Changes in Activity and Milk Components around the Onset of Peripartum Disease in Dairy Cows

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ABSTRACT

Activity and milk components were examined around disease onset in the periparturient period. Cows were monitored for daily rest bouts, rest duration, rest time, and step activity from -21 to +30 d relative to calving. Lactose concentration, fat to protein ratio (F:P), and milk yield were monitored. Diseases analyzed were assisted calving, mastitis, subclinical ketosis (SK), and a multiple disease category (MULTI) for animals who experienced more than one disease. Rest bouts decreased (d 0), step activity increased (d 0), and rest duration decreased (d -1) in animals that experienced assisted calving compared to controls. Mastitic cows showed fewer rest bouts (d -4, -3, -2, -1, and 0), increased activity (d -4, -2, and -1), decreased rest time (d -6, -5, -4, and -2), and decreased milk yield (d -4, -2, -1, 0) compared to controls. Cows with SK showed increased activity (d -5, -4, -3, and -2), decreased rest duration (d -7, -6, -5, -4, and -3), rest time (d -7, -6, -5, -4, -3, and -2), milk yield (d -4, -3, -2, -1, and 0) and lactose concentration (d -3, -2, -1, and 0) compared to controls. Animals categorized as MULTI showed increased activity (d -6, -5, -4, -3, and -2), increased F:P (d -2, -1 and 0), decreased rest time (d -5, -4, -3, and -2), and decreased lactose concentration (d -6, -5, -4, -5, -3, -2, -1, and 0) compared to controls. Deviations in activity and milk components could be used to proactively manage herd health.

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The 4-H flyer I received in February 2000 changed my life forever. Being the daughter of two naval officers and moving every couple of years to a new east coast city made me unfamiliar with agriculture. That was until we finally settled into Clark County Ohio, the birthplace of 4-H. Though I worked with many animal projects from rabbits to horses, I found that dairy was my true passion. Thanks to my 4-H advisor, Cathy Maine I had a wonderful experience and wanted to continue my education in dairy. My undergraduate work at The Ohio State University further sealed my fate in the dairy industry. It was great people like Bonnie Ayars, Dr. Maurice Eastridge, Dr. Joe Hogan, and Dr. Larry Smith that made me want to be a part of the industry. I thank you all for your enthusiasm about dairy and willingness to inspire the next generation, like me. Lastly, I must thank my friends Ashley Dietz and Katie Cole for line dancing, good advice, and margs whenever needed.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Animal welfare is a constant focus of the dairy industry in the United States as well as worldwide. With welfare as the motivation, the leading advancement in dairy health has been a shift from treatment of clinical illness to disease prevention and early detection methods (LeBlanc et al., 2006). The foremost area of interest in disease detection and prevention has been the transition period of dairy cattle. Approximately 75% of diseases in dairy cattle happen within this transition period, which is defined as 3 wk prepartum to 3 wk postpartum (Grummer, 1995, LeBlanc et al., 2006). During this time period, cows experience a negative energy balance (NEB) due to the increased energy demands for lactation along with decreased DMI (Huzzey et al., 2005); this in turn affects immune function and increases disease susceptibility (Grummer, 1995, Herdt, 2000).

All medium (100 to 499 cows) and large (≥ 500 cows) dairy operations along with 80% of small (<100 cows) operations in the United States have observed at least one case of clinical mastitis, lameness, retained placenta, infertility problems, or milk fever in 2006 (USDA, 2007). The diseases occurring most often on dairy operations, regardless of size, are clinical mastitis on 95%, lameness on 88%, and reproduction problems in addition to milk fever on 84% of operations (USDA, 2007). Furthermore, in a study on grazing dairy cattle, 35% of cows were diagnosed with subclinical ketosis, 15% with mastitis, and 9% with calving problems (Ribeiro et al., 2013). All of these diseases can develop from inadequate transition cow management and have detrimental effects on the profitability of dairy operations. The cost of disease is variable but on average a case of subclinical ketosis cost $78 (Sawall and Litherland, 2014) and a case of a Gram-negative mastitis infection would cost a producer over $200 (Cha et al., 2011). However, the true cost of disease is unknown because of detection limitations in diagnosing disease in the subclinical stages.

For this reason and the associated effect on animal well-being, recent research has been focused on assessing animal health with more sensitive indicators that are suitable for use before animals become clinically ill (von Keyserlingk et al., 2009).

Historically, physical observation, and urine or blood tests have been used as indicators of animal health but this can be costly, time consuming, and labor intensive, which indicates a need for a more rapid and continuous measure of health (von Keyserlingk et al., 2009). A potential measure to monitor animal health is the behavior or activity of each animal. Since activity is an automated measure, it does not require the presence of a human observer which can reduce labor cost as well as eliminate changes in behavior due to the presence of a human (Weary et al., 2009). Through the assessment of animal behavior, disease can be detected in both subclinical and clinical stages; this capability can improve cow comfort and animal well-being (Dawkins, 2003, von Keyserlingk et al., 2009).

The objectives of this review are to 1) discuss the technologies involved in monitoring dairy cattle behavior and the information that is obtained from these technologies, 2) address peripartum disease detection with the use of animal activity or behavior and 3) identify the physiological measurements that can be used to support disease detection along with behavioral measures.

CHAPTER 2: LITERATURE REVIEW

2.1 Behavioral Technologies in Dairy Cattle

Behavior is an important adjunct to the assessment of physical health, both as identifying clinical symptoms as well as giving early warning signs of health problems to come (Dawkins, 2003). Technologies that allow for the collection of data on behavior are increasing in popularity within the dairy industry. These technologies provide an array of data collection options including feeding and social behavior, milk production with component data, as well as animal activity on an individual cow basis. This type of data collection allows for Precision Dairy Farming. Which is focused on economically sound production as well as generating higher quality dairy products. The objective of Precision Dairy Farming is to maximize individual animal potential, detect disease sooner, and minimize the use of medication through preventative health measures (Bewley, 2013). The behavioral technologies within Precision Dairy Farming that are most commonly used include video observation and automated monitoring of animal activity. This section will discuss the literature pertaining to video observation and automated monitoring of animal activity to assess cow behavior.

2.1.1 Video Observation of Cattle Behavior

Video observation of behavior is considered the gold standard for behavioral research and has been used to evaluate an animal's natural behavior in addition to behavioral changes due to management decisions. In a study looking at the time budgets of dairy cattle in stanchion stalls, video was continuously recorded for 15 h/d to observe the natural behavior during this time (Hedlund and Rolls, 1977). Throughout the observation period, cows spent 45% of their time lying, 26% eating, and 22% ruminating (mostly during lying), while the remaining time consisted of drinking or socializing. Lying time has been shown to have a higher priority over eating time and social contact in early and late lactation animals thus indicating the importance of this behavior (Munksgaard et al., 2005). When looking at the time budget of cows in 16 freestall barns and the effect of external factors on these time budgets, video was observed for 1 s every 30 s for a 24 h period (Gomez and Cook, 2010). During observation, the average feeding time for all cows was 4.3 ± 1.1 h/d and lying time averaged 11.9 ± 2.4 h/d. Furthermore, when looking at lying bouts (n) the average was 12.9 ± 6.6 bouts and lying bout duration averaged 1.2 ± 0.4 h. This data gives a detailed look into how cattle spend their time naturally but how could management decisions affect cattle behavior?

A study looking at behavioral differences in cows housed in tie stalls with concrete (CON) flooring or mattress (MAT) flooring, observed cows for 4 periods of 24 h using time lapse video recording (Haley et al., 2001). Cows on MAT had increased total lying time by 1.8 h/d compared to CON and cows on CON spent more time standing without eating (35.2% vs. 28.1%) compared to MAT cows. Furthermore, when comparing cows housed in freestalls with a mattress (MAT) or bedded with sand (SAND), cows on MAT spent less time lying (11.5 vs. 12.66 h/d, $P<0.05$), had more lying bouts (14.44 vs. 10.22 bouts, $P = 0.049$), and shorter bout duration (1.02 vs. 1.30 h/d?, $P = 0.014$) than cows on SAND (Gomez and Cook, 2010). In contrast, another study looking at differences between MAT and SAND in 6 freestall barns saw no difference in lying bouts or lying time but cows on MAT did stand longer in stalls (1.61 h/d) compared to SAND ($P = 0.002$) and cows on SAND had more long lying bouts (>60 min) than cows on MAT ($P = 0.03$) (Cook et al., 2004). This data could be indicating different cow comfort levels and preference of bedding type within various housing systems. Although video observation has allowed for the identification of natural behaviors and effects of management decisions on cow comfort, it can be time consuming and labor intensive to analyze thus automated devices are becoming more common (Overton et al., 2002, Ledgerwood et al., 2010).

2.1.2 Automated Monitoring of Cattle Behavior

Automated monitoring of behavior essentially requires no human presence and is noninvasive to the animal as well as allows for a greater span of data collection compared to visual observation or video recording (von Keyserlingk et al., 2009, Weary et al., 2009). Automated monitoring is usually done by one of three ways, which includes a voltage data logger, accelerometer, or a pedometer. Many automated monitoring options are available and much research has been done to validate the different automated devices.

Of the automated devices available for behavioral monitoring, the most simple is a data logger looking at voltage changes between standing and lying. One such logger called TinyTag Plus® (Gemini Dataloggers Ltd., Chichester, UK), has been shown to correctly identifying standing and lying behavior in cattle. This logger stores data from a mercury tilt switch that has an open circuit (0V) when a cow is standing and a closed circuit (2.5 V) with lying behavior. A study in 2008 used Kappa results to look at the agreement between the TinyTag Plus® and direct observation (O'Driscoll et al., 2008). The study showed a Kappa value from screened data > 0.8 for all individual loggers and the overall concordance for lying and standing behavior was 96.3%. The TinyTag Plus® has also been used to evaluate the normal time budgets of dairy cattle within the transition period (Huzzey et al., 2005). During -10 to -2 d before calving cows spent an average of 12.3 ± 0.3 h/d standing and after calving (+2 to +10 d) the animals increased time standing to 13.4 ± 0.3 h/d ($P = 0.02$). While calving (d -1 to +1), cows had a significantly greater number of standing bouts (17.3 ± 1.08 bouts/d, $P = 0.002$) compared to before calving (11.7 ± 1.07 bouts/d) and after calving (13.1 ± 1.07 bouts/d). These studies indicate that discomfort from calving can be seen with activity changes and the TinyTag Plus® is accurate at determining lying and standing behavior.

More sensitive data loggers called accelerometers have been used and validated through research in identifying cattle behavior. A recent study looked at the accuracy of a 2-dimensional accelerometer compared to video recordings to monitor beef calf behavior after castration (White et al., 2008). This accelerometer had x- and y-axes and movement was determined by a micro-electromechanical system that took the constant pull of gravity into effect. The accelerometer was attached to the right hind limb just proximal to the metatarsophalangeal joint with the y-axis of the sensor perpendicular to the ground when the calves were standing. When looking at the agreement between the accelerometer and video images, standing and lying could be determined 98.3% of the time. Furthermore, steps taken were estimated with a reasonably low misclassification rate of 23.5%. Overall, it was determined that the 2-deminsional accelerometer was accurate in classifying animal posture as standing or lying and could be a replacement for video observation.

Another accelerometer called Actiwatch® activity monitoring system has been validated in dairy research to evaluate animal activity within a loose housing barn (Müller and Schrader, 2003). It was determined by comparing data to video observation that the number of high activity counts was correlated to locomotion r = 0.75 (P<0.001) and the number of low activity counts was correlated to lying r = 0.65 (P<0.001). These values were determined using dynamic thresholds that looked at the baseline activity of the individual animals. This shows the Actiwatch® is reliable at identifying different behavioral activities within cattle. In another study, using Actiwatch® it was found that high activity periods had a repeatability (REP) of 0.62 over 2 lactations which was greater than the REP of low activity periods REP = 0.40 and duration of low activity REP = 0.49 (Muller and Schrader, 2005). This study demonstrated that individual cow activity stays consistent through 2 lactations thus the possibility exist to have deviations from an animal's baseline activity that could be detected by automated devices.

The type of accelerometer that has been used most in animal research has 3-dimensions that record the x, y, and z axes, which has added sensitivity compared to a 2-dimensional accelerometer. A 3-dimensional accelerometer records the magnetic field shifts or force changes that occurs from cattle behaviors. One 3-dimensional accelerometer that is available is the IceTag® (IceRobotics, Edinburg, UK) system. A study comparing data collected from an IceTag® to visual observation showed the automated device could reliably estimate duration and number of moving and standing bouts in dairy cattle (Munksgaard et al., 2006). The IceTag® has also been validated in dairy calves with a sensitivity greater than 0.99 for lying behavior and greater than 0.92 for standing behavior as well as a specificity of 0.98 for lying and greater than 0.97 for standing behavior (Trenel et al., 2009). Overall, this automated device has been validated in recording behavior accurately and could be used as an alternative for video observation.

Another 3-dimensional accelerometer used in research is the GP1 SENSR (SENSR, Elkader, IA). A study in 2009 compared data collected from the GP1 SENSR attached to the right rear leg just proximal to the fetlock of beef cattle, to video recordings of these same cows (Robert et al., 2009). It was found that the GP1 SENSR had high agreement with the video recordings at 99.2% for lying classification, 98% for standing classification, and lower accuracy for walking classification at 67.8%. Furthermore, accuracy was found to be greatest in the recording intervals of 3 s (98.1%) and 5 s (97.7%) compared to 10 s (85.4%). Similar to the GP1 SENSR, the HOBO® Pendant G data logger has been validated in assessing animal behavior. When looking at the accuracy of the HOBO® at detecting lying and standing in 3 different sampling intervals, it was found that the predictability, sensitivity, and specificity was >99% for sampling intervals < 30 s

(Ledgerwood et al., 2010). The use of this data logger to assess lying behavior in calves has also been validated. When looking at the correlation coefficients between direct observation and data collected from a HOBO, the highest correlation for total lying time was found when the HOBO was attached to the right hind leg with a 60 s recording interval (r = 0.99, $P < 0.001$) (Bonk et al., 2013). Furthermore, the highest correlation for bout frequency was when the HOBO® was attached to the left hind leg with a 60 s recording interval (r = 0.85, $P < 0.001$). Finally, the HOBO® data logger has also been validated in assessing feeding behaviors like bin visits and feeding time (Krawczel et al., 2012). For feeding bin visits using screened data, no difference was found between video observation and the HOBO® data logger ($P = 0.57$) with 96% of the data points falling within the maximum allowable difference (MAD). Moreover, no difference was established for feeding time ($P = 0.13$), with 97% of data falling within the MAD. Overall, many 3-dimensional accelerometers have been shown to accurately define behavior in dairy cattle as well as claves.

The final automated behavioral monitoring device validated and used in research is a pedometer. A recent study has compared the IceTag® accelerometer which has already been validated in identify behavior in cattle, with Pedometer Plus© (S.A.E Afikim, Israel) (Higginson et al., 2010). In this study, cattle had an IceTag® and the Pedometer Plus© on different or the same hind leg, to see the correlation between the two automated devices. When the devices were on opposite hind legs the correlation for number of step between the two devices was r = 0.73 ($P < 0.0001$). This correlation is lower than expected which researchers thought could be explained by when a cow is lying the upper leg moves more than the bottom leg. Thus, the researchers looked at correlation values when the cattle had both automated devices on the same hind leg. This yielded much higher correlation values for number of steps (r = 0.82, $P < 0.0001$), number of lying bouts (r = 0.98, $P < 0.0001$) and duration of lying time (r = 0.90, $P < 0.0001$). These high correlation values validate Pedometer Plus© as a tool for monitoring behavior like steps, lying bouts, and lying duration in dairy cattle.

