The Application of Precision Dairy Technologies to Detect Disease in Group Housed Automatically Fed Preweaned Dairy Calves

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3 CHAPTER ONE

3.1 Economic and biologic impact of calf-hood disease

One factor influencing the future of the dairy industry is the ability of producers to successfully raise replacement animals. Therefore, the health of dairy calves during the pre-weaning period is important to dairy producers for the sustained well-being of the dairy enterprise. In the past half century, many advances have been made in how this period is managed but opportunities still exist for improvement. It is well known that calf-hood morbidity is associated with future impaired performance including reduced rate of gain, increased culling risk, increased age at first calving and reduced milk yield (Waltner-Toews et al., 1986; Correa et al., 1988; Virtala et al., 1996; Heinrichs and Heinrichs, 2011). Calf-hood nutrition is also important for future health and performance (Moallem et al., 2010; Soberon and Van Amburgh, 2013). The importance of health and growth aside, dairymen are often reluctant to make changes to calf rearing programs because the returns on the investment have a long time horizon. However, losses incurred from suboptimal calf management can be costly.

Morbidity and mortality incidence remains high during the preweaned period. Diarrhea and respiratory disease are the two most prevalent causes of morbidity and mortality in dairy calves (USDA, 2007). Diarrhea affects 23.9% of preweaned dairy calves, with 56.5% of affected calves dying (USDA, 2007) during the pre-weaning period (defined as 24hrs post birth to weaning), and usually occurs in the first two weeks of life (Curtis et al, 1988; Sivula et al, 1996). Respiratory disease affects approximately 12.4%

of preweaned dairy calves with 22.5% of affected calves dying (USDA, 2007). Respiratory disease usually occurs later during the pre-weaned period (Curtis et al, 1988; Sivula et al, 1996). The economic cost of calf-hood disease is substantial. Economic models that describe the association between calf hood morbidity and future performance are sparse, as changes in input costs are constant and dependent on many factors including size of farm, region, and year. One example, from a simple stochastic model, approximated that a mortality rate of 20% during the first five weeks of life resulted in a 38% net loss in profit for the dairy (Martin and Wiggins, 1973). Management practices that can reduce the risk of and aid in detection of these diseases are valuable to dairy producers.

The monetary value of a newly born heifer calf varies, partly due to the national dairy cow inventory (i.e. calves are worth more when there is a greater need for replacement animals) and market demands, but tends to average right around $200 (USDA NASS, Accessed 7/31/2017). The cost of rearing, and therefore the value of a heifer, increases as a she grows, as substantial inputs (e.g. feed, housing, and labor) are accrued long term. Average age at first calving in the United States is 25 months (USDA 2007) and it is well established that profitability of a heifer is not reached until she has her second calf. Producers also profit from an abundant heifer population, either through internal growth or sales of heifers to other dairies. Therefore, management strategies and tools that aid in the reduction or diagnosis of infectious disease and that help producers to wean healthy animals will ultimately lead to a more sustainable dairy farm.

3.2 Individual housing of dairy calves

One management strategy that producers have used to decrease the risk of infectious disease transmission throughout the pre-weaned period is the use of individual calf housing. Approximately 70% of US dairy calves (USDA, 2014) are housed individually. However, some argue that this calf housing system is one of the most contentious welfare issues in the dairy industry (Rushen et al, 2008) due to reduced opportunity for social contact and limited movement ability. However, this method is associated with a reduction in the of the spread of infectious disease. Calves housed individually have been shown to have a lower incidence of diarrhea (Waltner-Toews et al, 1986), and respiratory disease (Svennson et al, 2003; Svennson and Liberg, 2006) as compared to group housed calves (group size = 6 – 30 calves). Another advantage of this system is that calves housed individually are observed by calf workers multiple times a day, and that recognition of abnormal behavior, anorexia or digestive disorders is timely.

Despite the benefits of infectious disease control, there are some limitations to the individual calf housing strategy. The biggest limitation is the labor necessary to provide milk, water, grain, and bedding to each individual calf several times a day. Kung et al (1997) reported a 10 fold increase in labor needs when group housing and individual housing were compared. One economic model based on the Wisconsin dairy industry in 2007 estimated that the cost of labor represented almost 35% of the fixed and variable costs associated with raising a calf to weaning (Zwald et al, 2007). Another limitation to

individual housing as compared to other management strategies is a reduction in calf performance. Calves housed individually have been reported to have reduced grain intake and reduced growth rates as compared to calves housed in pairs (Costa et al, 2015). These authors found that calves that were pair housed early in life (6 ± 3d) ate more grain and had improved growth during the pre- and post-weaning period compared to calves pair housed later (43 ± 3d) or housed individually. Chua et al (2002) reported that calves housed in pairs did not experience a growth check at weaning, which the individually housed calves in the same study did. Additionally, recent work from Jensen et al (2015) reports that calves housed individually eat less grain and grow more slowly than calves housed in groups. It is still unclear as to why this relationship exists. Hypotheses include that calves housed together learn from each other (social facilitation) or that the higher rates of intake and gain are due to competition for resources.

