CTL was significantly greater than both. In a previous study, cows that were challenged with the same strain of *E.coli*, had a drop of 4% in DMI on the day of challenge and returned to normal levels by d 1-2 post-challenge (Todhunter et al., 1991). Similarly, Hogan et al. (1995) observed the most significant drop in DMI on d 1 post-challenge with recovery occurring by d 2. The decline in DMI of the EC infected cows in the current study is consistent with these findings where DMI declined 25.1% from d 0 to d 1 and returned to pre-challenge levels in all groups by d 3. While the effects were more dramatic in the current study, the previous studies were conducted following J5 immunization which likely reduced the severity of the symptoms.

In the current study, FM administration does appear to mitigate the negative impacts on DMI for 1 d post-injection. However, this was not maintained on d 2 post-injection. The half-life of FM is 8.1 hours (Lohuis et al., 1989) as compared to carprofen, an NSAID with a half-life of

30.7 hours (Vangroenweghe et al., 2005). The half-life of FM may explain the mitigation of DMI on d 1 but not on d 2. In future studies, the examination of a second dose of FM and the effects of DMI is warranted. While Milne (2003) showed no difference in the pain threshold whether animals were given one or three doses of meloxicam, the effect of DMI was not analyzed. Additionally, Zimov et al. (2011) found that neither the FM treatment nor the LPS infusion affected the DMI. However, it was acknowledged that time of feeding being 2.5 h prior to the challenge may have eliminated the expected and previously observed decline in intake as feeding activity is highest right after feed delivery (Zimov et al., 2011).

Due to the loss of milk from treatment and decreased production, a mastitis infection is arguably the largest cost to a dairy producer (Smith and Hogan, 2001). Milk of infected animals is excluded from the rest of the sellable product until the clinical signs are no longer displayed. However, cows do not return to normal milk production levels upon the lack of clinical signs. Therefore, there is a lag time between when milk appearance returns to normal and total milk production recovery is realized. This is a time period where FM treatment may have a positive impact (Anderson and Hunt, 1989, Wagner and Apley, 2004). In previous studies, cows challenged with *E.coli* 727 experienced between a 23 and 36% drop in milk production by 24 h post-challenge and were able to return to normal levels of production by d 4-5 without NSAID treatment (Hogan et al., 1999, Todhunter et al., 1991). Cows challenged with endotoxin, however, experienced much greater losses in milk production of 74% by 12 h post-challenge (Anderson and Hunt, 1989). This loss can be attributed to the inoculation of endotoxin as opposed to the live *E.coli* cell, as endotoxin is the actual damaging portion of *E.coli* released upon cell death from the inflammatory response (Hogan and Smith, 2003). In the present study, EC and ECF cows infected with *E.coli* had a 44.6% and 45.1% decline in yield from d 0 to d 1.

Recovery of milk production levels to that of the CTL cows occurred on d 4 for cows treated with FM (ECF). However, EC cows did not return to pre-challenge milk production levels during the 7 d study period. Wagner and Apley (2004) observed that full recovery of production levels was achieved by d 10 post-challenge using FM in an endotoxin challenge with some increase observed (no significance noted) by d 2 post challenge. Dosage of FM was equivalent between this and the current study, however, our findings showed a more rapid return to pre-infection yields which could be due to the previously stated difference in the bacterial component used for infection. Additionally, in the previous study, the timing of the FM administration ranged from 3-7 hours post-challenge (based on clinical signs) whereas in the current study, FM administration ranged between 18-24 hours post-challenge. Clinical mastitis was defined in Wagner and Apley (2004) by a combination of increased mammary surface area and a rectal temperature of $\geq 40^{\circ}$C. The current study based treatment upon clinical signs in milk appearance alone which may not be as sensitive to physiological changes in the animal, hence the later FM administration time point. In contrast, Anderson and Hunt (1989) found that cows infected with endotoxin followed by FM administration were able to return to 90% of the baseline production by 24 h after treatment was given. Again, the bacterial component of infection varied from the current study and FM was administered at 2 h post-challenge regardless of the expression of clinical signs of mastitis. Furthermore, two doses of 1.1 mg/kg of FM were administered at h 2 and h 10 post-infection. The pre-defined timing of multiple doses may have allowed for the cows administered FM to return to baseline production levels as was seen in Anderson and Hunt (1989). Additionally, DIM at the time of infection could further dictate the lasting impact on milk production as earlier in lactation cows are immunosuppressed, show more severe clinical symptoms, and may take longer to combat the infection. It is well documented that

immunosuppression causes animals to prioritize energy requirements into maintenance as opposed to a productive state. The animals used in the current study ranged in DIM from 35 to 170 days in milk with a median of 117 DIM whereas Wagner and Apley (2004) used cows from 30 to 60 DIM which could attribute to the longer lasting impact on milk production.

Previous research (Morkoc et al., 1993, Vangroenweghe et al., 2005, Wagner and Apley, 2004) has not shown a protective effect of treating mastitis with an NSAID on milk production, as seen in the current study. This difference may be largely due to the usage of endotoxin (Morkoc et al., 1993, Wagner and Apley, 2004) as compared to a live *E.coli* model, as mentioned previously. Further, different NSAIDs do vary in their physiochemical characteristics, which causes the differences in efficacy. NSAIDs can selectively inhibit prostaglandins, endoperoxides, and cyclooxygenase (COX) enzymes but it is unclear within the bovine model how that selectivity is determined (Vangroenweghe et al., 2005). For example, sodium salicylate is an anti-inflammatory analgesic but does not affect COX activity (Morkoc et al., 1993). By comparison, carprofen is a prostaglandin synthetase inhibitor and is able to block the prostaglandin synthetase through COX inhibition (Vangroenweghe et al., 2005). Therefore, due to this lack of specificity towards inhibition, it is difficult to delineate which NSAID is the most appropriate in alleviating the adverse effects of *E.coli* mastitis.

The parity by treatment by day interaction was also significant in the present study. Multiparous EC cows experienced reduced milk yield throughout the week of challenge, as compared to primiparous EC animals. A parity effect was not seen in the ECF or CTL treatment, indicating that FM may have mitigated the adverse milk loss associated with the *E.coli* infection in the FM animals. Upon identifying the significant three-way interaction, $\log_{10}$cfu and SCS were further analyzed as possible explanatory variables for these results. Log_{10}cfu was

significantly higher in multiparous animals throughout the challenge period. In contrast, parity by time was also significant for SCS, but no pairwise significance was observed at the same time point within parity. Furthermore, the parity by treatment by day interaction was not significant in either the log_{10}cfu or SCS models. These results indicate that while FM did not impact bacterial clearance or neutrophil recruitment, it may act upon the immune cascade associated with recognition of an LPS infection, thus allowing for the improvement in milk production of the ECF animals.

Toll-like receptor 4 (TLR4) is responsible for LPS recognition and induces inflammatory cytokine production along with the upregulation anti-microbial genes during infection (De Schepper et al., 2008, Werling and Jungi, 2003). Along with this production, the lipid-A portion of LPS is bound by lipopolysaccharide-binding protein (LBP). The LPS-LBP complex is then recognized by CD14 and stimulates mitogen-associated protein (MAP) kinases and the transcription factor (NF-κB) (De Schepper et al., 2008, Miyake, 2004). NF-κB activation occurs not only during a time of infection, but also in regular mammary gland development throughout the various stages of lactation (Connelly et al., 2010). When NF-κB was activated in fully functional murine mammary epithelial cells, a rapid reduction in milk production resulted with high rates of milk clearance and apoptosis. In the same study, mammary glands infused with LPS were assessed for their NF-κB activation capacity. Mastitis caused activation of NF-κB, which once again reduces milk production and also adversely affects milk protein levels (Connelly et al., 2010). NSAID administration, such as FM, results in the alteration of various cellular processes that block NF-κB signaling pathway. This is done through inhibition of IκB kinase which prevents the translocation of NF-κB to the nucleus of the cell and thus preventing the upregulation of target genes (Gupta and Dubois, 2001, Ulrich et al., 2006). In the current study,

it is likely that blocking the activation of the NF-κB protein complex is the reason for the lack of drop in milk production when FM was administered to the ECF animals. Therefore, further analysis of the ability of FM to act upon this cascade should be further targeted for mastitis therapy.

An additional component of the LPS inflammatory infection cascade is its ability to down-regulate milk fat globule epidermal growth factor-factor VII (MFG-E8) (Komura et al., 2009). MFG-E8 is contained within macrophages and dendritic cells and allows for the elimination of apoptotic cells by phagocytosis. However, when LPS is present, MFG-E8 is down-regulated. When mice were infected with LPS, mRNA expression of MFG-E8 decreased 43% when LPS dosage was 15 mg/kg of BW and by 80% when the dosage of 45 mg/kg of BW, thus greatly inhibiting the phagocytic ability of neutrophils. When Polymyxin B (an antibiotic shown to neutralize LPS activity) was administered to septic mice in the same study, the effect of LPS on the growth factor was alleviated (Komura et al., 2009). While FM is not an antibiotic, it does impact the inflammatory cascade through NF-κB regulation and therefore, may reduce the suppression of MFG-E8. This would provide a more rapid elimination of apoptotic cells. In turn, the epithelial cell turnover may be more efficient in the ECF animals, thus resulting in more rapid return to pre-infection milk yield.

There has been limited research focused on explaining the parity difference in recovery from infection and disease using NSAIDs. In one study, no parity effect was seen when cows were treated for metritis with FM (Drillich et al., 2007). In another study using an LPS mastitis challenge model, Zimov et al. (2011) used both primiparous and multiparous groups of animals and reported no effect on the described results. However, others have shown that primiparous cows indeed have a stronger immune response to intramammary *E.coli* infections by the rapid

clinical response they display when clearing the pathogen (Vangroenweghe et al., 2004). First lactation animals that were infused with 1 x 10^4 cfu of *E.coli* P4:O32 were compared to cows inoculated with a higher dosage of cfu at 1 x 10^6. Cows infected with the higher levels of bacteria showed a more pronounced and rapid elimination of the infection. This can be attributed to a quicker recruitment of neutrophils to the gland, higher rate of bacterial clearance, and faster recovery of quarter milk production levels. All of the primiparous animals used in this study were classified as moderate responders to the infection. A cow experienced a moderate response if milk production of the two non-infected quarters exceeded 50% of the pre-infection yield 2 days after challenge. The researchers concluded that resilience of primiparous animals can be partly attributed to better neutrophil function compared with older cows. Older animals have a decreased neutrophil responsiveness and less superoxide anion production as compared to younger cows in the first week post-calving (Gilbert et al., 1993). This indicates that there is a negative relationship between increasing parity and immune function, which impacts the ability of older animals to combat diseases, such as mastitis. Additionally, it has been hypothesized that the threshold for LPS sensing is compromised in multiparous cows which would delay the recognition of the pathogen (De Schepper et al., 2008). Each of these theories may support the increased recovery rate in primiparous milk production levels as compared to the multiparous group within the animals infected with *E.coli*.