Many automated devices have been shown to accurately assess cattle behavior. The behaviors that can be collected by automated monitors include standing and lying bouts, standing and lying time, bout duration, and step activity. Research has shown that dairy cattle behavior stays consistent over two lactations (Muller and Schrader, 2005). Thus, the consistency of individual cows could allow for differences from their baseline activity to be identified when a change in cow comfort occurs. Therefore, cow comfort changes that happen with disease could possibly be indicated by behavioral monitoring before detection from a display of clinical symptoms.

2.2 Behavioral Deviations of Diseased Dairy Cattle

The biggest advancement in dairy health has been a shift from treatment of clinical illness to disease prevention and early detection methods (LeBlanc et al., 2006). Research has been focused on using automated behavioral monitoring to detect disease particularly within the transition period. Much attention has been on the transition period because 75% of diseases occur within this time, due to the NEB cows experience around calving (Grummer, 1995, LeBlanc et al., 2006). Furthermore, the most prevalent diseases on all size dairy operations are clinical mastitis on 95%, lameness on 88%, and reproduction problems in addition to milk fever on 84% (USDA, 2007). For these reasons, it is easily understood why these diseases have received much attention and are the focus of many behavioral monitoring studies.

2.2.1 Mastitis

Mastitis is the most prevalent disease occurring on 95% of operations and is a leading cause of involuntary culling as well as death of dairy cattle (USDA, 2007). Not only is mastitis prevalent it also has a huge impact on cow comfort and farm profitability (Medrano-Galarza et al., 2012). Studies have looked at behavior during a mastitis infection to identify potential indicators of changes in cow comfort. Both an IceTag® and a HOBO® were affixed to the hind legs of 42 lactating dairy cows to assess activity, lying, and milking behavior with naturally-occurring mastitis (Medrano-Galarza et al., 2012). Cows that experienced mastitis spent less time lying down on the day after mastitis detection and had increased daily steps during days with mastitis compared to control cows. These results are in agreement with studies looking at cows with experimentally induced mastitis. It was found that cows with induced mastitis spent 73.3 fewer min lying down when compared to controls on the day of challenge (Cyples et al., 2012). Furthermore, cows with induced mastitis took more steps ($P = 0.02$), spent less time lying down overall and less lying on the side corresponding to the udder quarter with mastitis compared to controls (Siivonen et al., 2011). These results suggest the cows may be experiencing discomfort during mastitis. This pain could cause cows to spend less time lying due to pressure on the udder and more time taking steps because of being uncomfortable.

Efforts have been made to identify behaviors that could be influencing the acquisition of new intramammary infections and how management decisions could effect this. In a study in 2013, feeding manipulation relative to milking as well as standing and lying behavior after milking were observed (Watters et al., 2013). It was found that feed manipulation in the hour before milking and shortly after milking promoted the longest post-milking standing time. Furthermore, cows that laid down >90 min after milking had a lower risk of acquiring mastitis compared to cows that laid down <90 min after milking. This study showed that management decisions like feed manipulation has an effect on post-milking standing time and this can affect the risk of new intramammary infections.

Although management decisions can help to reduce the incidence of mastitis, complete prevention is not realistic. Thus, methods are needed to identify mastitis prior to onset of clinical signs to reduce treatment cost and lost milk production in addition to improving cattle well-being. A study has looked at the behavioral changes before the onset of naturally-occurring clinical mastitis during the transition period. This study examined daily rest time, rest duration, rest bouts and step activity, 7 d before the onset of mastitis to 7 d after mastitis diagnosis using Pedometer Plus©. Mastitis was defined as changes in milk appearance (flakes, clumps, or color) with or without inflammation as observed by milkers (Yeiser, 2011). Cows that experienced clinical mastitis showed reduced rest time 3 d before disease diagnosis but had no increase in daily steps. The data shown in this study could be indicating discomfort from mastitis and shows a potential in using animal behavior to detect mastitis in the subclinical stages. Detection of disease and consumer perception of cow comfort have a huge economic impact on dairy producers therefore behavioral monitoring of other diseases have been studied.

2.2.2 Lameness

Lameness is recognized as one of the most serious welfare and production concerns in the dairy industry due to how easily observable pain and gait alterations are of lame cows (Ito et al., 2010). A study in 2010 used HOBO® data loggers on 1,319 cows to record lying behavior as a possible diagnostic tool for lameness (Ito et al., 2010). Lameness was determined by one observer using a 5-point Numerical Rating System (NRS) with 1 = sound and 5 = severely lame. Cows were considered lame if the NRS was ≥ 3 and severely lame if the NRS = 4. This study found that cows with lying times >14.5 h/d in deep bedded stalls had 16.2 times higher odds of being severely lame.

Furthermore, cows with average lying bouts >90 min/bout were 3.0 times more likely to be severely lame. Another study used the IceTag®, video cameras, and weight distribution to assess behavior between lame and non-lame cows (Chapinal et al., 2011). It was found that lame cows (NRS>3) shifted weight between legs more often, had greater asymmetry in weight applied to rear legs, had longer lying bouts, and walked slower compared to non-lame cows. Correspondingly, another study using the HOBO® data loggers found that moderately lame cows (NRS = 3 or 4) displayed a greater lying bout duration (89.3 ± 3.89 min) than non-lame cows (NRS = 1; 80.7 ± 3.90 min) (Yunta et al., 2012).

One study has looked at lameness specifically during the transition period to see how it affects resting behavior in cattle (Calderon and Cook, 2011). Lameness was determined based on a locomotion score (LMS) where 1 = non-lame, 2= slightly lame, and 3 = moderate and severely lame. Like the studies previously discussed, moderate and severely lame cows had significantly longer lying times throughout the transition period compared to non-lame cows. In addition to longer lying bouts, moderate and severely lame cows had an increased number of lying bouts 3 d pre- and post-calving. All of these studies have indicated that lameness causes behavioral changes most likely due to pain and discomfort experienced after diagnosis. However, finding indicators of early signs of lameness to allow for detection of lameness prior to clinical onset is warranted.

A study in 2006 used changes in pedometry activity (steps/h) of -5%, to indicate lameness (Mazrier et al., 2006). Of the 38 cows diagnosed with clinical lameness, 55.3% of these cows could have been identified 7-10 days earlier using decreased activity. Furthermore, 92% of all lameness cases were identified if activity was reduced by >15%. This data shows that it is possible to detect lameness in the subclinical stages using pedometer activity. Although the most accurate threshold is still unknown, the opportunity is there for early detection at as much as 7 to 10 days before clinical signs. Behavioral monitoring has proven to be useful in the detection of mastitis as well as lameness and therefore should be useful to detect other disease process in dairy cattle.

2.2.3 Reproductive Problems

The occurrence of a reproductive problem can be detrimental to the longevity, production, and well-being of an animal. Common reproductive problems include dystocia, retained placenta, and metritis, which usually occur during the transition period. A study in 2009 looked at feeding, lying, and standing behavior of cows that experienced dystocia (Proudfoot et al., 2009). Calving ease was given a score of 1 to 3, where 1 = unassisted delivery, 2 = easy assistance (one person to pull), and 3 = difficult assistance (dystocia, 2 or more people to pull). Cows that experienced dystocia consumed less feed 48 h before calving occurred (14.3 ± 1.0 vs. 16.2 ± 1.0 kg, respectively) as compared to cows that had a calving ease score of 1. Furthermore, cows with dystocia transitioned from standing to lying positions more than cows without dystocia (10.9 ± 0.7 vs. 8.3 ± 0.7 bouts/d) beginning 24 h before calving. Similarly a study in 2011 found that cows that experience dystocia have an increased number of rest bouts (13.7 ± 1.3 bouts) as compared to cows who did not experience dystocia (10.5 ± 0.5 bouts) on the day of calving (Yeiser, 2011).

Metritis is a common postpartum disease that causes decreased milk production and has a negative effect on reproductive performance (Urton et al., 2005). Efforts have been made to determine behavioral changes that could identify cows at risk for metritis. A study looking at feeding behavior 2 wk before and 3 wk after calving, showed differences in cows diagnosed with metritis compared to those that did not experience metritis (Urton et al., 2005). Cows were diagnosed with metritis based on vaginal discharge (VD) scores (1 = clear to slightly cloudy mucous, 2 = mucopurulent and foul smelling, 3 = purulent and foul smelling, 4 = putrid) and rectal temperatures. Metritis was defined as a cow with a VD ≥ 2 along with a fever (>39.5°C). The study found that cows diagnosed with metritis spent 22 min/d less time at the feed alley during the experimental time. Moreover, for every 10 min decrease in feeding time the likelihood to be diagnosed with metritis doubled. An additional study looked at intake, feeding, drinking, and social behavior to identify cows with metritis (Huzzey et al., 2007). When weekly averages were obtained, cows diagnosed with severe metritis (VD = 4 and fever >39.5°C) spent less time feeding week -1 prepartum (146.0 ± 11.6 vs. 192.3 ± 8.9 min/d; $P < 0.01$) and week -2 prepartum (184.8 ± 10.9 vs. 214.1 ± 8.4 min/d; $P < 0.05$) compared to healthy herdmates. Additionally, these cows consumed less feed week -1 prepartum (11.2 ± 0.7 vs. 14.3 ± 0.5 kg/d; $P < 0.0$) and week -2 prepartum (13.1 ± 0.6 vs. 14.9 ± 0.5 kg/d; $P < 0.05$) compared to controls. Also for every 1 kg decrease in DMI the week before calving, cows were 3 times more likely to be diagnosed with metritis. Finally, cows experiencing severe metritis engaged in less aggressive displacements at the feed bunk (12.2 ± 1.58 vs. 16.8 ± 1.74 times/d) compared to healthy cows within the 2 wk before calving.

Reproductive problems like dystocia and metritis were shown to have behavioral alterations before the onset of clinical signs. The ability to detect these diseases as much as 2 wk before calving could allow for early intervention or treatment, which could improve milk production and reproductive performance on an individual cow or farm basis. Thus far, behavioral monitoring has been established as useful in identifying mastitis, lameness, and reproductive problems but one category of peripartum diseases remains and that is metabolic disorders.

2.2.4 Metabolic Disorders

Common metabolic disorders include clinical as well as subclinical milk fever and ketosis, displaced abomasum, and rumen acidosis. These diseases cause decreased milk production and make the animal at risk for other diseases (Edwards and Tozer, 2004, Jawor et al., 2012), which can affect farm profitability and the productive life of the animal. Therefore, research has focused on identifying these diseases in the subclinical stages using behavioral monitoring.

Behavioral changes have been examined as a means of identifying subclinical milk fever. A study in 2012, looked at standing behavior before subclinical hypocalcaemia (serum calcium concentration ≤1.8 mmol/L, without clinical signs) was diagnosed, around calving (Jawor et al., 2012). Cows that experienced subclinical milk fever stood 2.6 h ($P = 0.03$) longer than healthy cows during the 24 h period before calving. Moreover, animals experiencing subclinical hypocalcaemia spent 2.7 h ($P = 0.07$) less time standing the day after calving compared to healthy cows. A study looking at behavioral changes before clinical milk fever also saw changes in activity (Yeiser, 2011). Cattle with clinical milk fever (serum calcium concentration <7.5 mg/dl, plus clinical signs) had an increased number of rest bouts (14.2 ± 1.5 vs. 11.1 ± 0.5 bouts/d) and decreased daily steps (4242.4 ± 529.3 steps vs. 5475.0 ± 184.4 steps/d) compared to controls, the day before disease diagnosis. Furthermore, on the day before diseases diagnosis cows with milk fever spent more time resting (452.3 ± 54.4 vs. 319.0 ± 19.0 min) compared to cows without milk fever. These two studies agree that behavioral factors can help in identifying milk fever particularly the day before disease diagnosis.

Cows that experience ketosis, or hyperketonemia, cost producers an average of $211 per case (C. Guard, Cornell University, Ithaca, NY, personal communication, 2012) and make the animal more susceptible to develop a displaced abomasum or leave the herd within 30 DIM, as well as less likely to conceive at first service (McArt et al., 2012). Therefore, research has focused on classifying indicators to predict the occurrence of subclinical and clinical ketosis. A study has identified animals that are at the greatest risk of developing ketosis and important predictors are the sex of the calf (male), calving ease, and advanced parity (McArt et al., 2013). These factors can help producers pick cows to monitor during the transition period due to the increased risk of developing ketosis but cannot diagnose the disease before clinical onset.

As a result, studies have looked at feeding behavior and activity changes around the onset of ketosis. In 2009, a study looked at the feeding and social behaviors of animals diagnosed with subclinical ketosis (serum BHBA concentration ≥1000 µmol/L) during the first week of lactation (Goldhawk et al., 2009). Cows that experienced subclinical ketosis had 18% lower DMI compared to healthy controls in the week before calving. Furthermore, subclinically ketotic animals visited the feeder fewer times, spent less time at the feeder, and initiated fewer displacements the 2 wk prior to calving until 2 wk after calving, compared to controls. It was also found that for every 10 min decrease in time spent at the feeder and every 1 kg decrease in intake the risk of subclinical ketosis increased two-fold. An additional study looked at activity alterations in cows diagnosed with subclinical ketosis (serum BHBA concentration ≥ 1.3 mmol/L) (Yeiser, 2011). It was found that cows experiencing subclinical ketosis had increased rest bouts the day before diagnosis (15.6 ± 1.5 vs. 12.2 ± 0.6 bouts) and had decreased daily steps (4467.9 ± 447.5 vs. 5559.2 ± 174.7 steps/d) 6 d before disease diagnosis. These studies show that feeding behavior as well as activity alterations in the transition period can aid in early detection before the clinical diagnosis of ketosis.

The metabolic disorders that have been researched most include milk fever and ketosis but one study has looked at the behavioral changes prior to diagnosis of a displaced abomasum and general digestive problems (indigestion, acidosis, and reduced feed intake) during the transition period. For this study the walking activity, collected from a pedometer, was examined for 10 d before disease diagnosis until 10 d after (Edwards and Tozer, 2004). Cows that were diagnosed with a left displaced abomasum (LDA) showed increased activity from d -8 to -6 ($P < 0.01$) and on d -1 ($P < 0.01$) relative to clinical diagnosis (d 0), when compared to controls. Furthermore, cows diagnosed with a digestive problem had decreasing activity from d -8 to diagnosis of the disease. This study showed that walking activity could help to detect peripartum diseases, which could reduce treatment cost and revenue losses from decreased production.

Monitoring deviations in activity measures as well as feeding behavior has been proven effective in classifying animals with peripartum diseases prior to onset of clinical signs. The ability to identify diseased animals through automated detection could potentially reduce the discomfort an animal experiences with disease as well as increase farm profitability by cutting treatment cost and lost milk production (von Keyserlingk et al., 2009). Although behavior can indicate sickness before clinical signs it is not specific enough to diagnosis each peripartum disease independently. For this reason, research has also looked at physiological parameters to diagnose disease.