E.coli mastitis does alter animal activity, DMI, and milk production, which are all indications that this type of infection may have a negative impact on animal well-being. In the current study, the use of the NSAID, FM, improved DMI and milk production following administration. In turn, it can be concluded that FM could be used as supportive therapy in alleviating some of the adverse effects associated with *E.coli* mastitis. Further research to

determine if additional dosages may enhance the positive effects of FM as well as the immune response of these challenged animals should be explored.

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Treatment	Lying Time (minutes)		Standing Time (minutes)	
	Mean	**S.E.**	**Mean**	**S.E.**
CTL	78.0[a]	5.9[a]	102.0[a]	5.9[a]
EC	57.4[b]	5.3[b]	122.6[b]	5.3[b]
ECF	58.3[b]	5.6[b]	121.7[b]	5.6[b]

$P<0.05$

Table 2.1 Effect of flunixin meglumine administration on average lying and standing time (minutes/period) in animals challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). Each period is reflective of a 3 hr block of time. EC and ECF cows laid significantly less and stood significantly more than the CTL cows. Letters indicate significant differences between treatment groups.

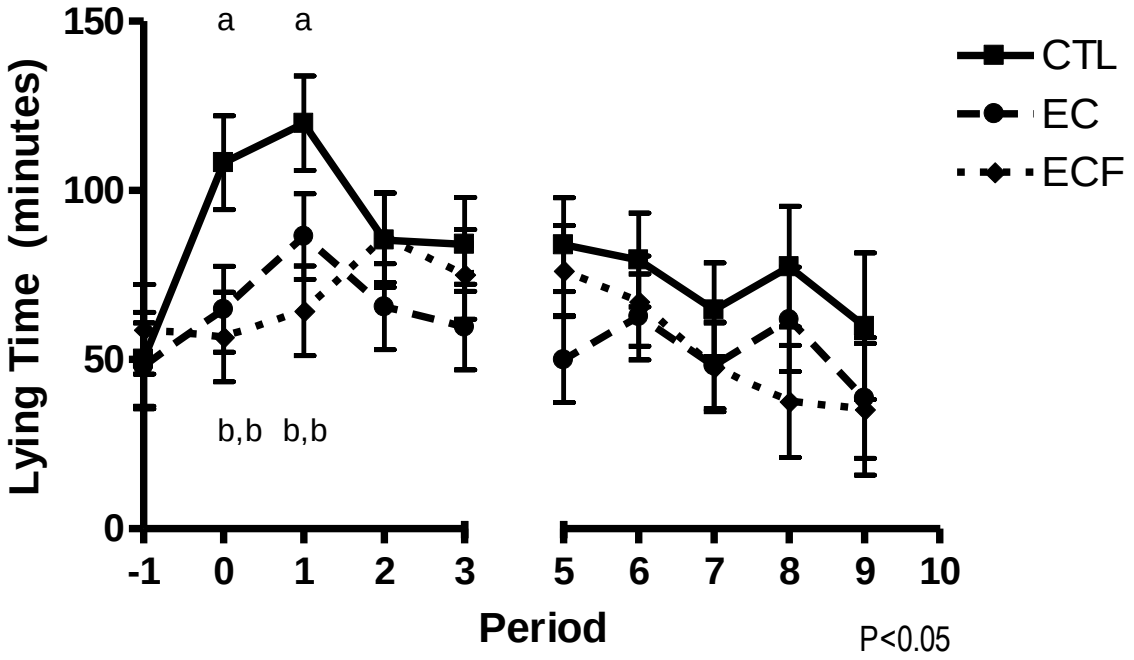


Figure 2.1 Effect of flunixin meglumine (administered at period 0 for ECF) on average lying time (minutes/period) in animals challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). Letters indicate significant differences between the treatment groups. At period 0 and 1, EC and ECF laid significantly less than the CTL. Period 4 was milking time, therefore activity was not influenced by the treatments at this period. Each period is reflective of a 3 hr block of time.

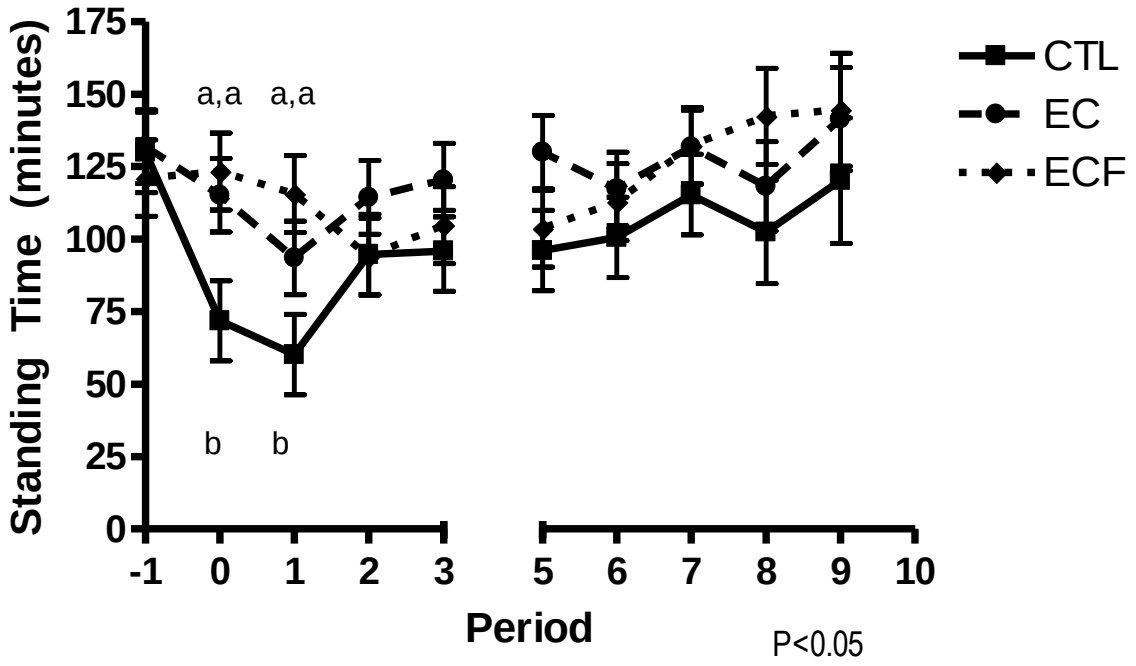


Figure 2.2 Effect of flunixin meglumine (administered at period 0 for ECF) on average standing time (minutes/period) in animals challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). Letters indicate significant differences between the treatment groups. At period 0 and 1, EC and ECF stood significantly less than the CTL. Period 4 was milking time, therefore activity was not influenced by the treatments at this period. Each period is reflective of a 3 hr block of time.

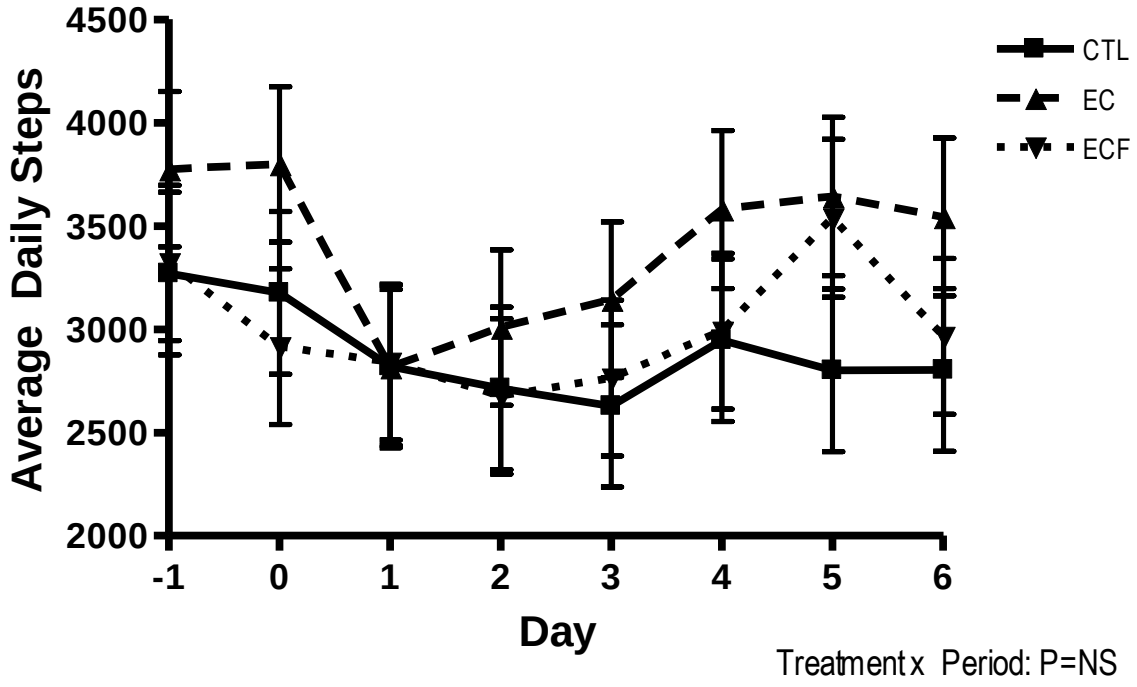


Figure 2.3 Effect of flunixin meglumine (administered on d 0 for ECF) on average steps/d on animals challenged with intramammary infusion of *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). There was no significant difference seen between the treatment groups

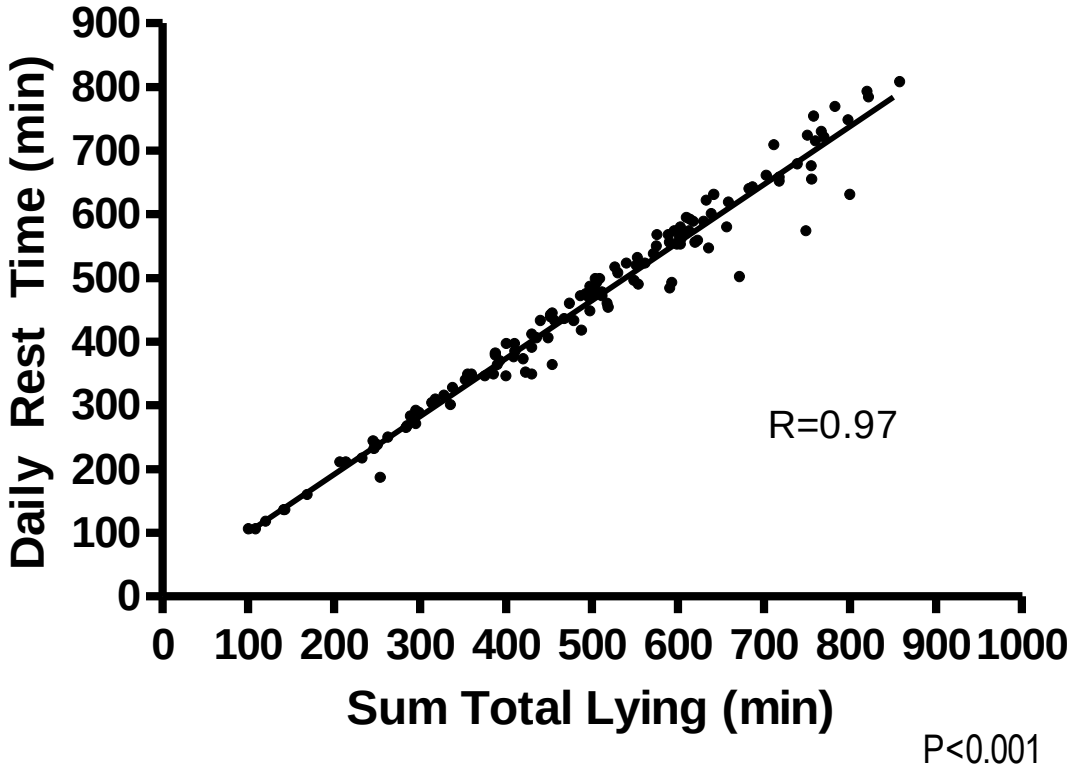


Figure 2.4 PedometerPlus© and HOBO© data loggers were used to quantify daily activities of animals challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). Daily rest time from the PedometerPlus© was highly correlated with the sum of total daily lying time from the HOBO© data loggers (R=0.97).

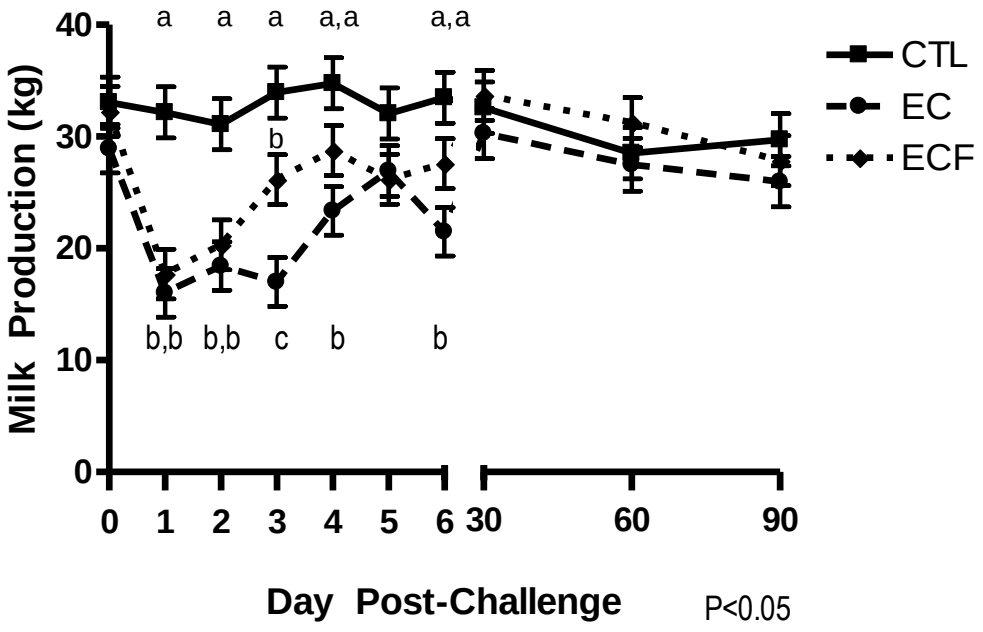
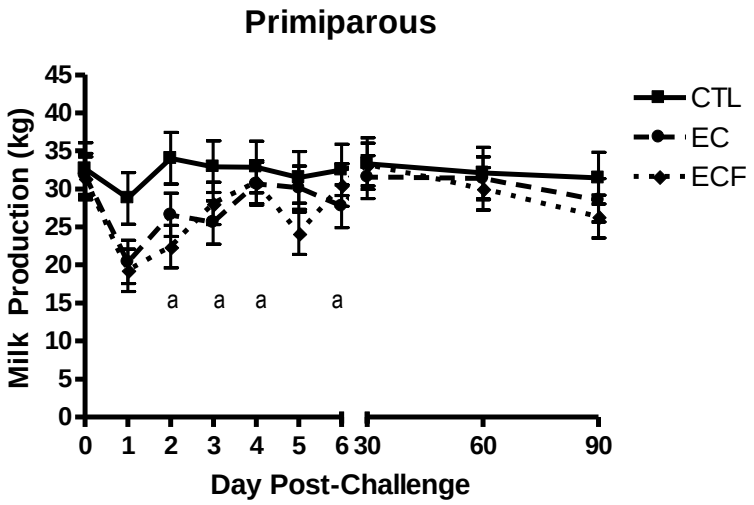
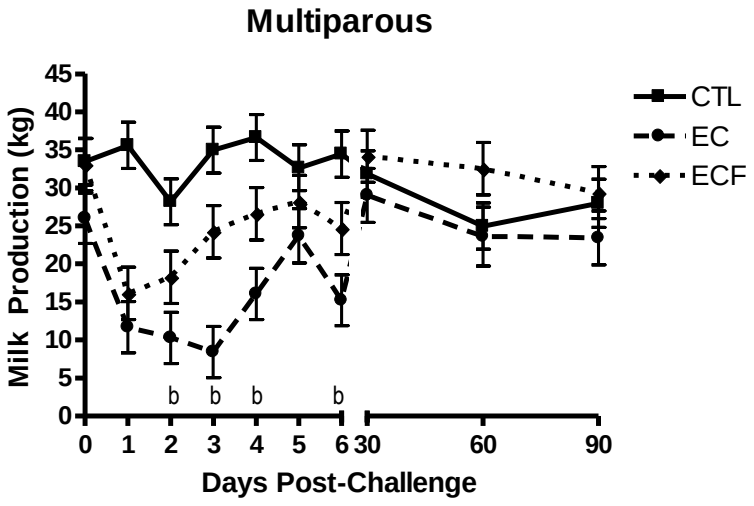


Figure 2.5 Effect of flunixin meglumine administration on daily milk production (kg) in animals challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). Letters indicate days on which significant differences between treatment groups existed. On d 1 and 2, EC and ECF animals had significantly lower milk production than CTL. On d 3, CTL cows had higher than EC and ECF. Additionally, on d 3, ECF has higher yield than EC cows. On d 4 and 6, CTL remained significantly higher in yield than the EC cows.





P<0.05

Figure 2.6 Effect of flunixin meglumine administration on daily milk production (kg) by parity in animals challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). A significant effect of parity was seen on milk yield. Letters indicate significant differences between the two parity groups. On d 2, 3, 4 and 6 post-challenge, EC multiparous cows produced significantly less milk than the EC primiparous cows. This parity difference was not observed for the ECF or CTL cows.

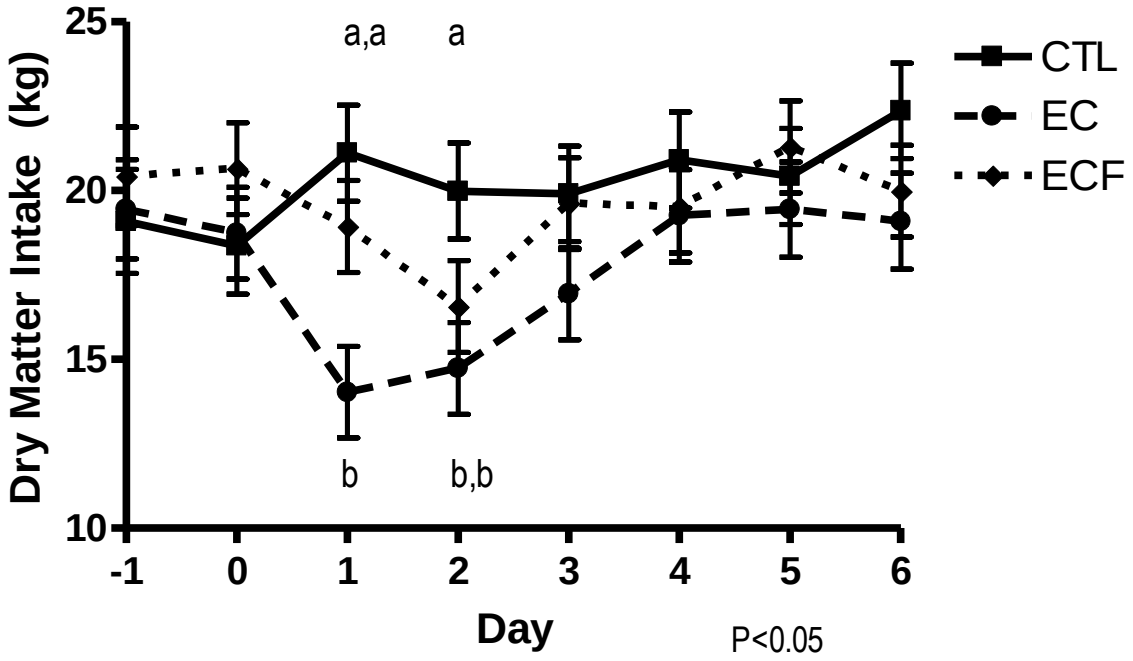
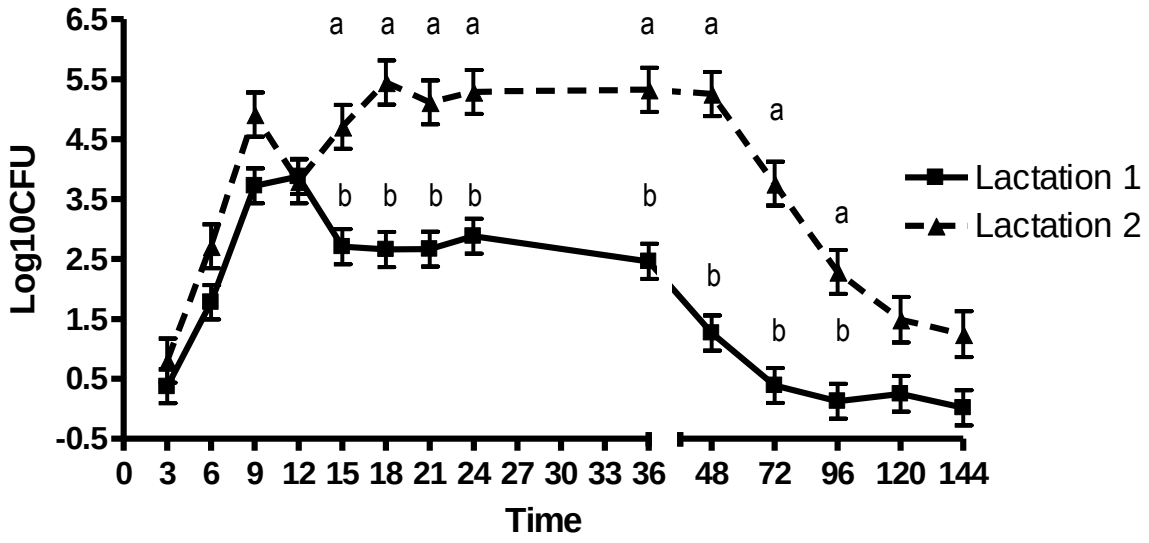
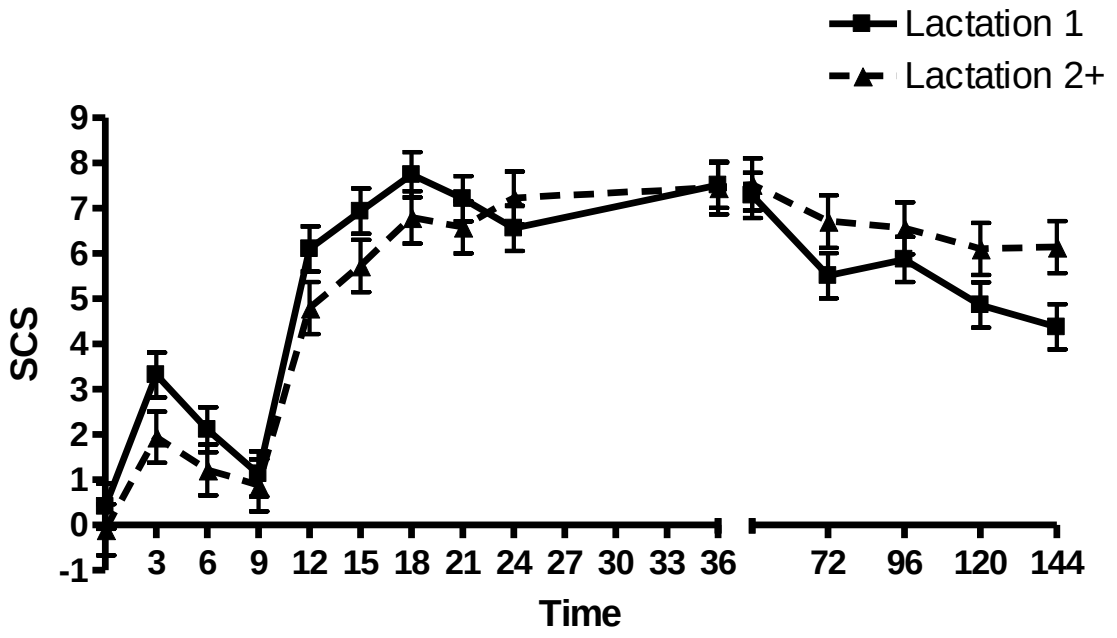