2.3 Physiological Deviations in Diseased Dairy Cattle

Physiological parameters that are used in disease detection include in-line milk component data such as yield, fat, protein and lactose %. Also blood, milk or urine metabolites including β-hydroxybutyrate (BHBA) are used for detection. In-line milk component data has the advantage of being collected at each milking, it allows for surveillance of changes on a daily basis, and does not require additional labor for collection. The use of a cow-side test that detects whole blood or urine BHBA concentrations has become increasingly popular over the last several years, in particular for the diagnosis of subclinical and clinical ketosis and displaced abomasum. Research has looked at the accuracy of these physiological parameters at diagnosing and detecting disease.

Milk yield has historically been used as a common indicator of disease and overall health. A study in 2004 looked at milk yield as a diagnostic tool for postpartum diseases, including ketosis, left displaced abomasum (LDA), and digestive disorders (Edwards and Tozer, 2004). In general, sick cows produced significantly less milk ($P < 0.01$) 10 d before diagnosis compared to healthy cows and the greatest reduction was seen starting 3 d before diagnosis. Milk yield was significantly decreased starting 6 d before diagnosis of ketosis compared to healthy cows. Similarly, cows diagnosed with an LDA and digestive disorders showed less yield 7 d and 5 d before diagnosis, respectively. Finally, cows diagnosed with subclinical hypocalcaemia (serum calcium < 1.8 mmol/L) after calving show increased milk yield during wk 2, 3, and 4 after calving (6 kg/d) compared to control cows (Jawor et al., 2012). This indicates that greater body reserves like calcium are being directed toward milk production in cows with subclinical hypocalcaemia. Although these studies show changes in milk yield with transitional diseases, milk production has a lack of specificity and can be affected by many factors including feed.

For this reason, research has looked into milk component changes with disease, as these could be a more specific indicator. One study has looked at early lactation fat and protein ratio (F:P) and its involvement in disease occurrence (Toni et al., 2011). It was found that cows with an F:P greater than 2 had increased incidence of postpartum diseases, which included retained placentas, LDA, metritis, and clinical endometritis. Another study showed that ketosis, mastitis, lameness, and LDA increase the likelihood that first test day F:P will be >1.5 (Heuer et al., 1999). Similarly, a study looking at different diagnostic tools for ketosis found that the F:P could diagnose ketosis with a specificity of 0.78 and a sensitivity of 0.63 (Krogh et al., 2011). F:P can be useful in disease detection but from these studies, it is seen that changes in F:P are not indicative of one specific disease but many. Lactose, somatic cell count (SCC), and electrical conductivity (EC) are additional components of milk that have been looked at for the detection of mastitis. A study in 2012 showed that cows diagnosed with mastitis, had a decreased lactose % compared to controls the day before clinical diagnosis (Tholen and Petersson-Wolfe, 2012). When looking at EC as a diagnostic tool for mastitis, it has been found that EC increases significantly as much a 7 d before clinical diagnosis of mastitis (Gaspardy et al., 2012). However, in-line EC has a low predictive value for mastitis at 35% and a sensitivity of only 25% (Lansbergen et al., 1994). A study looking at in-line SCC in combination with EC reported a positive predictive value of detecting clinical mastitis at 21.9 to 32.0% with a low false positive of 1.2 to 2.1%, which was found to be better than SCC or EC alone (Kamphuis et al., 2008). These studies show that there is potential in milk composition changes to help detect and diagnose postpartum diseases but more research is needed.

One final diagnostic tool has been used for the detection of subclinical or clinical ketosis and an indicator of a LDA. This tool is serum BHBA concentrations which has more accuracy compared to milk or urine BHBA (Iwersen et al., 2009). Serum BHBA can be taken by a simple cow-side BHBA meter that shows the concentration within 10 s. One such meter is the Precision Xtra® blood glucose and ketone monitoring system (Abbott Diabetes Care Inc., Alameda, California). This meter has been shown to have a correlation coefficient of 0.95 compared to BHBA concentrations determined in a lab photometrically (Iwersen et al., 2009). Furthermore, the sensitivity of this meter was determined to be 88 and 96% at 1,200 and 1,400 μmol of BHBA/L and the specificity was 96 and 97%. When looking at BHBA concentration for disease diagnosis, it has been found that cows with BHBA ≥ 1,200 μmol/L in the first week after calving had a 4.7 times greater risk of developing clinical ketosis (Seifi et al., 2011). Moreover, cows that tested ≥1,000 μmol/L in the first week after calving were 13.6 times more likely to develop a displaced abomasum. Similarly, another study found that cows with a BHBA ≥ 1,200 μmol/L in the first 7 d postpartum were 8 times more likely to acquire an LDA. Serum concentration of BHBA, especially during the first week post calving, has been shown to be a tool in predicting cows that will experience ketosis or a displaced abomasum.

Physiological parameters like milk yield, milk composition, and serum metabolite concentrations have been shown to have potential as well as drawbacks in diagnosing and detecting postpartum diseases. A drawback of physiological parameters like milk yield and F:P is, it is not specific to a single postpartum disease and can be affected by many factors. Furthermore, changes in activity are not specific in the diagnosis of postpartum diseases. However, limited research has been done looking at both activity in combination with physiological changes around disease in the peripartum period. Together, changes in both activity and physiological state could indicate specific postpartum diseases. By collecting and analyzing activity and physiological parameters, the hope is to be able to detect specific peripartum diseases in the subclinical stages. If a specific disease can be identified by combining activity and physiological changes, alert systems can be generated to tell the producers when a cow is coming down with a disease. Through early identification of animals, acquiring a disease a producer could treat the animal with antibiotic or therapeutic drugs, change feeding strategies, or give supplemental support like propylene glycol or calcium. These early intervention methods could have the potential to reduce treatment cost, lost milk production, and improve animal well-being.

2.4 Research Objective

Dairy cattle are susceptible to a host of postpartum diseases due to the NEB and immunosuppression a cow experiences during the transition period. While a majority of diseases are diagnosed in the clinical stage due to physical observation, animal behavior and physiological parameters have the possibility to identify disease in the subclinical stages. Detecting postpartum diseases in the subclinical stages will allow producer to treat animals sooner, improve animal well-being, and increase farm profitability by reducing treatment cost and lost milk production. For this reason, the objective of this research is to use animal activity and milk component data to determine if specific postpartum diseases can be identified in the subclinical stages.

CHAPTER 3: CHANGES IN ACTIVITY AND MILK COMPONENTS AROUND THE ONSET OF PERIPARTUM DISEASES

3.1 Introduction

The biggest advancement in dairy health over the past 25 yr, has been a shift from treatment of clinical illness to one of disease prevention and early detection methods (LeBlanc et al., 2006). This is important because all medium (100 to 499 cows) and large (≥ 500 cows) dairy operations along with 80% of small (<100 cows) operations in the United States observed at least one case of clinical mastitis, lameness, retained placenta, infertility, or milk fever in 2006 (USDA, 2007). The focus of disease detection and prevention has been primarily in the transition period (3 wk pre- to 3 wk post-calving) because 75% of diseases on dairy operations occur during this time frame (Grummer, 1995). During this time a cow experiences a state of NEB due to the increased energy needed for lactation as well as a decrease in DMI (Huzzey et al., 2005). This NEB initiates the mobilization of body fat that leads to ketone body (BHBA) production within the blood stream, as well as immunosuppression, which makes the animal susceptible to disease (Suriyasathaporn et al., 2000, van der Drift et al., 2012).

Historically, visual examination and/or a blood or urine test have been used to identify animals with disease but this can be time consuming and costly, which advocates a need for a more rapid and continuous measure of health (von Keyserlingk et al., 2009). One such continuous measure that has been developed is automated behavioral monitoring. These measurements have been shown to be useful in detecting diseases. One study showed that 92% of experimental cows with lameness had a 15 % reduction in pedometer activity 7 to 10 d before clinical diagnosis (Mazrier et al., 2006). Furthermore, cows experiencing dystocia (Proudfoot et al., 2009), metritis

(Huzzey et al., 2007), and subclinical ketosis (Goldhawk et al., 2009) show decreased DMI before disease diagnosis.

Milk yield and milk components have also proven useful in the detection and diagnosis of peripartum disease, including fat to protein ration (F:P), lactose %, somatic cell count (SCC), and electrical conductivity (EC). Studies have found that milk yield was reduced 7, 6, and 5 d before the onset of left displaced abomasum (LDA), ketosis, and digestive disorders, respectively (Edwards and Tozer, 2004). Ketosis, mastitis, lameness, and LDA has been shown to increase first test day milk F:P (>1.5) (Heuer et al., 1999). Furthermore, lactose % decreases a day before (Tholen and Petersson-Wolfe, 2012) and EC increases as much as 7 d prior to onset of clinical signs of mastitis (Gaspardy et al., 2012). Finally, in-line SCC in combination with EC has been shown to have some value in indicating mastitis (Kamphuis et al., 2008).

An examination of peripartum activity in combination with changes in daily milk components have not been fully evaluated as early indicators of naturally-occurring postpartum disease. Therefore, the objective of this study was to use activity measures in combination with milk component data to identify indicators of subclinical naturally-occurring postpartum disease. By identifying animals in the subclinical stages, dairy producers could have the ability to treat earlier and implement preventative measures, to improve cow comfort and increase farm profitability by reducing treatment cost and lost milk production.

3.2 Materials and Methods

From January 2013 to April 2014, all dry cows at the Virginia Tech Dairy Center (Blacksburg, Virginia) were enrolled in the study. The composition of cows in this study was, 205 Holstein (95 primiparous, 107 multiparous) and 65 Jersey (32 primiparous, 33 multiparous) with an average dry period of 62 d.

3.2.1 Prepartum Housing

All animals were moved from pasture to a bedded pack area (2,286 m^2) 21 to 27 d prior to expected calving. Cows stayed in this bedded pack area for the entirety of the close-up dry period and were allowed to calve in this location. A single feed bunk (80 ft) was provided for the dry cows and the animals were fed once daily, a ration consisting of corn silage, chopped hay, and dry cow pellets.

3.2.2 Postpartum Housing and Data Collection

Shortly after parturition, cows were moved into a freestall barn containing a fresh cow pen (960 m^2) with 34 to 36 stalls with rubber mattresses. The stalls were deep bedded with sawdust twice weekly. The fresh cows stayed in this section of the freestall barn for 3-4 wk postpartum. Animals were fed a TMR (bunk 75 ft) once daily that consisted of alfalfa hay, corn silage, alfalfa haylage, brewer's grain, and pellets. All cows were milked twice daily at 12-h intervals in a double-eight herringbone-milking parlor. During each milking, yield was determined by the milk meter. Furthermore, an in-line milk analysis system (AfiLab©, S.A.E Afikim, Israel) collected daily fat, protein, and lactose %, the F:P, and electrical conductivity.

A blood sample was taken 3 to 7 d postpartum, using a 1 mL syringe fitted with a 20-gauge needle. A minimum of 0.1 mL of blood was taken via venipuncture of the coccygeal vein. Immediately after the blood was drawn, a β ketone test strip (Precision Xtra®, Abbott Diabetes Care Inc., Alameda, CA) was inserted into a glucose and ketone meter (Precision Xtra®, Abbott Diabetes Care Inc., Alameda, California) and 0.1 mL of whole blood was placed on the strip. The meter gathered the BHBA information in 10 s and displayed a numerical BHBA value that was recorded immediately. The numerical BHBA value was used only for the diagnosis of subclinical ketosis. Cows with a BHBA concentration of ≥ 1.2 mmol/L were considered to have subclinical ketosis.

3.2.3 Activity Monitoring

From 21 d prepartum to 30 d postpartum, daily activity data was collected with a commercially available data logger (Afi PedometerPlus©, S.A.E. Afikim, Israel) that was affixed to a rear leg fetlock. The activity variables collected by the data logger included rest bouts, rest duration, rest time, and average daily steps summed to daily measurements. A rest bout was defined as the number of times the animal changed from a standing to a lying position. A bout was only counted if the animal was lying for a minimum of three minutes. Furthermore, rest time was defined as the amount of time, in minutes; a cow was lying within a 24 h period. Rest duration was the duration, in minutes, of lying time per rest bout. It was calculated by dividing the rest time by the number of rest bouts. Finally, average daily steps was defined as the total step activity for a 24-hr period. This was calculated by averaging the steps per hour between each milking session and multiplying that average by 24.

One entry/exit point was available for cows to get to and from the feed bunk in the dry pen, thus activity was collected at this time. Activity data was stored in the memory of the pedometer and when a cow walked through the feed bunk access, the stored data was transmitted through a reader box to herd management software program (AfiFarm©, S.A.E. Afikim, Israel). Activity data for postpartum cows was collected between milking sessions and stored in the memory of the pedometer. This data was collected through a reader box at each milking and transmitted from the milk parlor to the herd management software program.

3.2.4 Disease Recording

All health events were diagnosed and recorded by veterinarians on health event sheets and the data was transferred from these sheets to PC Dart (DRMS, Ames, Iowa) by the herd managers at the Virginia Tech Dairy Center. Treatment was administered by university veterinarians and herd managers. Displaced abomasum, assisted calving, mastitis, metritis, retained placenta, milk fever, and subclinical ketosis that occurred within the first 30 DIM were the health events of interest in this study. Animals that had more than one disease occurrence within this time were analyzed in a multiple disease category (MULTI).

The diseases of interest were defined as: a displaced abomasum (LDA or RDA) - the abomasum filled with gas and shifted from the normal position to the left or right side of the abdomen; assisted calving - calving required assistance and had a score of ≥ 2 (1=no assistance, 2=slight assistance, 3=average assistance, 4=considerable force, 5=extreme difficulty); mastitis - a visual change in milk appearance (flakes, chunks, or color) in one or more quarters as identified by the milkers; metritis - inflammation of the layers of the uterus with clinical symptoms like decreased rumen contractions and milk production as well as a fever; retained placenta - failure to separate and expel the placenta within 24 h of calving; milk fever – clinical symptoms like weakness, loss of appetite, and flaccid paralysis, within the first few days of lactation; subclinical ketosis (SK) – whole blood BHBA concentration ≥ 1.2 mmol/L in the first 7 DIM. Healthy animals were defined as any animal that did not experience any type of clinical disease or illness within the first 30 DIM.

3.2.5 Data Analyses

Activity and milk component data were extracted from the herd management software program daily and stored in a database (Microsoft Excel 2013for Windows©; Microsoft Corporation, Redmond, Washington). Disease information (type and date diagnosed) was obtained from PC Dart (DRMS, Ames, Iowa) or directly collected in the case of subclinical ketosis and entered into the same database as the activity and milk component data. This data was entered by cow and date of disease diagnosis.

Statistical analyses were performed in SAS v. 9.3 (SAS Institute Inc., Cary, North Carolina) on all diseases with greater than 10 occurrences. A power analysis was conducted to determine the number of disease occurrences needed to achieve the desired significance. Activity and milk component models were created to assess differences between animals with and without disease from d -7 to d 7 relative to day of disease diagnosis. Depending on the DIM at disease diagnosis, some or all of the milk component data for the days before disease were missing (for example, animals who experienced dystocia did not have milk component data for the 7 d prior to diagnosis). The milk from a fresh cow entered the bulk tank on d 3, at which point milk component data was collected. All diseased cows were assigned a control group based on cows in the same group (dry or fresh), parity (1 or ≥ 2), breed (Holstein or Jersey), and date as the diseased cow. Thus, day relative to disease for a control group was based on the same date as the diseased cow to account for day-to-day pen and management changes. In the dystocia model, the disease day (d 0) was considered to be the day of calving.