Figure 2.7 Effect of flunixin meglumine administration DMI (kg) in animals challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). Letters indicate significant differences between treatment groups. On d 1, DMI for ECF and CTL cows were not different. ECF and CTL animals consumed a greater amount of DM as compared to the EC animals. On d 2 post-challenge, DMI for the CTL cows was significantly greater than the EC and ECF animals.



P<0.0001

Figure 2.8 Effect of flunixin meglumine administration on log_{10}cfu in animals by parity challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). Letters indicate significant differences between parities. From 9 h through 96 h post-challenge, log_{10}cfu was greater in multiparous cows than the primiparous counterparts. There was no significant difference by treatment observed (P>0.05).



P<0.05

Figure 2.9 Effect of flunixin meglumine administration on SCS in animals by parity challenged by intramammary infusion with *Escherichia coli* (EC), *E.coli* followed by flunixin meglumine treatment at the onset of clinical signs (ECF) or sterile saline (CTL). While the interaction was significant, Tukey's pairwise comparison showed no significant differences between the parities at the same h time points.

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Chapter 3: The use of peripartum activity measures and blood metabolites as indicators of naturally occurring postpartum diseases

ABSTRACT

Activity measures and the blood metabolites, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA), were collected during the periparturient period to determine the likelihood of disease occurrence. Primiparous and multiparous Holstein, Jersey, and crossbred dairy cows were monitored for rest bouts, rest duration, rest time, and average daily steps throughout the pre and postpartum periods from -21 d to +30 d relative to calving. Activity measurements were collected using Afi PedometerPlus© monitors. During the pre-partum period, blood samples were obtained from the coccygeal vein and serum was analyzed for NEFA concentration 1 wk prior to calving. After calving, BHBA levels were analyzed between 3 and 10 DIM. If a disease occurred greater than 10 cows during the study period, it was considered for analysis. Therefore, the diseases analyzed were dystocia, subclinical ketosis, clinical mastitis, and milk fever. NEFA had a positive association with activity for subclinical ketosis, mastitis and milk fever prior to the onset of disease. On the day of calving, rest bouts increased in animals that experienced dystocia over those who did not experience dystocia. Further, cows experiencing subclinical ketosis displayed higher rest bouts on d -1 and decreased daily steps on d -6. Additionally, cows experiencing clinical mastitis had decreased rest times beginning on d -3 prior to the onset of clinical disease and continued through d -1 as compared to animals without mastitis. Cows with milk fever displayed increased rest bouts with decreased daily steps on d -1 and d 1 relative to disease diagnosis and increased overall rest duration and time after the clinical diagnosis of disease compared to cows that were not diseased. Therefore, it can be concluded that activity changes and deviations from healthy herdmates could allow producers to utilize activity monitoring to proactively manage herd health.

Key Words: animal activity, periparturient, disease, non-esterified fatty acids, β-hydroxybutyrate

INTRODUCTION

Over the past 25 years, the mentality of dairy producers throughout the world has shifted from disease treatment to and proactive management strategies for the prevention of disease (LeBlanc et al., 2006). All medium and large size dairy operations annually experience at least one case of clinical mastitis, lameness, retained placenta, reproductive problems or milk fever (USDA, 2007). Greater than 75% of these diseases incidents have been identified to occur in the transition period, which has been defined to as 3 wk before to 3 wk after parturition (Drackley, 1999). During this critical time, a cow will go from a pre-calving state of positive energy balance to a state of substantial negative energy balance (NEB) immediately after calving. This NEB in combination with a pre-calving decline in DMI (Huzzey et al., 2005) initiates the mobilization of fat energy storage which results in the release of non-esterified fatty acids (NEFA) into the bloodstream. If NEFA are not completely metabolized by the liver, ketone bodies in the form of β-hydroxybutyrate (BHBA) are produced (Hammon et al., 2006, Suriyasathaporn et al., 2000). Increases in blood concentrations of both NEFA and BHBA have been associated with the occurrence of displaced abomasums (DA) (LeBlanc et al., 2005, Ospina et al., 2010, Stengarde et al., 2010), ketosis (Ospina et al., 2010, Suriyasathaporn et al., 2000), metritis (Hammon et al., 2006, Ospina et al., 2010), and retained placentas (RP) (Ospina et al., 2010).

While blood measures can serve as extremely valuable tools in determining the likelihood of such disease occurrences, often times, these tests are difficult to conduct on a dairy farm due to timeliness of the test or labor availability. In response to these limitations, advanced technology tools for monitoring herd health have been developed. The tracking of animal activity

and behavior has proven to be another valuable way of identifying animals that are at risk for periparturient diseases. It has been recognized that the percent of lying bouts lasting longer than 1 h and the percent of standing bouts shorter than 15 min were both increased in the dry period, possibly due to the discomfort associated with pregnancy (Dechamps et al., 1989). It is plausible that these frequent changes in position and activity, both pre and postpartum, may provide further evidential support for proactive health management. Furthermore, animals that experience a metabolic or digestive disorder in the prebreeding stage of lactation, walk an average of 8 to 14 steps/h less than healthy cows following calving. While the overall activity was less, there was an increase in steps taken 8 to 9 d prior to clinical diagnosis with a gradual decline until the day of diagnosis (Edwards and Tozer, 2004). Similarly, prepartum feeding time and DMI were indicators for cows at risk for metritis (Huzzey et al., 2007). Cows experiencing metritis spent less time feeding and consumed less feed beginning 2 wk before clinical signs were expressed (Huzzey et al., 2007). In another study, cows that experienced dystocia altered both their eating and drinking habits prior to calving while also having more changes in position from standing to lying. Specifically, cows having greater than 30 bouts in the 24 h before calving were at a greater risk for dystocia (Proudfoot et al., 2009).

The use of peripartum activity measures in combination with blood metabolites to assess the risk of postpartum diseases has not been previously reported. Therefore, the objectives of this study were to utilize activity measures in combination with prepartum NEFA concentrations and postpartum BHBA concentrations to identify animals at risk for developing naturally occurring postpartum diseases. In doing so, dairy producers may be able to better identify cows at risk of experiencing subclinical and clinical diseases in the transition period, while also addressing cow

comfort and animal welfare concerns associated with diseased animals (Dawkins, 2003, von Keyserlingk et al., 2009).

MATERIALS AND METHODS

Prepartum Data Collection

From September 2010 through May 2011, 216 dry cows, that consisted of 103 Holstein (42 primiparous, 61 multiparous), 43 Jersey (20 primiparous, 23 multiparous), and 70 Crossbred (18 primiparous, 52 multiparous), were enrolled in the study. These animals were moved into a bedded pack area (2,286 m^2) at the Virginia Tech Dairy Center (Blacksburg, Virginia) 21 to 27 d prior to expected calving. Cows were housed in this location for the duration of the close-up dry period and allowed to calve in this same area. One feed bunk area was provided for these animals and animals were fed a ration consisting of corn silage, grass hay, soybean meal and corn grain.

To assess prepartum changes in body weight, cows were walked to the holding pen of the milking parlor (approximately 0.40 km) each week prior to calving where the body weight scales (DeLaval, Kansas City, Missouri) were located. Each animal crossed the scale, being held on the scale for at least 10 s to ensure the accurate body weight was recorded. Body weights were transferred into the AfiFarm© (S.A.E. Afikim, Israel) Herd Management software program through the transponder box attached to the scale.

After body weights were collected, animals returned to the close-up dry cow area and were held in a small freestall area to obtain a blood sample. At this time, a 10 mL evacuated sterile Vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey) was used to collect a blood sample from the coccygeal vein. Once the samples were collected, the cows were returned to the bedded pack area. The samples were allowed to clot and the blood was then centrifuged at 2000 rpm for 30 min at 15°C using the IEC CL31R Multispeed Centrifuge

(Thermo Electron Corporation, Waltham, Massachusetts) to obtain serum. Within 5 h of collection, the serum samples were separated into two 1 mL aliquots and stored at -20°C until further analysis.

For each animal, the serum from the sample collected within 1 wk prior to calving (range: d -8 to d-2) was sent to the Virginia Maryland Regional College of Veterinary Medicine Diagnostic Lab (Blacksburg, Virginia) for analysis of NEFA concentrations. If the sample was from the day of calving, the previous week's sample was used for the analysis. All biochemical tests were conducted using the colorimetric procedure with Beckman Coulter AU480 chemistry analyzer (Beckman Coulter, Inc., Brea, California) with the use of reagents (Randox Laboratories, Antrim, United Kingdom). Reagent, distilled water, standard and the serum sample were mixed in a test tube and incubated for 10 min at 37°C. An additional reagent was added with another 50 µl of the serum sample and the subsequent mixture was once again incubated for 10 min at 37°C. An absorbance reading was determined as compared to the reagent blank at 550 nm of wavelength. The NEFA concentration was then determined using the equation: mmol/L = (absorbance of the sample/absorbance of the standard) x concentration of the standard.

Postpartum Data Collection

Immediately after parturition, cows were moved into a freestall pen (960 m^2) containing 34 stalls with rubber mattresses. Stalls were bedded with sawdust twice weekly. These recently fresh animals remained in this area of the freestall barn for 3-4 wk postpartum. Cows were milked twice daily at 12-h intervals in a double-eight milking parlor and body weights were collected at each milking time. Animals were fed a TMR consisting of alfalfa hay, corn silage, corn grain, cottonseed, and soybean meal.

Between d 3 and d 10 post-calving, a minimum of 0.1 mL of blood was retrieved from the coccygeal vein using a 1 mL syringe fitted with a 20 gauge needle. This amount of blood was then placed on a Precision Xtra® Blood β ketone test strip that was inserted into the Precision Xtra® blood glucose and ketone monitoring system (Abbott Diabetes Care Inc., Alameda, California). The monitoring system gathered the BHBA information in 10 s and provided a numerical BHBA value. BHBA values were used only to diagnose subclinical ketosis.

Animal Activity Monitoring

Activity data was collected from 21 d prepartum to 30 d postpartum. Each of the animals had an Afi PedometerPlus© affixed to a rear leg fetlock, which collected activity data on a daily basis. When cows were in the close-up dry cow area, cow activity was collected each time the animal exited or entered the bedded pack area. Only one entry/exit point was available for the cows, which allowed them to access feed. The activity data was stored in the memory of the pedometer and then transmitted through a reader box to the AfiFarm© (S.A.E. Afikim, Israel) Herd Management software program. Postpartum cow activity was collected between milking sessions and stored in the memory capacity of the pedometer. The activity information collected was then transmitted at each milking through a reader box in the milking parlor to the AfiFarm© (S.A.E. Afikim, Israel) Herd Management software program. Activity variables collected by the Afi PedometerPlus© included rest bouts, rest duration, rest time, and average daily steps summed for daily measurements of these activities. A rest bout was defined as the number of events the animal changed from a standing position to a lying position. A bout is only counted as such if the animal was lying for greater than three minutes. Rest time is the amount of time, in minutes, that the cow was lying down in a day. The rest duration is the duration, in minutes, of the lying time during a session. It was calculated by dividing the rest time by the number of rest

bouts. Average daily steps were calculated by averaging the steps per hour for the two collection sessions and multiplying that average by 24.

Disease Recording

Herd managers and veterinarians collectively recorded disease events on health event sheets and the data were transferred into PC Dart (DRMS, Ames, Iowa). Disease events of interest included displaced abomasum, dystocia, clinical ketosis, subclinical ketosis, mastitis, metritis, milk fever, and retained placenta that occurred in the first 30 DIM. Ketosis animals were not examined because it is likely that these animals originated in a state of subclinical ketosis.

The definitions of the diseases were as follows: DA is the displacement of the abomasum from its normal position to either the right or left side of the abdomen. Dystocia occurred when a calving required assistance with a dystocia score of 2 or greater (1=no assistance, 2=slight assistance 3=average assistance 4=considerable force 5=extreme difficulty). Clinical ketosis occurred with the acute onset of inapetance, decrease in milk production with positive urine ketone test. Subclinical ketosis was defined as a BHBA level of 1.3 mmol/L or greater with no other signs of illness, inapetance, decrease in milk production and was found by the postpartum blood testing. Mastitis was defined as an inflammation of the mammary gland along with clinical signs of alteration of the milk found at the time of milking by those individuals responsible for milk harvest. Metritis was classified as an inflammation of all the layers of the uterus presented with systemic clinical signs of inapetence and decreased milk production. Milk fever was the acute flaccid paralyses or somnolence occurring within 72 h after calving with a response to calcium treatment or with a calcium serum level below 7.5 mg/dl. Retained placenta was quantified as a failure to separate and expel the placenta within 12 h of calving. Cows were

not eligible for more than one disease occurrence. Non-diseased animals were defined as any animal that did not experience any type of disease, including but not limited to the aforementioned diseases, within the first 30 DIM.

Data Management and Analyses

Activity and disease information were extracted from the AfiFarm© (S.A.E. Afikim, Israel) Herd Management software program and PC Dart (DRMS, Ames, Iowa) and imported into SAS version 9.2 (SAS Institute Inc., Cary, North Carolina). All NEFA and BHBA data were stored in a database (Microsoft Excel 2007 for Windows©; Microsoft Corporation, Redmond, Washington) until analysis. Diseases with n greater than 10 observations were considered for analysis. Greater than 10 observations allowed for estimates to be produced from the models. Activity models were created to assess activity differences between diseased and non-diseased animals from d -7 to d 7 relative to the day of diagnosis. This 15 d period was selected for analysis since most of the diseases occurred within the first 10 d of calving. This timeline allowed for a portion of the dry period to also be included in the activity analysis. Any animal that did not experience a disease was input into the specific disease datasets to serve as non-diseased controls for activity comparisons. For these animals, the day of disease diagnosis was equal to that of the average of the diseased animals. In the dystocia model, disease day was considered day of calving. Variables offered into the respective activity models were lactation number, day of disease, disease status and NEFA with associated interactions. Season was not considered in the model due to the reduced time frame of data collection. The interaction between NEFA and day of disease for the dystocia model was not included, since dystocia disease day was always on d 0 (the day of calving). BHBA was not entered into the models, as it was collected after the onset of the diseases, and could not be used as a predictor. Odds ratios

were determined using PROC GLIMMIX with NEFA offered into the model. Significance was determined at a level of $P<0.05$. Bonferroni adjusted slice differences were used to identify significant days within interactions.

RESULTS

Out of the original eight diseases that were of interest, dystocia (n=16), subclinical ketosis (n=26), mastitis (n=15), and milk fever (n=12) met the criteria for analysis. The breed and parity of the diseased animals is shown in Table 3.1. The mean, median and standard deviations for the day of disease diagnosis analyzed diseases are summarized (Table 3.2).

Body weights were not consistent due to scale malfunctioning and, therefore, were not included in the analyses. Of the total cows sampled, five were not in dry cow pen long enough to obtain a pre-calving blood sample and were therefore dropped from the study. Furthermore, two animals that were sampled prepartum died before d 3 post-calving and did not have BHBA values, and they were also dropped from the study.

Dystocia

Disease day was significant for each activity in relation to dystocia incidence ($P<0.05$, Figure 3.1). On d 0, cows who experienced dystocia had increased number of rest bouts (13.7 ± 1.3 bouts) as compared to animals who did not experience a difficult birth (10.5 ± 0.5 bouts). However, the interaction between day of diagnosis and disease status was not significant for any of the activity models.

Subclinical Ketosis

Day of disease and NEFA by day of disease diagnosis were significant variables in the rest bout, rest time, and daily steps models. An increased serum NEFA concentration in the days prior to the diagnosis of subclinical ketosis was positively associated with the number of bouts

from d -7 to d -3. Additionally, the same relationship was observed on d -5 and d -4 for rest duration (Table 3.3). Further, an increased serum NEFA in animals that did not experience subclinical ketosis was negatively associated with the average number of steps these animals took in a day as compared to their diseased counterparts. The interaction between day of disease diagnosis and disease status was only significant for the average number of steps taken (Figure 3.2, $P<0.05$). Cows that went on to have subclinical ketosis took a decreased number of steps in a day (4467.9 ± 447.5 steps) as compared to the cows who did not experience subclinical ketosis (5559.2 ± 174.7 steps) Rest bouts for animals who later presented subclinical ketosis were increased on d -1 (15.6 ± 1.5 bouts) as compared to animals without detectable disease (12.2 ± 0.6 bouts). Additionally, for every one unit increase serum NEFA concentration prior to calving, an animal was 6.6 (1.6-27.3 95% CI, $P<0.05$) times more likely to be subclinically ketotic after calving. Further, the interaction between NEFA and the disease status was significant ($P<0.05$).

Clinical Mastitis

Relative to the day of clinical mastitis diagnosis, there was a significant interaction of day of disease diagnosis by disease status for rest bouts, rest time, and average daily steps (Figure 3.3, $P<0.05$). Rest time increased (397.5 ± 43.3 min) 5 d prior to onset of signs as compared to non-mastitic cows (307.0 ± 16.6 min). However, on d -2 and -1, cows who later presented mastitis decreased their resting time (348.5 ± 42.5 min and 390.6 ± 42.5 min, respectively) as compared to healthy herdmates (480.5 ± 16.5 min and 488.2 ± 16.4 min, respectively). On d-5 and d -4, the daily steps of cows who later displayed signs of mastitis were significantly reduced (3267.3 ± 465.0 steps and 3002.7 ± 459.7 steps, respectively) as compared to the non-diseased group (5553.4 ± 182.6 steps and 4573.3 ± 174.5 steps, respectively). Rest duration was not significantly impacted by disease day, but animals with mastitis did show longer durations of rest

(93.5 ± 17.1 min) than those without mastitis (51.6 ± 6.5 min) on d -5. The interaction between NEFA and day of disease diagnosis was also significant for both rest bouts and rest time. Significant positive associations for this interaction existed on d -2, -3, -5, and -6 for resting bouts. A positive association for NEFA concentration by day of disease interaction also existed on d -3 for the rest duration (Table 3.3).

Milk Fever

The interaction of the day of diagnosis by disease status of the animal was significant for all four activities (Figure 3.4, $P<0.05$). On d -1 and d 1 relative to milk fever diagnosis, animals who presented with signs of the disease displayed an increased number of rest bouts (14.2 ± 1.5 bouts and 14.6 ± 1.5 bouts, respectively) compared to those without milk fever (11.1± 0.5 bouts and 10.9 ± 0.5 bouts, respectively). On those same days relative to diagnosis, daily steps of animals that showed milk fever signs took fewer steps (4242.4 ± 529.3 steps and 2548.7 ± 513.5 steps, respectively) as compared to those who did not experience milk fever (5475.0 ± 184.4 steps and 3812.3 ± 174.0 steps, respectively) On d 6 after disease onset, non-milk fever cows increased their bouts (13.2 ± 0.5 bouts) compared to those with milk fever (10.1 ± 1.5 bouts). Rest duration was significantly increased in cows who did not go on to experience milk fever on d -5 and d -4 (105.3 ± 5.5 min and 108.4 ± 5.5 min, respectively) compared to those animals who did (67.3 ± 15.7 min and 56.7 ± 16.3 min, respectively). However, the rest duration for diseased animals was significantly greater (128.1 ± 15.1 min) than their non-diseased counterparts (94.6 ± 5.2 min) by d 3 following diagnosis. Furthermore, the amount of time milk fever cows spent resting (452.3 ± 54.4 min and 610.4 ± 56.2 min, respectively) was significantly greater than non-milk fever cows (319.0 ± 19.0 min and 455.2 ± 18.8 min, respectively) on d -1

and d 1. Additionally, rest duration was significantly impacted by the interaction between NEFA and day of disease on d -6 with a positive association (Table 3.3).