Variables that were offered into the activity and milk component models included parity (1 or ≥ 2), day relative to disease, and disease status (diseased or control) with associated interactions. For the model assessing animals in the MULTI category, day relative to disease was based on the first disease diagnosed. In addition, the MULTI model included the first disease diagnosed and a covariate the number of days a cow was sick. First diseases considered in the model were milk fever, SK, dystocia, multiple (MUL; more than one disease was diagnosed on the same day), and other (OTH). If an animal's first disease was OTH it was diagnosed with a disease other than milk fever, SK, dystocia, or MUL. To calculate days sick, the date the final disease was diagnosed was subtracted from the date the first disease was diagnosed. PROC GLIMMIX was used to examine the difference between disease and control animals. Data was analyzed for outliers by removing means outside 3 standard deviations from the overall mean of each parameter. However, removal of the outliers had not effect on the p values examined and therefore, all data was left unfiltered. Repeated measures were accounted for in a random statement which included day relative to each disease and the subject of disease status by lactation nested within cow. Finally, slice differences were used to identify significant pairwise comparisons within day. For instance, slice differences of day relative to disease and parity would show day relative for each parity (1 or ≥ 2) allowing differences to be determined between primiparous and multiparous cows. Significance was declared at $P \leq 0.05$.

3.3 Results

Of the original seven diseases of interest, dystocia (n = 16), mastitis (n = 22), subclinical ketosis (n = 42), and a multiple disease category (n = 44) met the criteria for analysis. A summary of breed and parity by disease is shown in Table 3.1. The mean, median, and standard deviations for the day of disease diagnosis are summarized Table 3.2.

3.3.1 Assisted Calving

Day relative to assisted calving was significant for each behavioral measure examined (Figure 3.3.1.A-D). The interaction between day relative assisted calving and parity was significant for activity and rest time. In addition, the interaction between day and disease status was significant for rest bouts, activity, and rest time. On d 0 and 1, cows that experienced assisted calving had fewer rest bouts (6.9 ± 1.5 and 8.8 ± 1.6 bouts, respectively) compared to cows that did not experience any disease within the first 30 DIM (13.6 ± 1.5 and 14.3 ± 1.5 bouts, respectively). Cows that experienced assisted calving showed increased activity on d 0 (5276.7 ± 557.5 steps) compared to controls (3676.3 ± 557.6 steps). Moreover, on d -4, -3, and -2, primiparous cows took fewer steps (3135.2 ± 227.3, 3427.9 ± 224.2, and 3278.9 ± 224.2 steps, respectively) compared to multiparous cows (5470.0 ± 868.3, 5316.0 ± 868.3, and 6396.0 ± 868.3 steps, respectively). For the behavioral measure of rest duration, the d -1 slice showed assisted calving cows had decreased rest duration (38.2 ± 8.9 min/bout) compared to controls (55.1 ± 8.9 min/bout). Furthermore, on d -6, 5, 6, and 7 relative to assisted calving, diseased animals showed increased resting time (486.5 ± 64.8, 627.9 ± 65.3, 622.5 ± 64.8, and 612.6 ± 64.8 min, respectively) compared to non-diseased animals (271.5 ± 65.2, 489.1 ± 65.7, 416.6 ± 65.2, and 449.0 ± 65.2 min, respectively). Finally, the interaction of parity and disease status was significant for rest time (Figure 3.3.1.E). Primiparous animals experiencing assisted calving (n =

15) had a reduced mean rest time (384.8 ± 16.6 min) compared to primiparous controls (438.7 ± 16.9 min). In contrast, diseased multiparous animals (n =1) spent more time resting (565.0 ± 63.8 min) compared to non-diseased multiparous animals (367.7 ± 63.8 min).

3.3.2 Mastitis

The interaction between day relative to mastitis, parity, and disease status was significant for milk lactose % (Figure 3.3.2.A). On d -1, 0, 1, 2, and 4, multiparous cows diagnosed with mastitis had decreased lactose concentration (4.5 ± 0.1, 4.5 ± 0.1, 4.5 ± 0.1, 4.6 ± 0.1, and 4.7 ± 0.1 %, respectively) compared to healthy multiparous cows (5.0 ± 0.1, 4.9 ± 0.1, 4.9 ± 0.1, 4.9 ± 0.1, and 4.9 ± 0.1 %, respectively). Lactose concentration in healthy and diseased primiparous animals did not differ. Additionally, multiparous cows with mastitis had reduced lactose concentration on d -1, 1 and 2 (4.5 ± 0.1, 4.5 ± 0.1, and 4.6 ± 0.1 %, respectively) compared to primiparous animals with mastitis on the same days (4.8 ± 0.1, 4.9 ± 0.1, and 4.9 ± 0.1 %, respectively). The interaction between day and disease status was significant for rest bouts, activity, and daily yield (Figure 3.3.2.B, C and E). On d -4, -3, -2, -1, 0, 2, 3, 4, 5, 6, and 7 relative to diagnosis, cows with mastitis showed fewer rest bouts compared to cows that did not experience a disease. The interaction between parity and disease status was significant for rest bouts (Figure 3.3.2.F). Primiparous animals diagnosed with mastitis showed fewer rest bouts (9.5 ± 1.0 bouts) over the 15 d relative to diagnosis, compared to controls (15.4 ± 1.0 bouts). There was no difference between multiparous animals. In addition on d -4, -2, and -1, cows diagnosed with mastitis showed increased activity (4487.3 ± 323.0, 4399.8 ± 318.1, and 4117.2 ± 318.1 steps, respectively) compared to controls (3297.4 ± 323.0, 3180.2 ± 322.7, and 3189.0 ± 318.1 steps, respectively). The slice for d -6, -5, -4, and -2 showed that mastitic animals spent less time resting (402.8 ± 32.8, 390.9 ± 32.9, 403.6 ± 33.5, and 376.9 ± 32.8 min, respectively) compared to controls (498.7 ± 33.5, 483.7 ± 33.5, 517.8 ± 32.9, and 494.2 ± 32.8 min, respectively) (Figure 3.3.2.D). Finally on d -4, -2, -1, 0, 1, 2, 3, and 7 relative to mastitis diagnosis, diseased cows had decreased milk yield compared to controls.

3.3.3 Subclinical Ketosis

The interaction between day relative subclinical ketosis onset, parity, and disease status was significant for rest bouts (Figure 3.3.3. A). On d -4 and -3, primiparous animals diagnosed with SK showed fewer rest bouts (4.4 ± 2.1 and 11.8 ± 2.1 #/d, respectively) compared to controls (11.7 ± 2.1 and 18.9 ± 2.1 #/d, respectively). Moreover, primiparous animals with SK had more rest bouts (21.4 ± 2.1 #/d) on d 3, compared to controls (15.0 ± 2.1 #/d). First lactation animals with SK had more rest bouts on d -6, 1, 2, 3, and 4 (12.6 ± 2.1, 19.6 ± 2.1, 18.8 ± 2.1, 21.4 ± 2.1, and 20.0 ± 2.1 bouts, respectively) compared to multiparous animals with SK (7.3 ± 0.8, 14.4 ± 0.8, 14.1 ± 0.8, 13.5 ± 0.8, and 14.5 ± 0.8 bouts, respectively). In contrast, primiparous animals with SK showed fewer rest bouts on d -4 (4.4 ± 2.1 bouts) compared to multiparous animals with SK (11.3 ± 0.8 bouts).

The interaction between day and disease status was significant for activity, rest duration, rest time, daily yield, and lactose % (Figure 3.3.3.B-F). Parity was significant for activity, rest duration, and daily yield. The interaction between day relative subclinical ketosis and parity was significant for rest time. On d -5, -4, -3, and -2 relative to diagnosis of SK, diseased animals showed increased activity (4289.0 ± 228.4, 5584.5 ± 228.4, 5115.3 ± 228.4, and 4605.9 ± 232.8 steps, respectively) compared to non-diseased animals (3601.3 ± 234.4, 3447.7 ± 228.4, 3424.0 ± 229.9, and 3499.4 ± 234.4 steps, respectively). In contrast, on d 7 diseased cows had decreased activity (3150.5 ± 228.4 steps) compared to control animals (3745.1 ± 229.9 steps). Moreover, on d -7, -6, -5, -4, and -3 relative to disease diagnosis, animals who experienced SK showed a shorter rest duration (43.2 ± 2.8, 40.6 ± 2.9, 36.4 ± 2.8, 22.7 ± 2.9, and 29.5 ± 2.8 min/bout, respectively) compared to animals that did not experience any disease (64.2 ± 3.0, 65.5 ± 3.0, 54.1 ± 2.9, 48.9 ± 2.8, and 37.0 ± 2.9 min/bout, respectively). Animals diagnosed with SK also spent less time resting (338.3 ± 30.5, 302.4 ± 30.0, 94.1 ± 31.1, and 358.1 ± 30.0 min, respectively) on d -6, -5, -4, and -3, compared to non-diseased animals (431.1 ± 31.7, 417.8 ± 30.4, 419.4 ± 30.4, and 527.7 ± 30.1 min, respectively). Furthermore on d -4, -3, -2, -1, 0, 1, 2, 3, 6, and 7, primiparous animals spent less time resting (209.0 ± 49.0, 373.0 ± 47.3, 376.7 ± 47.3, 367.8 ± 47.3, 425.0 ± 47.3, 452.4 ± 47.3, 451.7 ± 47.3, 446.3 ± 47.3, 449.2 ± 47.3, and 386.0 ± 47.3 min, respectively) compared to multiparous animals (404.6 ± 17.4, 512.8 ± 17.5, 542.1 ± 18.0, 563.7 ± 17.5, 561.0 ± 17.5, 573.3 ± 17.5, 557.9 ± 17.5, 544.6 ± 17.5, 553.1 ± 17.5, 554.9 ± 17.5 min, respectively). The results also showed an effect of parity within the healthy animals. Animals with SK produced less milk on d -3, -2, -1 and 0 (14.5 ± 2.5, 22.5 ± 2.1, 24.1 ± 2.0, and 27.1 ± 1.9 kg, respectively) compared to controls (32.0 ± 2.0, 32.9 ± 1.9, 32.6 ± 1.9, and 32.0 ± 1.9 kg, respectively). Moreover On d 0, 1, 2, 3, 4, 5, 6, and 7, primiparous animals produced less milk compared to multiparous animals. Finally on d -3, -2, -1, 0, 1, and 2, dairy cattle diagnosed with subclinical ketosis had decreased milk lactose concentration (4.4 ± 0.1, 4.6 ± 0.1, 4.8 ± 0.0, 4.8 ± 0.0, 4.8 ± 0.0, and 4.8 ± 0.0 %, respectively) compared to controls (4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, and 4.9 ± 0.0 %, respectively).

The interaction between day relative subclinical ketosis onset, parity, and disease status was significant for F:P (Figure 3.3.3.G). Primiparous animals with SK also showed a greater F:P on d 2, 3, 5, and 6, (1.5 ± 0.1, 1.6 ± 0.1, 1.7 ± 0.1, and 1.6 ± 0.1 F:P, respectively) compared to controls (1.2 ± 0.1, 1.2 ± 0.1, 1.1 ± 0.1, and 1.1 ± 0.1 F:P, respectively). Furthermore, multiparous animals with SK showed a greater F:P on d -1, 1, 2, 3, 4, 5, and 6 (1.4 ± 0.1, 1.5 ±

0.0, 1.5 ± 0.0, 1.6 ± 0.0, 1.5 ± 0.0, 1.5 ± 0.0, and 1.5 ± 0.0 F:P, respectively) compared to controls (1.3 ± 0.0, 1.3 ± 0.0, 1.3 ± 0.0, 1.3 ± 0.0, 1.3 ± 0.0, 1.3 ± 0.0, and 1.3 ± 0.0 F:P, respectively). Finally, primiparous animals with SK showed a greater F:P on d 5 (1.7 ± 0.1 F:P), compared to multiparous animals with SK (1.5 ± 0.0 F:P).

3.3.4 Multiple

The interaction between day relative to first disease diagnosis, the first disease an animal experienced and disease status was significant for rest bouts (Figure 3.3.4.A-B), rest duration (Figure 3.3.5.A-C), and daily yield (Figure 3.3.6.A-E). Animals that experienced SK first showed fewer rest bouts 7 d after diagnosis (11.7 ± 1.2 bouts) compared to animals that did not experience any disease (15.0 ± 1.2 bouts). When an animal’s first disease was dystocia, rest bouts were fewer on d 1, 2, and 4 after the event (8.5 ± 1.6, 10.0 ± 1.6, and 11.2 ± 1.6 bouts, respectively) compared to controls (14.6 ± 1.8, 14.7 ± 1.8, and 15.1 ± 1.2 bouts, respectively). When looking at rest duration for the multiple disease category, when an animal’s first disease occurrence was milk fever, subclinical ketosis, or dystocia there was an interaction between disease status and day for rest duration. On d -5 and 7 animals that had milk fever as the first disease showed increased rest duration (80.9 ± 11.4 and 74.1 ± 11.4 min/bout, respectively) compared to their healthy herdmates (48.7 ± 11.4 and 42.1 ± 11.4 min/bout, respectively). Furthermore, animals with a first disease of SK showed a shorter rest duration on d -7 and -6 (49.2 ± 5.6 and 44.7 ± 5.5 min/bout, respectively) compared to controls (72.4 ± 5.6 and 61.4 ± 5.6 min/bout, respectively) and a longer rest duration on d 2 and 3 (57.6 ± 5.4 and 64.1 ± 5.4 min/bout, respectively) compared to controls (42.4 ± 5.4 and 45.5 ± 5.4 min/bout, respectively). If an animal’s first disease was dystocia shorter rest duration was observed on d -1 (42.9 ± 7.6 min/bout) compared to controls (61.3 ± 8.1 min/bout). Finally for daily yield, cattle diagnosed with milk fever, SK, dystocia, MUL, and OTH as their first disease showed a significant interaction between disease status and day. Animals that first experienced milk fever showed reduced milk yield on d 2, 3, and 5 (4.1 ± 6.5, 11.5 ± 4.8, and 16.4 ± 5.3 kg, respectively) compared to controls (30.4 ± 4.2, 30.1 ± 4.2, and 31.2 ± 4.2 kg, respectively). When SK was diagnosed first, reduced milk yield was shown on d -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, 6, and 7 (13.3 ± 5.6, 12.2 ± 3.4, 19.5 ± 2.9, 19.0 ± 2.6, 22.0 ± 2.6, 24.5 ± 2.6, 25.3 ± 2.6, 25.4 ± 2.6, 25.8 ± 2.6, 22.8 ± 2.6, 22.8 ± 2.7, and 25.1 ± 2.7 kg, respectively) compared to controls (35.5 ± 2.5, 34.6 ± 2.4, 35.4 ± 2.3, 36.3 ± 2.3, 34.0 ± 2.3, 34.6 ± 2.3, 33.2 ± 2.3, 34.5 ± 2.3, 34.1 ± 2.3, 34.2 ± 2.3, 33.9 ± 2.3, and 34.8 ± 2.3 kg, respectively). Moreover, animals that first experienced dystocia also showed reduced milk yield on d 1, 2, 3, 4, 5, and 6 (15.6 ± 5.7, 16.2 ± 3.2, 17.0 ± 3.0, 23.3 ± 2.9, 23.0 ± 2.8, and 25.6 ± 3.0 kg, respectively) compared to controls (32.7 ± 3.0, 35.3 ± 3.1, 34.2 ± 3.0, 35.6 ± 3.0, 33.1 ± 3.0, and 35.4 ± 3.0 kg, respectively). Animals diagnosed with MUL produced less milk on d 1 (7.0 ± 6.5 kg) compared to controls (36.0 ± 3.5 kg). Lastly, animals first diagnosed with OTH showed decreased milk production on d -2, -1, 0, 1, 2, 3, and 4 (18.5 ± 4.6, 16.8 ± 4.6, 18.9 ± 4.3, 21.0 ± 4.6, 22.6 ± 4.6, 22.5 ± 4.6, and 15.9 ± 4.6 kg, respectively) compared to controls (34.1 ± 3.3, 35.0 ± 3.3, 36.2 ± 3.2, 33.9 ± 3.2, 33.5 ± 3.2, 34.4 ± 3.2, and 35.2 ± 3.2 kg, respectively).