DISCUSSION

The time immediately leading up to and following calving, a dairy cow undergoes significant changes that affect her, not only physiologically but behaviorally as well. Elevated blood BHBA and NEFA levels, specifically postpartum, have been indicative of cows at risk for metritis (Duffield et al., 2009, Hammon et al., 2006), DA (Duffield et al., 2009, LeBlanc et al., 2005, Stengarde et al., 2010), and ketosis (Duffield et al., 2009, Seifi et al., 2010) along with a variety of other clinical diseases. Higher NEFA and BHBA levels have also been shown to inhibit the ability of the neutrophil to phagocytize thus increasing the risk of a mastitis infection (Sordillo et al., 2009, Suriyasathaporn et al., 2000). The association between NEFA and the day of disease diagnosis was significant for all the diseases analyzed, with the exception of dystocia, for the rest bouts and time (subclinical ketosis and mastitis), rest duration (milk fever), and average daily steps (subclinical ketosis). It is evident from these significant interactions that increased blood NEFA prior to disease diagnosis will result in a greater impact on the activity, especially rest bouts, around the time of the specific disease.

While blood parameters may not be valuable in identifying animals at risk for dystocia, activity prior to and after calving is altered. Dystocia is an extremely subjective disease to quantify. Therefore, the use of activity measures to identify cows that may experience dystocia would be a helpful management tool as producers could aid these animals that are known to be predisposed to calving difficulties. In the current study, cows that did experience dystocia had an increased number of rest bouts (13.7 ± 1.3 bouts/d) when compared to the animals that did not (10.5 ± 0.5 bouts/d) on the day of calving. This is consistent with the studies that analyzed

activity around the time of calving. Proudfoot et al. (2009) found that cows with dystocia had an increased number of bouts in the 24 h prior to calving at 10.9 ± 0.7 bouts/d as compared to 8.3 ± 0.7 bouts/d in the non-diseased group. The higher number of bouts/d observed in the current study is likely due to broader day definition, whereas Proudfoot et al. (2009) used video evidence along with hourly timestamps to identify the precise start of calving and 24 hours prior. In general, standing bouts (the interval between two lying events) during the calving period do increase significantly despite the disease state (Huzzey et al., 2005). Standing and lying bouts are dependent on each other and therefore, this additionally shows that changes in positions at the time of calving can indicate restlessness. For those animals that go on to experience dystocia, these results indicate a heightened state of discomfort. Additionally, cows have been shown to have more general total locomotor activity (feeding, drinking, walking, grooming, and ruminating) in the dry period (Piccione et al., 2011). In the present study, total locomotor activity was unable to be tracked due to the lack of video observation. However, in combining both rest time and locomotor activity quantification in the dry period and during the lactation, an even greater understanding of cow time budget requirements will be realized.

Upon diagnosis of clinical ketosis, the subclinical state of ketosis is one that often goes undetected (LeBlanc et al., 2006). The amount of rest bouts around the time of disease diagnosis was not significant in the predicting the subclinical ketosis disease status of the cows. However, on d -1 prior to subclinical ketosis, non-diseased animals had reduced rest bouts (12.2 ± 0.6 bouts/d) as compared to subclinically ketotic animals (15.6 ± 1.5 bouts). The average daily steps was also reduced in animals that later were diagnosed with subclinical ketosis (4467.9 ± 447.5 steps) when compared to their non-diseased herdmates (5559.2 ± 174.7 steps). Additionally, rest time and rest duration were not significantly impacted by the disease status of the animal. It is

possible, dependent on when subclinical ketosis is identified postpartum, that a portion of this activity deviation occurred in the dry period. Additionally, many of the postpartum diseases are interrelated and therefore, a relationship between dystocia and subclinical ketosis could be linked (LeBlanc et al., 2006). While activity around subclinical ketosis has not been extensively researched, activity prior to clinical ketosis in a Florida field study has shown an increase in total steps taken 8 to 9 d before (Edwards and Tozer, 2004). After that point, activity began to decline up to d 5 prior to diagnosis as compared to non-diseased cows. A rapid increase in steps taken was observed at d 0 with equal to higher activity than healthy counterparts after diagnosis The measurement of activity versus clinical status of the disease varied between the current study and that of Edwards and Tozer (2004). The findings related to daily steps observed in current study was in contrast to previous findings, where daily steps were reduced in diseased animals versus the non-diseased controls. This once again supports the traditional sickness behavior of decreased activity. Furthermore, the subtleties of the changes in behavior associated with subclinical diseases are not as easily distinguishable, as shown in the current study, when compared to those of clinical status. Therefore, the degree of activity change at d -9 or d -8 may not be sufficiently robust to be predictive for subclinical diseases such as subclinical ketosis.

Similar to subclinical ketosis, rest time in cows that did display clinical mastitis was significantly less, beginning at d -2 prior to the disease diagnosis with a decreased number of daily steps taken beginning at d -5. Cows with clinical mastitis have been shown to alter their rest patterns (Hänninen et al., 2007, Zimov et al., 2011). Animals infused with endotoxin spent 40.7 ± 4.0% of the day that they were infected lying in the stall compared to 47.9 ± 3.4% for control animals (Zimov et al., 2011). Similarly, lying time of cows infected with endotoxin was shortened from 2 h through 13 h post-infection as compared to the day prior to infection

(Hänninen et al., 2007). Both of these studies were controlled experiments. However, as seen in the present study, there was a disruption in the expected rest activity of the infected animals when compared to the non-infected both prior to and after the disease was diagnosed. Therefore, deviations from normal lying behavior in combination with reduced steps taken may be indicative of the discomfort associated with the mastitis infection and be associated with a higher likelihood of cows showing signs of clinical mastitis.

Lastly, activity of animals that experienced milk fever was distinguishable from non-diseased herdmates. Classic signs of clinical milk fever directly indicate that behavior would be altered at time of diagnosis, as it causes muscle weakness and possible paralysis. It was observed that on d -1 and d 1 relative to diagnosis that rest bouts (14.2 ± 1.5 and 14.6 ± 1.5 bouts, respectively) were significantly greater than their healthy counter parts (11.1 ± 0.5 bouts and 10.9 ± 0.5 bouts, respectively). Again, this increase in position change may indicate discomfort caused by the onset of disease. Upon the diagnosis of disease, milk fever cows had a greater length of rest duration over the non-diseased herdmates. Rest time also increased over the healthy herdmates immediately prior to disease, and in subsequent days after onset. In theory, the observed decrease in activity for milk fever may be occurring so that energy can be conserved for a full immune response to take place (Aubert, 1999). Additionally, the daily steps taken immediately prior to and after disease diagnosis were reduced in the diseased animals. This deviation in normal behavior for milk fever animals in the current study may provide further support for this theory of increased rest time to enhance recovery and address any discomfort associated with the disease state.

The ability to use activity monitoring in combination with blood parameters pre and postpartum to identify animals at risk for periparturient diseases had not been previously

explored. NEFA measurements precalving in combination with rest bouts, rest duration, and rest time as provided by activity monitoring systems may help in identifying animals at risk for developing dystocia, subclinical ketosis, mastitis and milk fever when compared to healthy herdmates. Through the use of such a system showing an alteration in activity, producers will be able to practice a more proactive management strategy in these particular animals where a disease is impending. The precise disease that a particular cow is at risk for may not be able to be determined solely from activity. However, activity indicators can encourage producers to further investigate an animal and collect blood, milk, and/or urine for physiological parameters to more specifically identify the disease and, in turn, the management practices needed for the improvement of cow health.

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Disease	Incidence by Breed, Parity, and Overall			
Dystocia				
	Breed	**1**	**2+**	**Total**	**Percentage**
	Holstein	9	4	13	13.8%
	Jersey	0	0	0	1.1%
	Mixed	1	2	3	3.2%
	Total	10	6	16	18.1%
Subclinical Ketosis				
	Breed	**1**	**2+**	**Total**	**Percentage**
	Holstein	1	14	15	17.4%
	Jersey	2	1	3	7.6%
	Mixed	1	7	8	10.9%
	Total	4	22	26	35.9%
Mastitis				
	Breed	**1**	**2+**	**Total**	**Percentage**
	Holstein	4	4	8	8.7%
	Jersey	1	0	1	2.2%
	Mixed	1	5	6	8.7%
	Total	6	9	15	19.6%
Milk Fever				
	Breed	**1**	**2+**	**Total**	**Percentage**
	Holstein	0	2	2	3.2%
	Jersey	0	8	8	8.6%
	Mixed	0	2	2	4.3%
	Total	0	12	12	16.1%

Table 3.1 Incidence of naturally occurring postpartum diseases within the Virginia Tech Dairy herd by breed and lactation from September of 2010 through May of 2011. Percentage of incidence was determined by the total number of infected animals out of the total number of non-infected animals for the specific disease.

	Mean Day Diagnosis	SD	Median Day Diagnosis
Disease			
Dystocia	0.0	0.0	0.0
Subclinical Ketosis	5.5	1.9	6.0
Mastitis	4.9	3.0	5.5
Milk Fever	0.88	1.0	0.7

Table 3.2 The mean day of diagnosis, standard deviation, and median day of diagnosis relative to calving for naturally occurring postpartum diseases within the Virginia Tech dairy herd from September 2010 through May 2011.

A

Subclinical Ketosis		
Rest Bouts		
	Day	**Estimate ± S.E.**
	-7	6.8 ± 2.4
	-6	5.7 ± 2.3
	-5	4.9 ± 2.3
	-4	5.0 ± 2.3
	-3	4.4 ± 2.3
	0	0.0 ± 0.0
Rest Time (min)		
	-5	150.6 ± 69.9
	-4	149.8 ± 69.9
	0	0.0 ±0.0

B

Mastitis		
Rest Bouts		
	Day	**Estimate ± S.E.**
	-6	5.0 ± 2.5
	-5	5.6 ± 2.4
	-3	4.8 ± 2.4
	-2	5.1 ± 2.4
	0	0.0 ± 0.0
Rest Time (min)		
	-5	165.7 ± 74.5
	0	0.0 ±0.0

C

Milk Fever		
Rest Duration (min)		
	Day	**Estimate ± S.E.**
	-6	61.2 ± 27.4
	0	0.0 ± 0.0

Table 3.3 The interaction between non-esterified fatty acids (NEFA) and day of disease diagnosis estimates and standard error (S.E.) by day for rest time, rest bouts, and rest duration for subclinical ketosis (A), mastitis (B), and milk fever (C) ($P<0.05$). For every unit increase in NEFA, the specific activity was increased as compared to d 0.

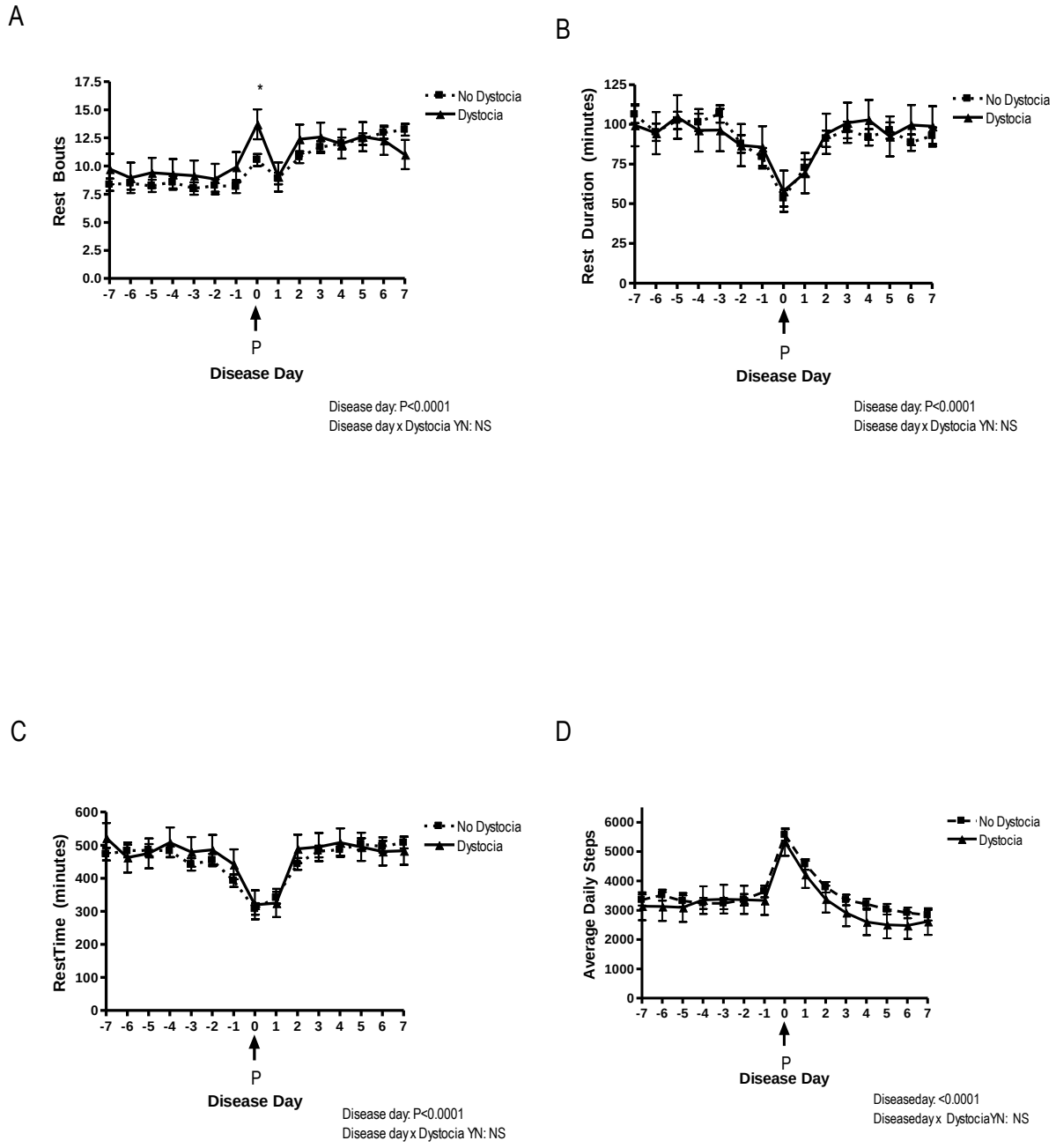

Figure 3.1 A comparison of rest bouts (#/d) (A), rest duration (min/bout) (B), rest time (min/d) (C), and average daily steps (D) -7 d prior to and 7 d after the onset of disease between cows that experienced dystocia (——, n=16) and those who were not diseased (- - -, n=94). (*) are indicative of days where slices adjusted by Bonferroni were significantly different (P<0.05) between diseased and non-diseased animals. "P" indicates where parturition occurred.

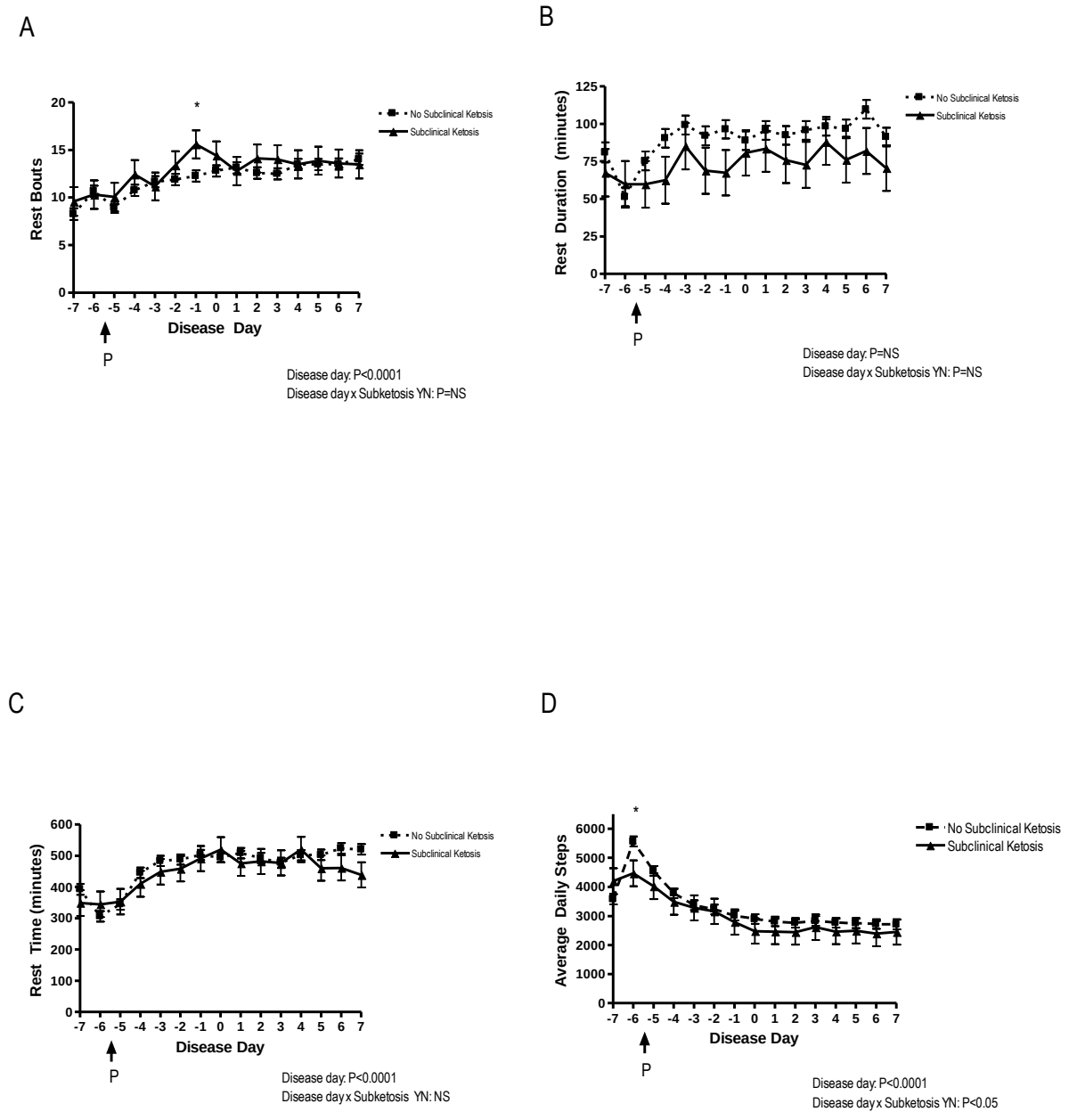

Figure 3.2 A comparison of rest bouts (#/d) (A), rest duration (min/bout) (B), rest time (min/d) (C), and average daily steps (D) -7 d prior to and 7 d after the onset of disease between cows that experienced subclinical ketosis (—— , n=26) and those who were not diseased (- - -, n=92). (*) are indicative of days where slices adjusted by Bonferroni were significantly different (P<0.05) between diseased and non-diseased animals. "P" indicates where parturition occurred.