The interaction between day and disease status was significant for activity, rest time, lactose %, and F:P (Figure 3.3.7.A-D). Animals in the MULTI category showed increased activity on d -6, -5, -4, -3, and -2 (4139.1 ± 203.5, 4191.6 ± 201.7, 4149.3 ± 201.7, 3949.6 ± 201.7, and 3929.2 ± 201.7 steps, respectively) compared to healthy animals (3462.0 ± 210.1, 3233.1 ± 210.1, 3351.7 ± 208.0, 3284.8 ± 204.0, and 3229.7 ± 204.0 steps, respectively) and reduced activity on d 5, 6, and 7 (2459.3 ± 203.5, 2398.1 ± 201.7, and 2381.9 ± 203.5 steps, respectively) compared to controls (3196.4 ± 208.0, 3271.1 ± 206.0, and 3258.2 ± 206.0 steps, respectively). Furthermore, MULTI animals spent less time resting on d -5, -4, -3, and -2 (343.1 ± 29.2, 342.2 ± 29.3, 350.8 ± 29.0, and 381.9 ± 29.7 min, respectively), compared to controls (441.9 ± 30.8, 440.9 ± 30.8, 434.0 ± 29.7, and 458.0 ± 29.8 min, respectively). Also, the interaction between parity and day was significant for rest time. On d -3, -2, -1, 0, 1, 2, 3, 4, and 5 all primiparous animals showed reduced resting time (325.5 ± 41.3, 359.4 ± 42.3, 377.0 ± 41.3, 369.8 ± 42.4, 342.1 ± 42.4, 392.8 ± 42.4, 427.6 ± 42.4, 414.5 ± 42.4, 443.3 ± 42.4 min, respectively) compared to all multiparous animals (459.3 ± 20.3, 480.5 ± 20.3, 535.0 ± 20.5, 561.8 ± 20.5, 604.8 ± 20.7, 610.3 ± 20.5, 590.6 ± 20.3, 597.0 ± 20.3, and 600.5 ± 20.6 min, respectively). Animals in the MULTI category showed decreased lactose concentration on d -6, -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 5, 6, and 7 (4.4 ± 0.1, 4.6 ± 0.1, 4.5 ± 0.1, 4.1 ± 0.1, 4.5 ± 0.1, 4.6 ± 0.0, 4.6 ± 0.0, 4.7 ± 0.0, 4.8 ± 0.0, 4.8 ± 0.0, 4.8 ± 0.0, 4.8 ± 0.0, 4.8 ± 0.0, and 4.8 ± 0.0 %, respectively) compared to controls (4.6 ± 0.1, 4.9 ± 0.1, 5.0 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, 4.9 ± 0.0, and 4.9 ± 0.0 %, respectively). On d -2, -1, 0, 1, 2, 3, 4, 5, 6, and 7, MULTI cows showed increased F:P (1.4 ± 0.1, 1.4 ± 0.1, 1.4 ± 0.1, 1.5 ± 0.1, 1.5 ± 0.1, 1.5 ± 0.1, 1.6 ± 0.1, 1.6 ± 0.1, 1.5 ± 0.1, and 1.4 ± 0.1 F:P, respectively) compared to controls (1.2 ± 0.1, 1.2 ± 0.1, 1.2 ± 0.1, 1.1 ± 0.1, 1.2 ± 0.1, 1.2 ± 0.1, 1.2 ± 0.1, 1.1 ± 0.1, 1.2 ± 0.1, and 1.2 ± 0.1).

3.4 Discussion

Dystocia is a subjective disease to quantify, thus the use of behavioral measures to identify animals at risk could be a beneficial tool for producers. If these animals at risk can be identified early, producers could provide aid or assistance to help relieve the short and long-term effects of assisted calving. In the current study, animals that experienced assisted calving showed fewer rest bouts (d 0 and 1), increased activity (d 0), and shorter rest duration (d -1) compared to non-diseased animals. These results are in contrast to a previous study (Proudfoot et al. 2009) where the researchers reported an increase in standing bouts the 24 h prior to calving. Standing and rest bouts both are measures of position change and therefore should be interchangeable. The difference in bouts could be due to the method of data collection, where Proudfoot et al. (2009) used video evidence with hourly timestamps to recognize calving and the current study did not identify the exact time of calving. The current method did not take rest bouts that are less than 3 min into account. Furthermore, the pervious study matched controls based on calving time and in the current study controls could or could not be calving at the same time as the animals that experienced assisted calving. Therefore, it is hard to make direct comparisons between the pervious and current study. Although results from the current study showed fewer rest bouts on d 0 and 1, activity was increased on d 0 and rest duration decreased on d -1. No other research has observed changes with these variables in cattle experiencing assisted calving. These behavioral measures indicate that an animal with assisted calving could be experiencing restlessness or discomfort as much as a day before experiencing calving difficulties.

A previous study reported a decrease in lactose concentration on d -1, 0, 1, 2, 3, and 4 around the onset of clinical mastitis but no parity differences were observed (Tholen and Petersson-Wolfe, 2012). In the current study, a reduction in lactose concentration was also found, and the results showed this effect was attributed to a change in multiparous animals on d -1, 0, 1, 2, and 4, compared to controls. Moreover, multiparous cows with mastitis had a reduced lactose concentration on d -1, 1, and 2 compared to primiparous cows with mastitis. This indicates that lactose may be a better indicator of mastitis in multiparous animals in early lactation. The previous study did not see changes within or between lactations and looked at mastitis throughout lactation, as the current study only examined mastitis in early lactation. Furthermore, the current study showed cattle with naturally occurring mastitis displayed fewer rest bouts (d -4, -3, -2, -1, and 0) compared to controls. This is the first time changes in rest bouts with naturally-occurring mastitis has been identified. Moreover, cows with mastitis rested less (d -6, -5, -4, and -2) and showed increased activity (d -4, -2, and -1) compared to controls. Similarly, studies looking at experimentally induced mastitis saw increased standing on the d of inoculation and 2 d after (Fogsgaard et al., 2012) as well as less lying time the day of challenge (Cyples et al., 2012). The previous studies were performed using an experimentally-induced mastitis model and did not take into account time that clinical symptoms were observed. This may be why in the current study changes in behavior can be seen before the previous studies and the differences in experimental design make comparisons difficult. The changes observed in the behavioral measures may be attributed to pain or discomfort of the infected quarter before clinical symptoms arise. This is the first study to show behavioral changes along with milk component fluctuations in cattle with naturally-occurring clinical mastitis.

Subclinical ketosis often goes undetected but it has been shown to have detrimental effects on cattle and thus early identification is important (LeBlanc et al., 2006). Before the onset of SK, primiparous animals showed decreased rest bouts (d -4 and -3) compared to healthy primiparous animals. There was no difference in diseased or non-diseased multiparous animals.

Furthermore, on d -4 diseased primiparous animals had reduced rest bouts and on d -6 increased rest bouts compared to diseased multiparous animals. This data indicates changes in rest bouts before the onset of subclinical ketosis are more specific to primiparous animals. Overall, cattle diagnosed with SK had increased activity on d -5, -4, -3, and -2 compared to controls. Moreover, rest duration was decreased (d -7, -6, -5, -4, and -3) as well as rest time (d -6, -5, -4, and -3) compared to non-diseased animals. The changes observed in activity, rest duration, and rest time before d -3 could be due to normal calving behavior or possibly cows that will experience SK struggle more with the calving process. Since the average DIM SK was diagnosed was 4, d -4 relative to SK diagnosis would have been on average the day of calving. After d -4 the changes in behavior could be due to the cow adjusting to a new environment (fresh pen) or the oncoming SK. When looking at F:P, multiparous animals diagnosis with SK had increased F:P (d -1) compared to non-disease multiparous cattle. This indicated that F:P changes before subclinical ketosis onset could be more specific for multiparous cattle. Elevated F:P is correlated with clinical ketosis (Koeck et al., 2013) and increases the risk of occurrence when looking at historical data (Heuer et al., 1999). Previous research showed a relationship between elevated F:P (>1.4) and ketosis as indicated by blood metabolites, in fresh cows (Sawall and Litherland, 2014). However, specific changes in F:P in the days leading up to ketosis have not been identified. In the current study, lactose % and daily yield was decreased (d -3, -2, -1, and 0) for SK animals compared to controls. These results are consistent with previous research that has identified decreased milk yield in animals diagnosed with clinical ketosis 6 d before onset (Edwards and Tozer, 2004). One reason for the difference in yield could be that the previous study was looking at clinical ketosis instead of SK. It could be that the animals with ketosis previously had SK, which is where the current study identifies the animal explaining the difference in day that reduced yield is observed. Finally, this is the first study to show changes in lactose concentration before the onset of subclinical ketosis. A possible reason animals with SK would be showing reduced lactose concentration is because these animals are adapting poorly to the negative energy balance they experience when entering into lactation.

The current study is the first to look at behavioral and milk component changes in cows that experience multiple diseases within the first 30 DIM. Differences in rest bouts were due to cows that experienced subclinical ketosis or dystocia as their first disease. No differences were found before disease onset, only after. This is in contrast with animals just diagnosed with SK or dystocia. An animal that experienced dystocia only showed increased rest bouts (d 0 and 1), while an animal that experienced multiple diseases displayed fewer rest bouts (d 1, 2, and 4). Furthermore, MULTI cows with the first disease of SK showed decreased rest bouts only on d 7 as compared to primiparous animals that only experienced SK differences in rest bouts prior to disease onset. The differences seen between MULTI animals and those diagnosed with only one disease could be due to increased sickness behavior caused by multiple on coming diseases.

When looking at rest duration the differences between diseased and non-diseased animals were due to animals first diagnosed with milk fever, subclinical ketosis, and dystocia. Cows first diagnosed with milk fever had decreased rest duration on d -5 compared to controls. This data is similar to research on subclinical milk fever which found that diseased animals stood longer than healthy animals the day before calving (Jawor et al., 2012). Since there were no differences in rest bouts for milk fever in this study, a decrease in rest duration indicates longer time standing. The d that an increase in standing is seen could be different between the pervious and current study because the current study was on clinical milk fever and did not take into account calving time only the d of diagnosis. Furthermore, cattle diagnosed with SK as the first disease had decreased rest duration (d -7 and -6) compared to controls, which is consistent with cattle just diagnosed with subclinical ketosis in the current study, who also had decreased rest duration (d -7 -6, -5, -4, and -3). When an animal’s first disease was dystocia it showed decreased rest duration (d -1) compared to controls, which is the same as animals just diagnosed with dystocia in this study. Differences in daily yield could be contributed to the first disease being milk fever, subclinical ketosis, dystocia, MUL, or OTH. Daily yield was effected only after disease diagnosis (d 1 – d7) for animals with milk fever, dystocia, and MUL. If an animal’s first disease was SK it had reduced milk yield (d -4, -3, -2, -1 and 0) compared to non-diseased animals. This is similar to animals just diagnosed with SK which showed decreased yield (d -3, -2, -1 and 0) also. A possible reason for why MULTI animals showed decreased yield on d -4 is because the body is experiencing more stress from multiple diseases as compared to a single disease. An animal experiencing an OTH first disease showed decreased milk yield (d -2, -1, and 0) compared to controls. These animals could be following a similar pattern to animals just diagnosed with mastitis which had decreased yield (d -4, -2, -1 and 0) because the majority of animals in the OTH category were first diagnosed with mastitis.

The behavioral measures of activity and rest time as well as the milk components of lactose concentration and F:P were independent of the first disease diagnosed in animals in the MULTI category. Cows that experienced multiple diseases showed increased activity (d -6, -5, -4, -3, and -2) compared to healthy cows. Also rest time was reduced (d -5, -4, -3, and -2) for animal’s diagnosed with multiple diseases compared to controls. As for milk components, MULTI animals showed reduced lactose concentration (d -6, -5, -4, -3, -2, -1, and 0) as well as reduced F:P (d -5) compared to non-diseased animals. This is the first study to show changes in activity and milk components in cows diagnosed with multiple disease within the first 30 DIM.

Since activity, rest time, lactose concentration, and F:P was not affected by the first disease diagnosed these parameters could be specific to animals that will contract multiple diseases.

Disease	**Incidence by Breed, Parity, and Overall**				
Dystocia					
	Breed	**1**	**2+**	**Total**	**Percentage**
	Holstein	14	1	15	5.6%
	Jersey	1	0	1	0.3%
	Total	15	1	16	5.9%
Subclinical Ketosis					
	Breed	**1**	**2+**	**Total**	**Percentage**
	Holstein	2	24	26	9.6%
	Jersey	3	13	16	5.9%
	Total	5	37	42	15.6%
Mastitis					
	Breed	**1**	**2+**	**Total**	**Percentage**
	Holstein	6	11	17	6.3%
	Jersey	2	3	5	1.9%
	Total	8	14	22	8.1%
Multiple					
	Breed	**1**	**2+**	**Total**	**Percentage**
	Holstein	9	26	35	12.9%
	Jersey	1	8	9	3.3%
	Total	10	34	44	16.3%

Table 3.1 Incidence of naturally occurring peripartum diseases in the Virginia Tech dairy herd by breed and lactation from January 2013 through May 2014. 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	4.4	1.0	4.0
Mastitis	13.0	10.5	10.1
Multiple	3.5	3.0	2.1

Table 3.2 The mean day of diagnosis, standard deviation, and median day of diagnosis relative to calving for naturally occurring peripartum diseases in the Virginia Tech dairy herd from January 2013 through May 2014.

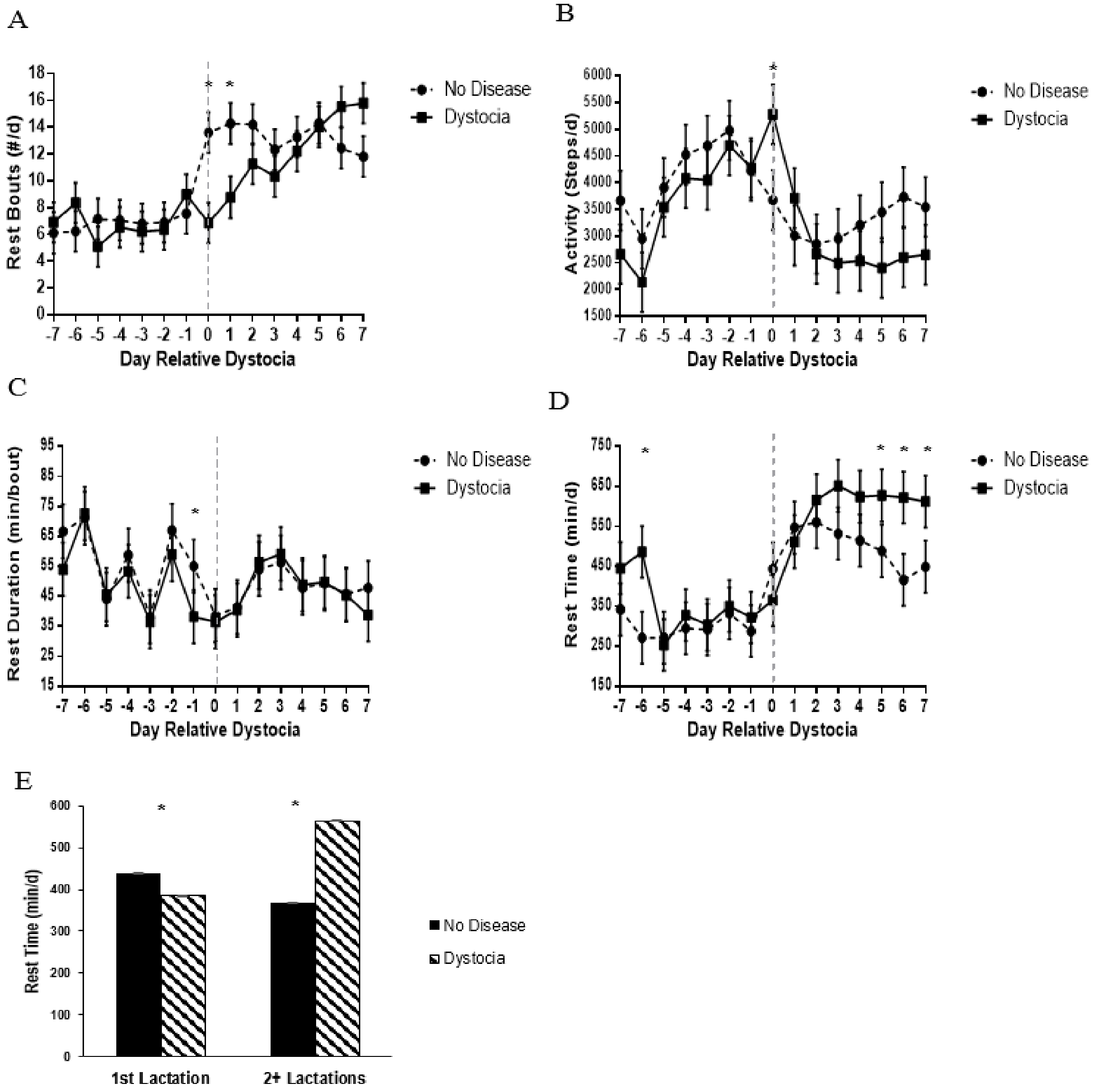

Figure 3.3.1 A comparison of (A) rest bouts (#/d), (B) activity (steps/d), (C) rest duration (min/bout), and (D) rest time (min/d) -7 d prior to and 7 d after the onset of disease between cows that experienced dystocia (——, n = 16) and those who did not experience disease (- - -, n = 1- 10). (*) are indicative of days where the slice was significantly different ($P \leq 0.05$) between diseased and non-diseased animals. A comparison of (F) mean rest time between disease status and parity, from -7 d prior to 7 d after the onset of dystocia. (*) are indicative of differences ($P \leq 0.05$) within lactations between diseased and non-diseased animals.

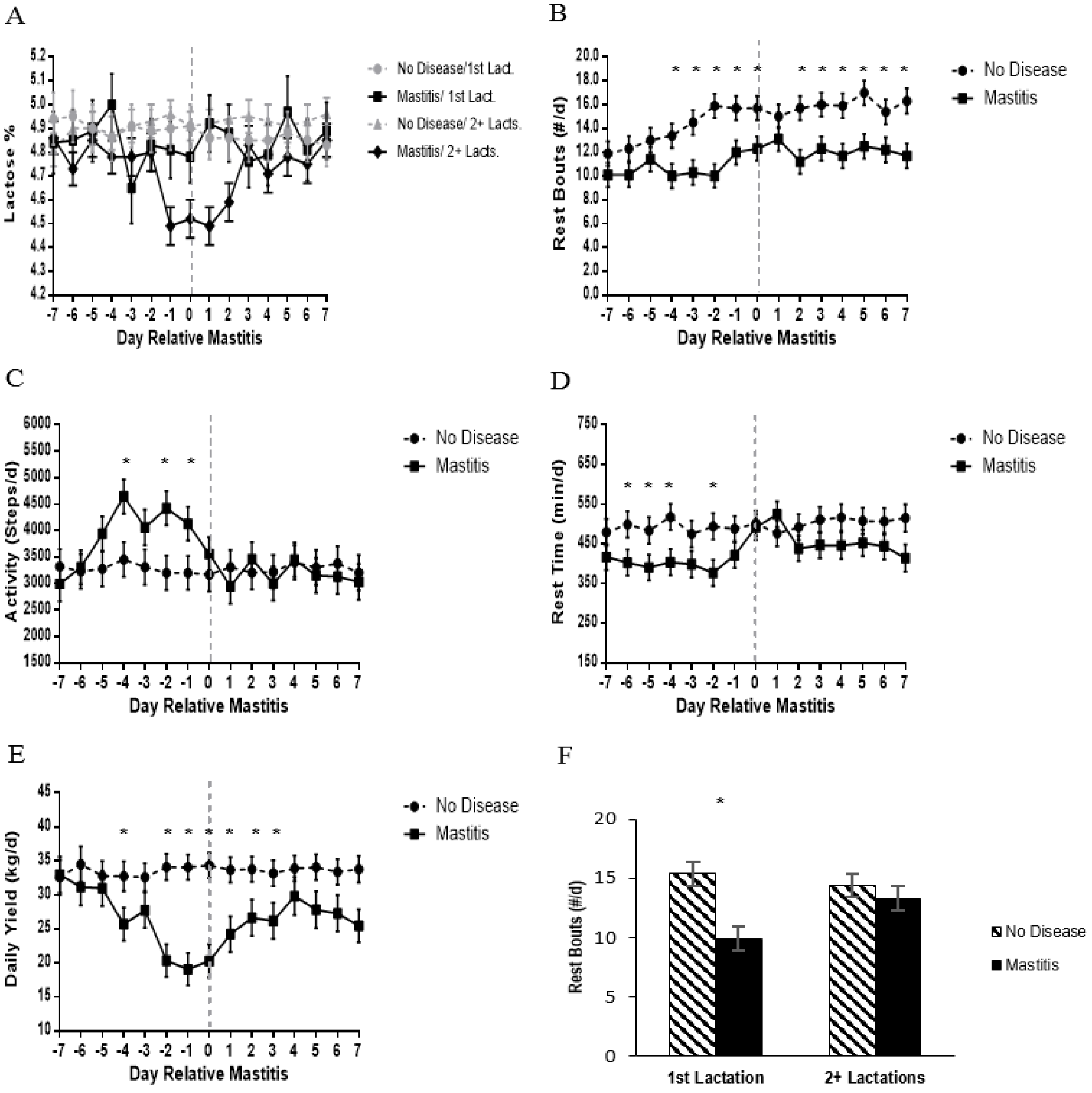

Figure 3.3.2 A comparison of (A) lactose concentration (%) and lactation -7 d prior to 7d after the onset of disease between cows diagnosed with mastitis (——, n = 22) and those who did not experience disease (- - -, n = 1-10). A comparison of (B) rest bouts (#/d), (C) activity (steps/d), (D) rest time (min/d), and (E) daily yield (kg/d) -7 d prior to and 7 d after the onset of disease between cows that experienced mastitis (——, n = 22) and those who did not experience disease (- - -, n = 1- 10). (*) are indicative of days where the slice was significantly different (*P* ≤ 0.05) between diseased and non-diseased animals. A comparison of (F) mean rest bouts between disease status and parity, from -7 d prior to 7 d after the onset of mastitis. (*) are indicative of differences (*P* ≤ 0.05) within lactations between diseased and non-diseased animals.

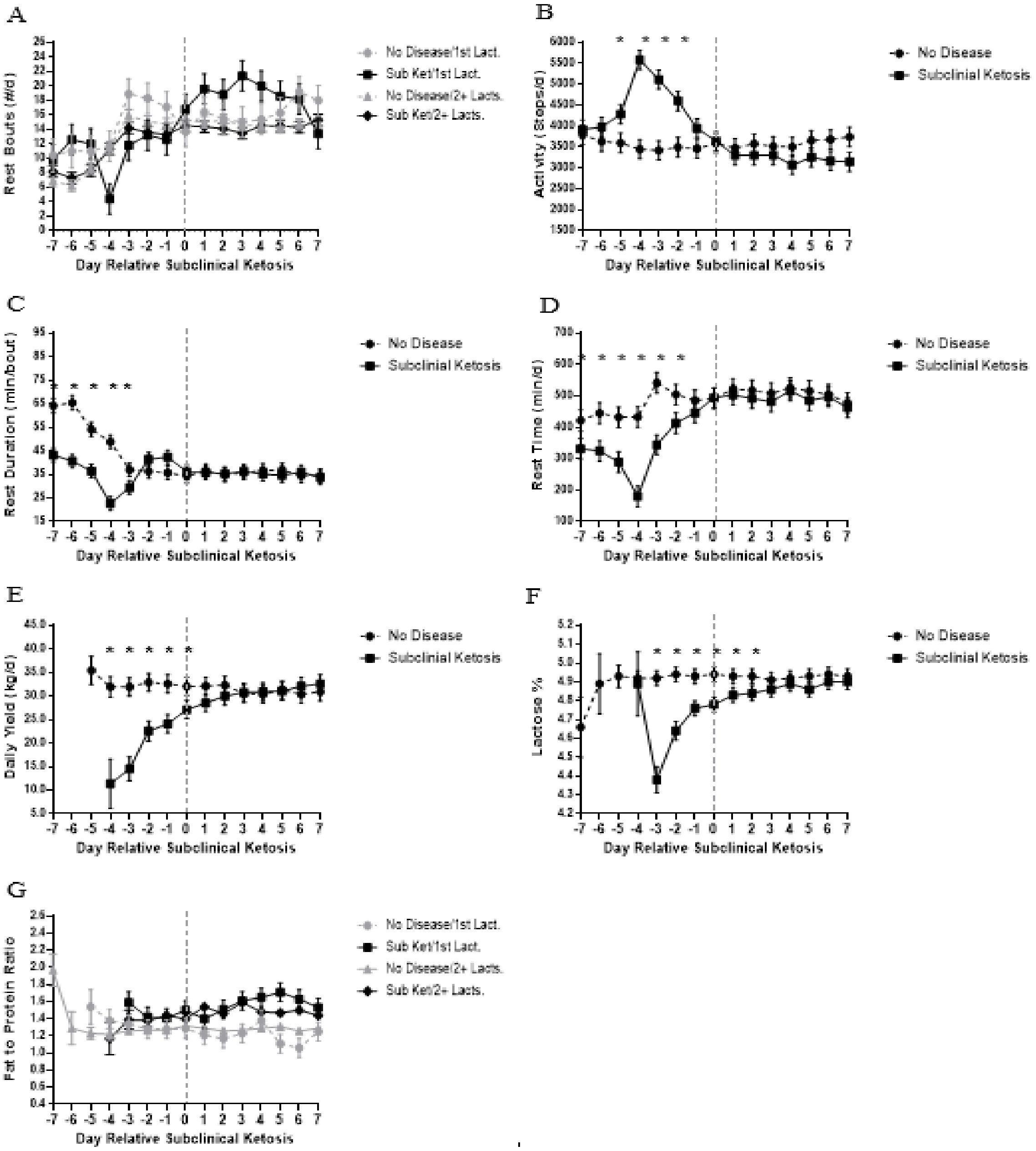

Figure 3.3.3 A comparison of (A) rest bouts (#/d), (B) F:P and lactation, -7 d prior to 7d after the onset of disease between cows diagnosed with subclinical ketosis (——, n = 42) and those who did not experience disease (- - -, n = 1-10). A comparison of (C) activity (steps/d), (D) rest duration (min/bout), (E) rest time (min/d), (F) daily yield (kg/d), and (G) lactose concentration (%) -7 d prior to and 7 d after the onset of disease between cows that experienced subclinical ketosis (——, n = 42) and those who did not experience disease (- - -, n = 1- 10). (*) are indicative of days where the slice was significantly different ($P \leq 0.05$) between diseased and non-diseased animals.

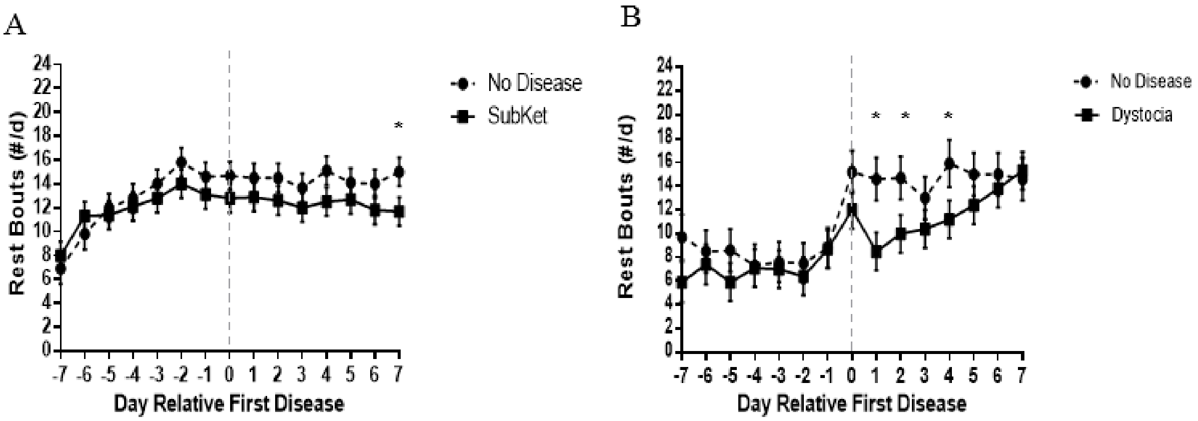

Figure 3.3.4 A comparison of rest bouts (#/d) for the multiple disease category were the first disease was (A) subclinical ketosis or (B) dystocia, from -7 d prior to 7 d after the onset of disease between cows that experienced multiple diseases (——, n = 44) and those who did not experience disease (- - -, n = 1-10). (*) are indicative of days where the slice was significantly different ($P \leq 0.05$) between diseased and non-diseased animals.