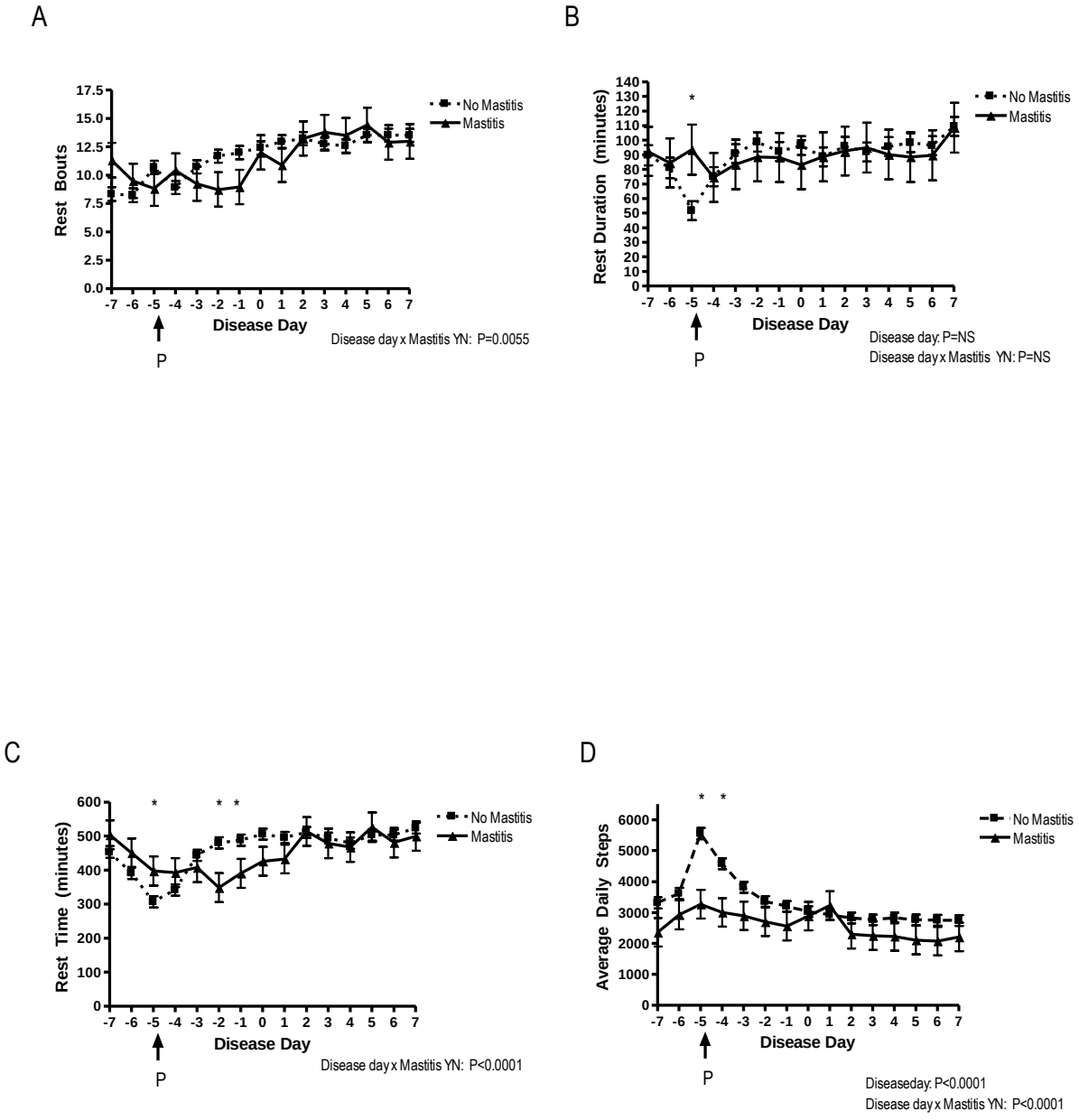

Figure 3.3 A comparison of rest bouts (#/d) (A), rest duration (min/bout) (B), rest time (min/d) (C), and average daily steps (D) -7 d prior to and 7 d after the onset of disease between cows that experienced mastitis (—— , n=15) and those who were not diseased (- - -, n=92). (*) are indicative of days where slices adjusted by Bonferroni were significantly different (P<0.05) between diseased and non-diseased animals.

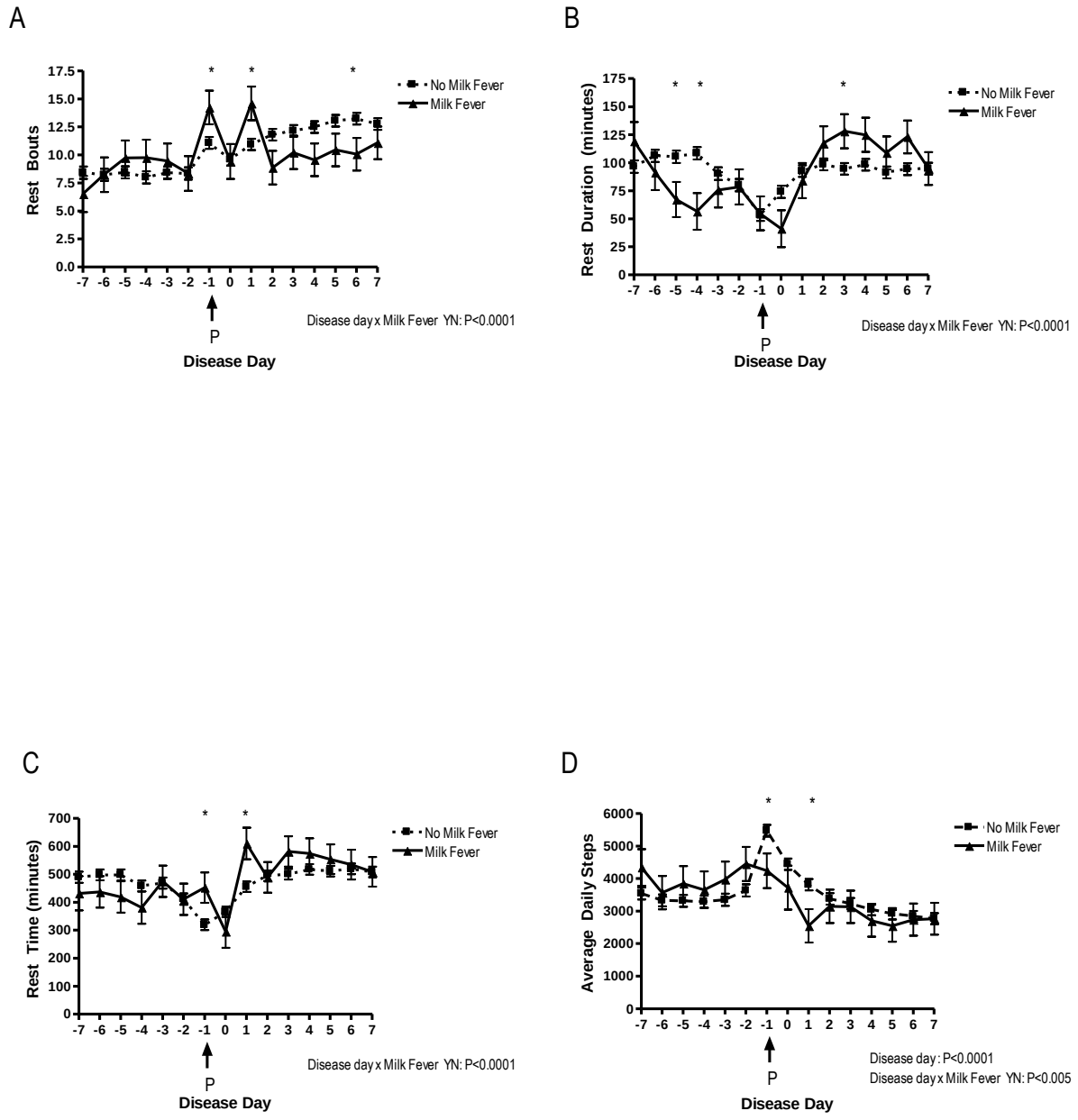

Figure 3.4 A comparison of rest bouts (#/d) (A), rest duration (min/bout) (B), rest time (min/d) (C), and average daily steps (D) -7 d prior to and 7 d after the onset of disease between cows that experienced milk fever (——, n=12) and those who were not diseased (- - -, n=93) in multiparous animals. (*) are indicative of days where slices adjusted by Bonferroni were significantly different (P<0.05) between diseased and non-diseased animals.

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Chapter 4: General Conclusions

A dairy herd is routinely afflicted with a multitude of diseases that must be managed to maintain a high level of herd health. By maintaining sound herd health, dairy producers can ensure a higher level of productivity from their animals and, in turn, realize increased profitability from their herd. The indirect benefit of improved herd health and reduction of disease is the improvement of animal well-being and consumer perception of the dairy industry. There has recently been a shift from disease treatment to disease prevention. As such, the identification of animals that are at a higher risk of such diseases is critical. This identification can be accomplished through the use a number of available management tools. One tool that has enhanced the overall herd health has been activity monitoring systems to track animal behaviors, yet the full potential of these systems has been under explored.

As a result of the need to better understand the capabilities of these types of activity monitoring systems, the main objectives of the studies were to determine the impact of both experimentally induced *E. coli* mastitis and naturally occurring periparturient diseases on various activity measures along with physiological and production measures. Any disease an animal experiences is costly both economically and to the overall health of the cow making it an important aspect to herd management. However, the impact of the disease prior to clinical expression of symptoms is not easily identifiable without specific physiological measures. However, it is accepted that animals do alter their normal behaviors but the quantification of this change in behavior is necessary.

The first aim of the study was to assess the activity, along with feed intake and milk production changes that occur with experimentally induced *E.coli* mastitis. The secondary aim in this research was to determine the effects of flunixin meglumine (FM), a non-steroidal anti-

inflammatory drug (NSAID), administration on those same measures. Lactating dairy cows were challenged with *E.coli* 727 in one quarter. Of the *E.coli* challenged animals, half were administered FM (0.002 µg/45.5kg of body weight) at the onset of clinical mastitis signs. The remaining half of *E.coli* challenged animals were untreated. An additional group cows were infused with 1 mL of sterile phosphate-buffered saline and served as the control group. Activity measures, dry matter intake (DMI), and milk production were collected on all animals. Activity measurements were collected using both Afi PedometerPlus© pedometers and HOBO® data loggers. Differences between the three treatment groups were analyzed.

The experimentally induced mastitis study data showed that *E.coli* mastitis did have an adverse effect on animal activity, where infected cows, regardless of treatment, stood for increased amounts of time and laid a decreased amount of time as compared to the uninfected controls. However, FM treated animals did show an improvement in DMI and milk production. Therefore, it was concluded that mastitis does negatively impact the normal behavior of animals. However, by using NSAID therapy, alleviation of the reduction of DMI and milk production in infected animals is possible. Future studies may investigate administering FM in multiple doses to enhance the advantageous effects observed in this study.

A second study was conducted to determine activity changes in the periparturient period. The aim of this study was to determine if those changes in combination with blood parameters could aid in the prediction of naturally occurring diseases. Activity measures and blood metabolites, non-esterified fatty acids (NEFA) prepartum and β-hydroxybutyrate (BHBA) postpartum were collected during the periparturient period. Dairy cows from the Virginia Tech dairy herd were monitored for activity measures including rest bouts, rest duration, and rest time throughout the pre and postpartum periods from -21 d to +30 d relative to calving. Activity

measurements were collected using Afi PedometerPlus© activity meters. Each of the activities monitored were affected by the onset of disease. NEFA levels were also significant in prediction of disease occurrence. Therefore, it can be concluded that activity changes in combination with deviation of activity from healthy herdmates could allow producers to utilize activity monitoring to proactively manage herd health. Additional studies should associate the possible interrelationships between diseases. It may also be beneficial to match cows by parity and DIM for diseased and non-diseased populations to have a more specific comparison of the two demographics of animals.

Based upon the results of these two studies, the ability to use activity monitoring to detect individual cows that may be at risk for disease is feasible in current dairy production. While the activity changes in both studies were observed in relatively small numbers, it provides further validation for more targeted investigation. Additionally, with this enhanced understanding of activity changes around disease, deviation percentages and thresholds could be developed so that dairy producers' attention could be brought to at risk animals. Therefore, once the animal is identified, various treatment strategies could be developed. For example, if an animal has been determined to be at risk for dystocia, it is possible for early induction of calving to be assessed as a treatment. A comparison between activity the at-risk animals that were hormonally induced and those that were not could provide the tool necessary to prevent such disease. Additionally, for animals determined to be at risk of being subclinical ketosis, ration changes when activity begins to deviate could be assessed to determine if it may aid in prevention. Those diseases that are both the most costly and most prevalent in a dairy herd, such as mastitis and ketosis, should be those in which early intervention strategies should be developed from activity monitoring. In regards to mastitis, it would be advantageous to assess the impact of flunixin meglumine has on non-

infected animals. This would determine if the effects observed is not only beneficial to sick animals but to their non-diseased counterparts as well.

The proactive herd health management strategy that is likely to develop from the use of activity monitoring will greatly benefit the overall dairy industry through proper identification and possible preventative methods against common dairy diseases. In turn, a greater animal well-being and farm profitability will be able to be realized.