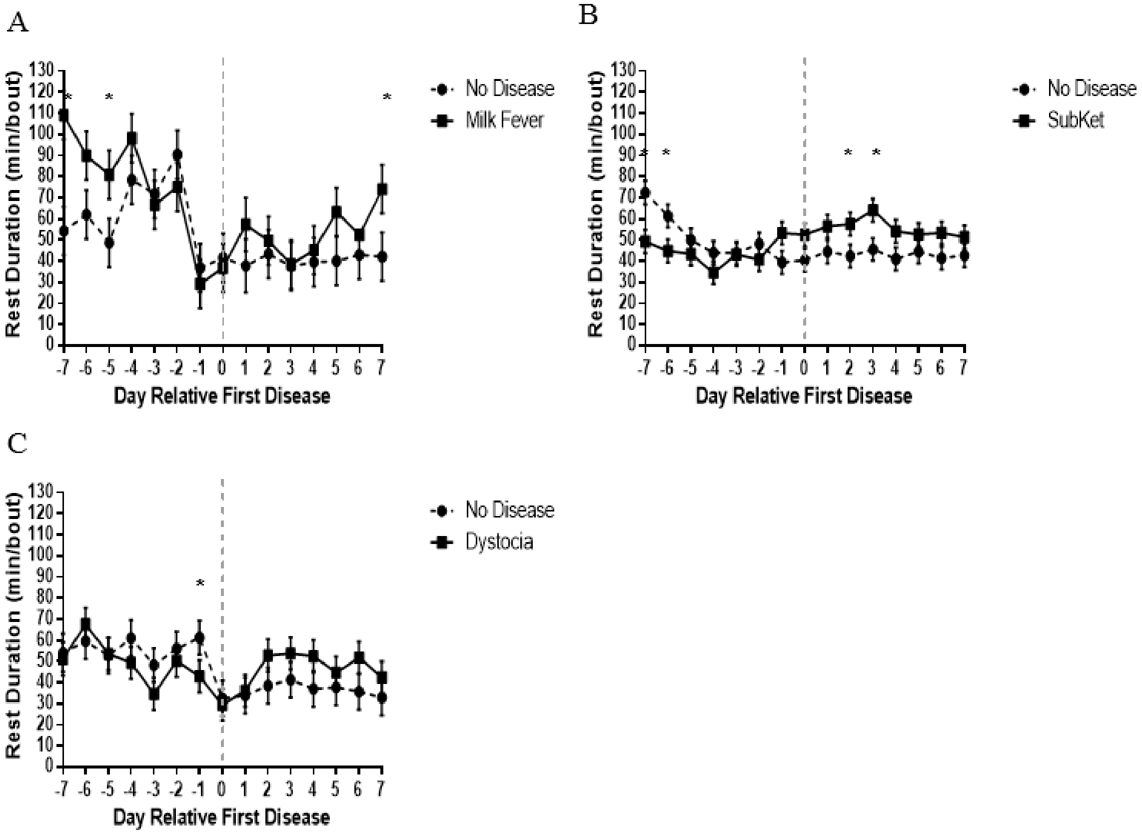

Figure 3.3.5 A comparison of rest duration (min/bout) for the multiple disease category were the first disease was (A) milk fever, (B) subclinical ketosis, and (C) dystocia, from -7 d prior to 7 d after the onset of disease between cows that experienced multiple diseases (——, n = 44) and those who did not experience disease (- - -, n = 1-10). (*) are indicative of days where the slice was significantly different ($P \leq 0.05$) between diseased and non-diseased animals.

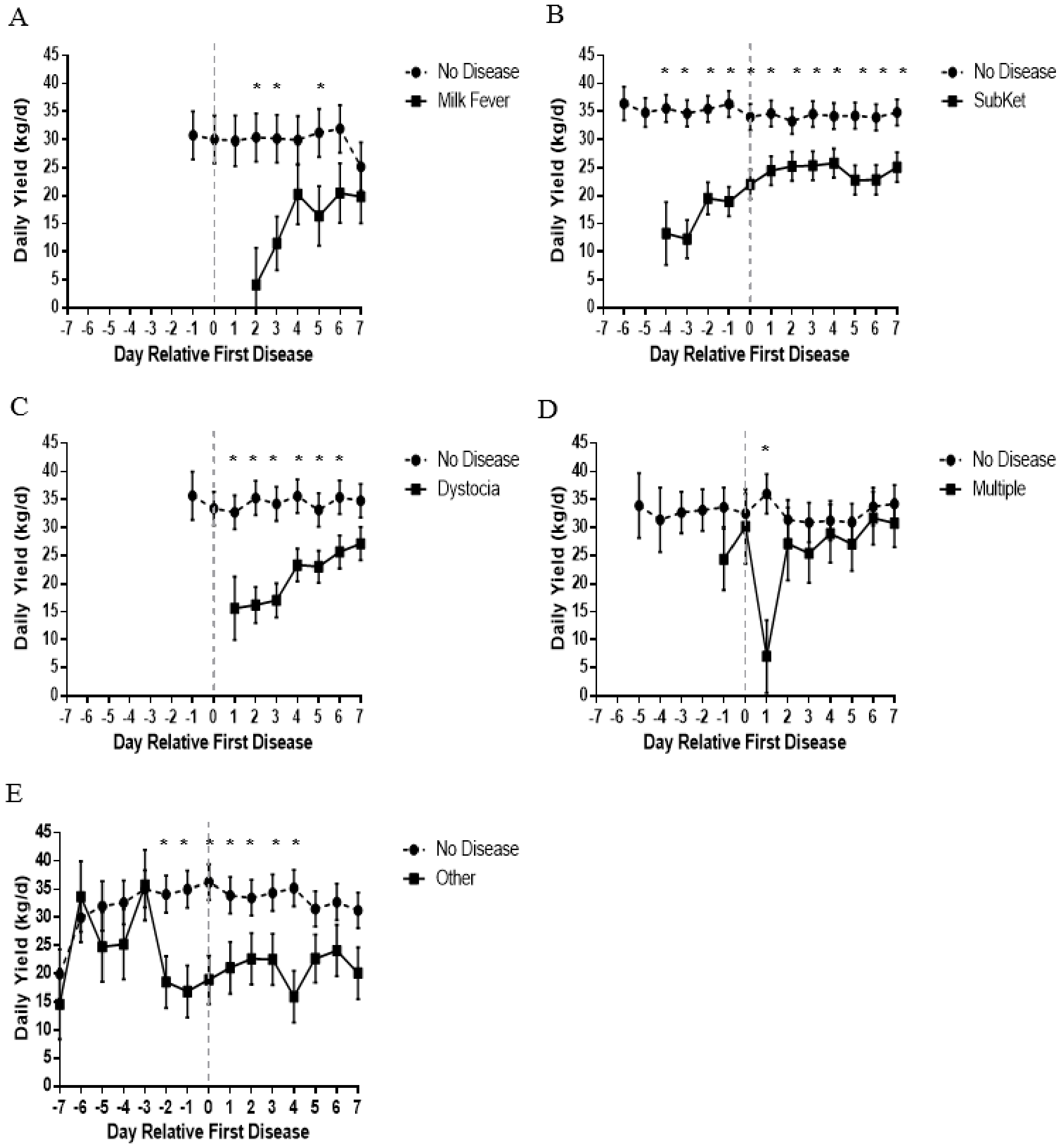

Figure 3.3.6 A comparison of daily yield (kg/d) for the multiple disease category were the first disease was (A) milk fever, (B) subclinical ketosis, (C) dystocia, (D) multiple diseases in one day, and (E) animals diagnosed with a disease other than milk fever, subclinical ketosis, dystocia, or multiple diseases, from -7 d prior to 7 d after the onset of disease between cows that experienced multiple diseases (——, n = 44) and those who did not experience disease (- - -, n = 1-10). (*) are indicative of days where the slice was significantly different ($P \leq 0.05$) between diseased and non-diseased animals.

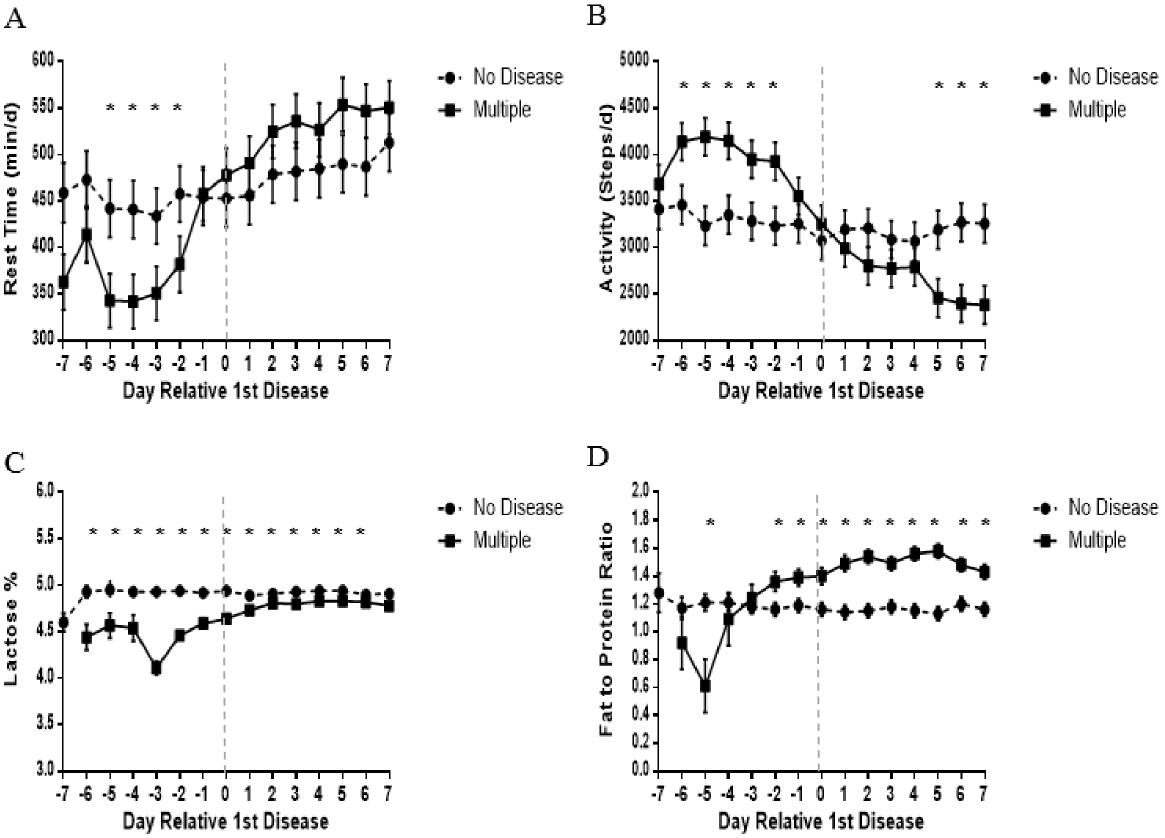

Figure 3.3.7 A comparison of (A) rest time (min/d), (B) activity (steps/d), (C) lactose concentration (%), and (D) F:P, -7 d prior to and 7 d after the onset of disease between cows that experienced multiple diseases (——, n = 44) and those who did not experience disease (- - -, n = 1- 10). (*) are indicative of days where the slice was significantly different (*P* ≤ 0.05) between diseased and non-diseased animals.

CHAPTER 4: GENERAL CONCLUSIONS

Dairy herds are faced with a multitude of diseases that must be managed carefully to maintain a high level of herd health. By managing herd health, dairy producers can ensure higher production of the animals and consequently increase the profitability of their herd. Management of herd health not only increases production and in turn profitability but it also improves animal well-being. The improvement of animal well-being can also improve public perception and acceptance of the dairy industry. There are a variety of management tools to identify and diagnose dairy cattle diseases. Two tools that could improve identification and overall herd health include behavioral measures and milk component data but their potential has not been fully explored.

For this reason, the main objective of this study was to use animal activity and milk component data to determine if specific postpartum diseases can be identified in the subclinical stages. Since the majority of disease occurs in the transition period, this was our focus. Detection of disease is an important aspect of herd management due the economic burden disease implores and the decrease in animal well-being that results from disease. Although clinical disease is fairly easy to identify, the impact of disease in the subclinical stages is harder to identify and quantify. It is accepted that animals change their behavior as well as milk components around disease but the quantification of these changes are needed.

In the current study daily animal activity and milk components were collected throughout the periparturient period. Dairy cows at the Virginia Tech dairy herd were monitored for behavioral measures including rest bouts, rest duration, rest time, and step activity from -21 d to +30 d relative to calving Furthermore, daily milk components including yield, lactose, and F:P were monitored after calving to 30 DIM. All the activity measures were affected by assisted calving but the measures that could be specific to assisted calving are rest bouts, rest duration, and activity. The possibility exists to identify animals that will experience difficult calving as much as a day before calving. When examining animals that will experience mastitis; activity, rest time, and daily yield could have the potential to identify these animals as much as six days before disease onset. Furthermore, rest bouts change specifically in first lactation animals that experience mastitis and lactose concentration changes specifically in multiparous animals. In addition cows that experience subclinical ketosis have distinct changes in activity, rest duration, rest time, and lactose concentration that have the ability to indicate disease as much as 7 days before clinical onset. Moreover, changes in rest bouts in subclinical ketosis animals are more specific to primiparous animals and F:P changes are more specific to multiparous animals. As for animals that will experience multiple diseases within the periparturient period, the behavioral measures of activity and rest time as well as milk components of lactose and F:P could be used to identify these animals starting six days before the first disease id diagnosed.

Changes in behavior and milk components could be used by producers to identify animals at risk for postpartum diseases. Future research could look at combining activity and milk component parameters into algorithms that will alert producers of a specific diseases. By combining multiple parameters higher sensitivity and specificity can be reached. Furthermore, future research could examine percentage changes of activity or milk components that accompany each disease. By looking a percentage changes thresholds could be created to alert producers based on individual cow changes from the individual baseline. Each disease or parameter (activity or milk components) could have thresholds that are specific enough to alert producers of an oncoming disease. If thresholds are reached companies that supply the tools for behavioral and milk component monitoring could incorporate them into their systems to give accurate and quick warnings to dairy producers. Finally, with risk factors in mind producers might be able to pin point the specific disease and apply proactive measures to treat or subdue the oncoming disease.

The proactive management of herd health is a focus of dairy industries worldwide. With the use of animal activity and milk components identification of common dairy diseases can be realized. Through earlier identification, preventive measures can be taken to improve animal well-being and increase farm profitability through reduced treatment cost and increased productivity.

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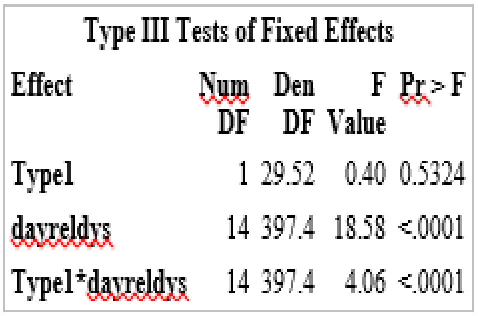
APPENDIX A –FINAL MODELS

Appendix A1. Dystocia

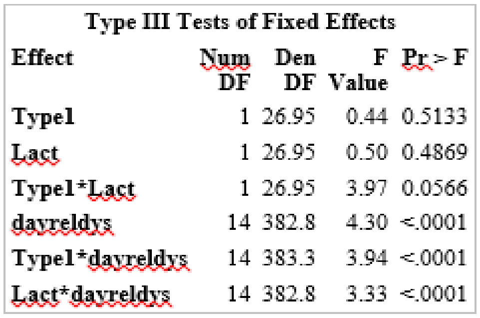
A



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	29.52	0.40	0.5324
dayreldys	14	397.4	18.58	<.0001
Type1*dayreldys	14	397.4	4.06	<.0001

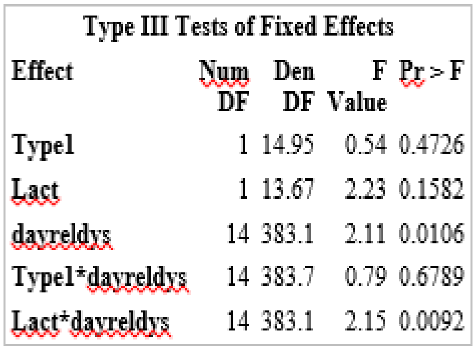
B



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	26.95	0.44	0.5133
Lact	1	26.95	0.50	0.4869
Type1*Lact	1	26.95	3.97	0.0566
dayreldys	14	382.8	4.30	<.0001
Type1*dayreldys	14	383.3	3.94	<.0001
Lact*dayreldys	14	382.8	3.33	<.0001

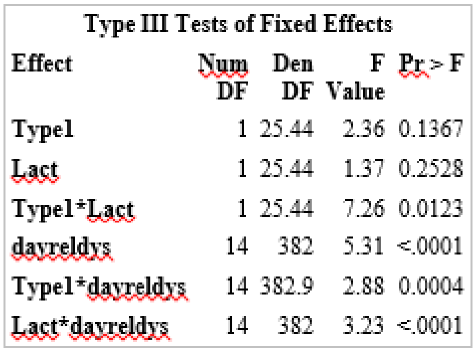
C



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	14.95	0.54	0.4726
Lact	1	13.67	2.23	0.1582
dayreldys	14	383.1	2.11	0.0106
Type1*dayreldys	14	383.7	0.79	0.6789
Lact*dayreldys	14	383.1	2.15	0.0092

D



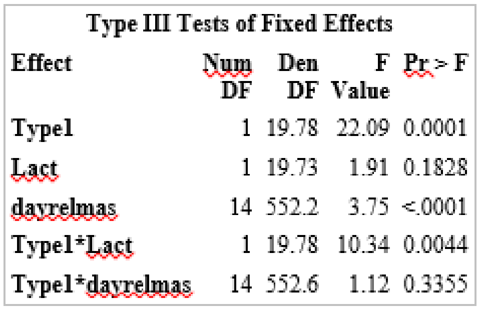
Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	25.44	2.36	0.1367
Lact	1	25.44	1.37	0.2528
Type1*Lact	1	25.44	7.26	0.0123
dayreldys	14	382	5.31	<.0001
Type1*dayreldys	14	382.9	2.88	0.0004
Lact*dayreldys	14	382	3.23	<.0001

Table A.1 Final SAS models for changes in activity and milk components arounf the onset of dystocia, following backwards elimination, for rest bouts (A), activity (B), rest duration (C), and rest time (D).

Appendix A2. Mastitis

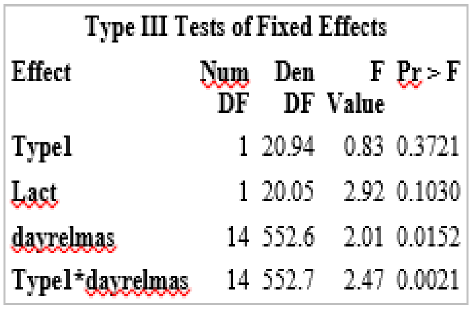
A



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	19.78	22.09	0.0001
Lact	1	19.73	1.91	0.1828
dayrelmas	14	552.2	3.75	<.0001
Type1*Lact	1	19.78	10.34	0.0044
Type1*dayrelmas	14	552.6	1.12	0.3355

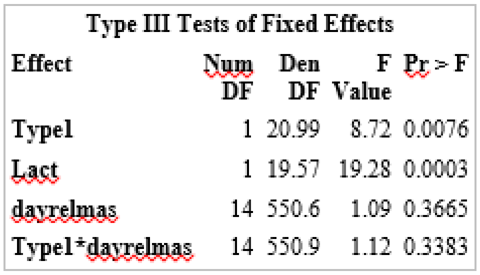
B



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	20.94	0.83	0.3721
Lact	1	20.05	2.92	0.1030
dayrelmas	14	552.6	2.01	0.0152
Type1*dayrelmas	14	552.7	2.47	0.0021

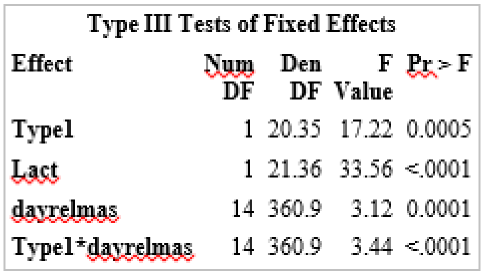
C



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	20.99	8.72	0.0076
Lact	1	19.57	19.28	0.0003
dayrelmas	14	550.6	1.09	0.3665
Type1*dayrelmas	14	550.9	1.12	0.3383

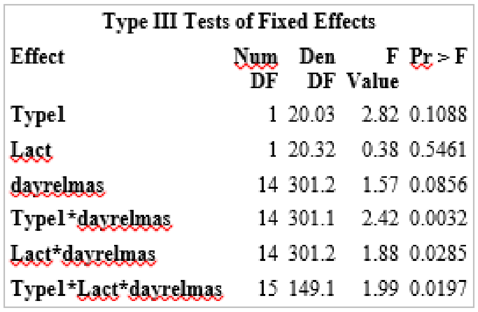
D



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	20.35	17.22	0.0005
Lact	1	21.36	33.56	<.0001
dayrelmas	14	360.9	3.12	0.0001
Type1*dayrelmas	14	360.9	3.44	<.0001

E



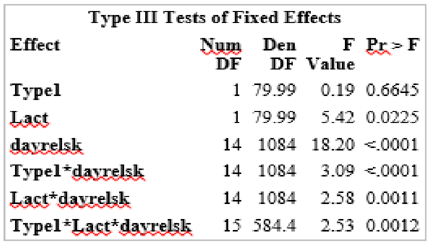
Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	20.03	2.82	0.1088
Lact	1	20.32	0.38	0.5461
dayrelmas	14	301.2	1.57	0.0856
Type1*dayrelmas	14	301.1	2.42	0.0032
Lact*dayrelmas	14	301.2	1.88	0.0285
Type1*Lact*dayrelmas	15	149.1	1.99	0.0197

Table A.2 Final SAS models for changes in activity and milk components around the onset of mastitis, following backwards elimination, for rest bouts (A), activity (B), rest time (C), daily yield (D), and lactose (E).

Appendix A3. Subclinical Ketosis

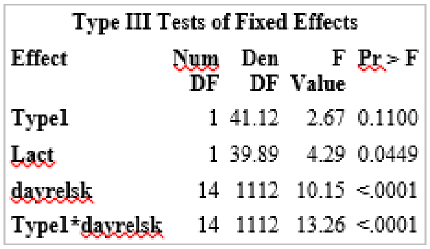
A



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Typel	1	79.99	0.19	0.6645
Lact	1	79.99	5.42	0.0225
dayrelsk	14	1084	18.20	<.0001
Typel*dayrelsk	14	1084	3.09	<.0001
Lact*dayrelsk	14	1084	2.58	0.0011
Typel*Lact*dayrelsk	15	584.4	2.53	0.0012

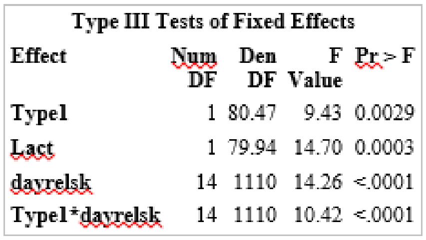
B



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Typel	1	41.12	2.67	0.1100
Lact	1	39.89	4.29	0.0449
dayrelsk	14	1112	10.15	<.0001
Typel*dayrelsk	14	1112	13.26	<.0001

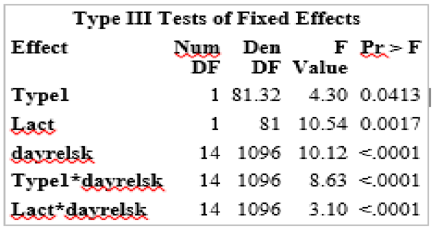
C



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Typel	1	80.47	9.43	0.0029
Lact	1	79.94	14.70	0.0003
dayrelsk	14	1110	14.26	<.0001
Typel*dayrelsk	14	1110	10.42	<.0001

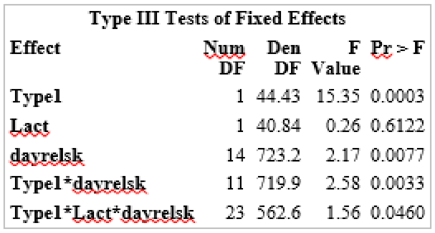
D



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Typel	1	81.32	4.30	0.0413
Lact	1	81	10.54	0.0017
dayrelsk	14	1096	10.12	<.0001
Typel*dayrelsk	14	1096	8.63	<.0001
Lact*dayrelsk	14	1096	3.10	<.0001

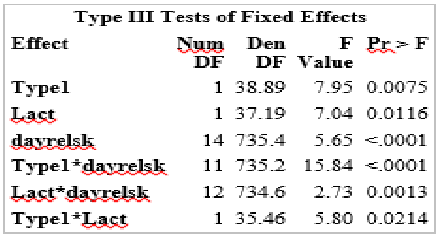
E



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Typel	1	44.43	15.35	0.0003
Lact	1	40.84	0.26	0.6122
dayrelsk	14	723.2	2.17	0.0077
Typel*dayrelsk	11	719.9	2.58	0.0033
Typel*Lact*dayrelsk	23	562.6	1.56	0.0460

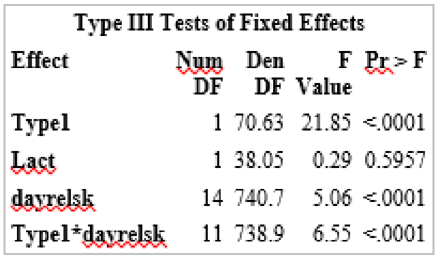
F



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Typel	1	38.89	7.95	0.0075
Lact	1	37.19	7.04	0.0116
dayrelsk	14	735.4	5.65	<.0001
Typel*dayrelsk	11	735.2	15.84	<.0001
Lact*dayrelsk	12	734.6	2.73	0.0013
Typel*Lact	1	35.46	5.80	0.0214

G



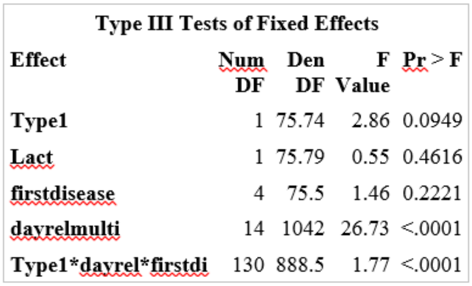
Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Typel	1	70.63	21.85	<.0001
Lact	1	38.05	0.29	0.5957
dayrelsk	14	740.7	5.06	<.0001
Typel*dayrelsk	11	738.9	6.55	<.0001

Table A.3 Final SAS models for changes in activity and milk components around the onset of subclinical ketosis, following backwards elimination, for rest bouts (A), activity (B), rest duration (C), rest time (D), daily yield (E), F:P (F), and lactose (G).

Appendix A4. Multiple Diseases

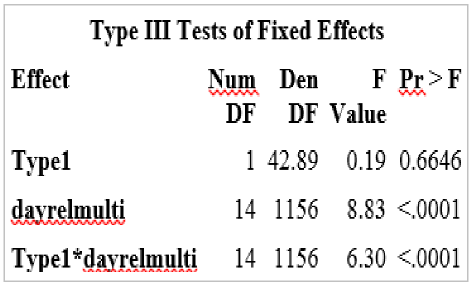
A



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	75.74	2.86	0.0949
Lact	1	75.79	0.55	0.4616
firstdisease	4	75.5	1.46	0.2221
dayrelmulti	14	1042	26.73	<.0001
Type1*dayrel*firstdi	130	888.5	1.77	<.0001

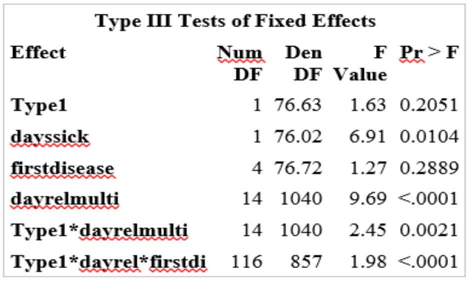
B



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	42.89	0.19	0.6646
dayrelmulti	14	1156	8.83	<.0001
Type1*dayrelmulti	14	1156	6.30	<.0001

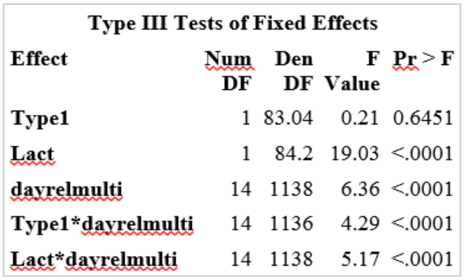
C



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	76.63	1.63	0.2051
dayssick	1	76.02	6.91	0.0104
firstdisease	4	76.72	1.27	0.2889
dayrelmulti	14	1040	9.69	<.0001
Type1*dayrelmulti	14	1040	2.45	0.0021
Type1*dayrel*firstdi	116	857	1.98	<.0001

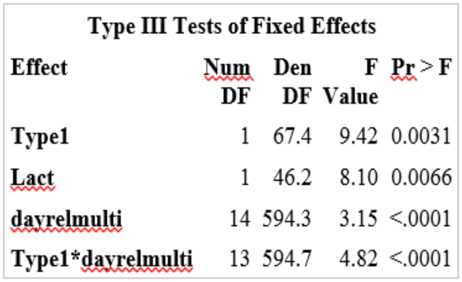
D



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	83.04	0.21	0.6451
Lact	1	84.2	19.03	<.0001
dayrelmulti	14	1138	6.36	<.0001
Type1*dayrelmulti	14	1136	4.29	<.0001
Lact*dayrelmulti	14	1138	5.17	<.0001

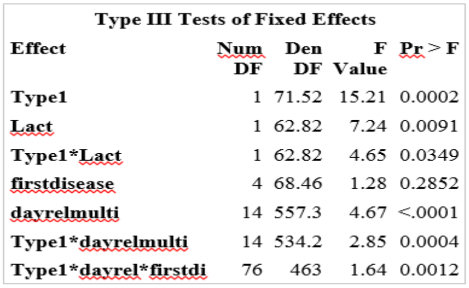
E



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	67.4	9.42	0.0031
Lact	1	46.2	8.10	0.0066
dayrelmulti	14	594.3	3.15	<.0001
Type1*dayrelmulti	13	594.7	4.82	<.0001

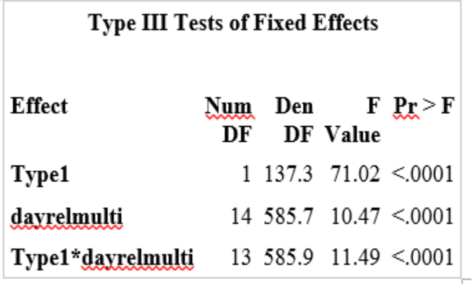
F



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	71.52	15.21	0.0002
Lact	1	62.82	7.24	0.0091
Type1*Lact	1	62.82	4.65	0.0349
firstdisease	4	68.46	1.28	0.2852
dayrelmulti	14	557.3	4.67	<.0001
Type1*dayrelmulti	14	534.2	2.85	0.0004
Type1*dayrel*firstdi	76	463	1.64	0.0012

G



Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Type1	1	137.3	71.02	<.0001
dayrelmulti	14	585.7	10.47	<.0001
Type1*dayrelmulti	13	585.9	11.49	<.0001

Table A.4 Final SAS models for changes in activity and milk components around the onset of the first disease of multiple, following backwards elimination, for rest bouts (A), activity (B), rest duration (C), rest time (D), daily yield (E), F:P (F), and lactose (G).