Another important disadvantage to housing calves individually is the inability of these calves to express their natural social behaviors. The welfare of calves and their housing situation will become important as consumers and the public become more aware of traditional agricultural practices. After 6 weeks of individual housing, calves are less likely to approach an unfamiliar calf, and have higher heart rates when exposed to a novel situation that calves housed in pairs for the same time period (Jensen and Larsen, 2014). These responses, both physiological and behavioral, can be associated with fear (Rushen et al, 1999). Gaillard et al (2014) reported an association between housing and the ability of a calf to learn a task using a combination of initial discrimination learning and reversal

learning. There were no differences between calves in group or individual housing systems in the initial discrimination learning task (time to learn). However, individually housed calves showed slower learning on the reversal task, less able to adapt to a change. The authors hypothesize that this learning deficit could potentially have downstream effects (i.e. the ability to cope with novel experiences and learning later in life), however this hypothesis remains to be evaluated.

3.3 Group housing of dairy calves

For some of these reasons, some producers are beginning to house calves in groups and provide milk automatically through the pre-weaning period. 15% of calves in the United States and 16% of calves in Canada are being housed in a group during the pre-weaning period (USDA, 2014; Medrano-Galarza et al, 2017), mostly due to the labor savings of these systems (Kung et al., 1997). Several management factors heavily influence the success or failure of these systems. Two of the most important are calf space and age at introduction to the group. Recommended minimum space per pre-weaned calf is 34ft² (DCHA Gold Standards; Graves et al, 2008) regardless of housing situation. Smaller group sizes and more space per calf is preferred both for reasons of feeding competition at the teat (Jensen, 2004) and for infectious disease control (Svensson et al, 2003; Jorgensen et al., 2017). Calves are typically introduced to the group feeder anywhere from 1 to 14d of age, mostly due to producer preference and calving pressure. Jensen (2007) reported that a younger age at introduction (6d vs 14d) results in a longer time lag to find the feeder, and more labor necessary to help guide the

calf to her milk meal. Recent work has indicated that calves that experience a morbidity event in the first month of life tend to have drank less milk in the first days of life (de Passielle et al, 2014), indicating that early age at introduction and failing to successfully find the feeder in a timely manner could have detrimental downstream effects.

While group housing provides perceived labor savings for the producer (Kung et al, 1997) and the potential benefit of earlier socialization for the calf (Jensen et al., 1999; Vieira et al., 2012), like individual housing, this management strategy has some important limitations. Housing calves in large groups (> 7 calves per group) can result in higher morbidity and mortality as compared to calves housed individually or in smaller groups. Reasons for this are multi-factorial but most importantly include a younger age at introduction to the group pen and a large group size (Losinger and Heinrichs, 1997; Svensson et al, 2003; Svensson et al, 2006). Automatic feeding systems can represent a high initial capital investment for the producer, both through the purchase of the robotic feeder itself ($13,000 – 20,000) and the cost of retro-fitting existing space or building entirely new calf facilitates, which is common (Jorgensen et al., 2017; Medrano-Galarza et al., 2017). Often, because of these high costs, or because of producer naivety to dealing with these systems, stresses occur (ie large group sizes), often to the detriment of calf health.

3.4 Welfare considerations for the housing of dairy calves.

When producers choose a housing and feeding management strategy their biggest concern is often economic with great consideration for system cost and potential labor savings. However, it is also important to consider other factors such as calf welfare. Fraser (2008) has proposed three general criteria for animal welfare; is the animal functioning well (good health), is the animal feeling well (positive affective states), and is the animal able to live a relatively natural life (natural living). The focus in animal production systems is generally on animal function; asking if the animal is healthy and growing well, reproductively successful and free of injury (Fraser, 2008). More recent focus has been on ensuring that animals experience a positive affective (emotional) state. This is typically categorized into negative (fear, hunger) and positive (play) affect (Neave et al., 2013; Daros et al., 2014). Additionally, and of great importance to the public and the consumer are if animals are able to live a relatively natural life, and are able to express their natural behaviors in production settings (von Keyserlingk et al, 2009). Either individual or group housing strategies could fulfill these requirements for adequate calf welfare.

Compared to group housing, individual housing is associated with better calf health (Losinger and Heinrichs, 1997; Svensson et al., 2003; Svensson and Liberg 2006). The next two general welfare criteria (positive affective states and natural living) are more complicated. Group housing may be the better calf management system for natural living requirements. Social isolation is detrimental to growth, grain consumption (Warnick et al, 1977), and later social ranking (Broom and Leaver, 1978) as compared to group and

individual housing. However, it is not clear when (ie early in life or at weaning) group housing becomes important for the social needs of the calf. There have been no long term prospective studies to look at learning and social dynamic differences in calves housed in groups or individually. Group housing and automatic feeding can provide the calf with a more natural feeding schedule (Berberich and Grimm, 2013). Healthy calves housed in these systems have been shown to visit the feeder for a milk meal an average of 7 times per day (Svensson and Jensen, 2007), which is similar to what has been observed in beef calves paired with their dam on the range (Odde et al, 1985).

Affective state and animal consciousness are new areas of research among production animal welfare scientists. Jensen et al (1998) explored the association between housing type, space allowance and play behavior in pre-weaned dairy calves. Continual video monitoring showed that during weeks 4 and 6 of life, calves housed with reduced space (14.5ft^2) had reduced locomotor play compared to calves with a larger space allowance (58ft^2). Calves housed individually were also less active overall than calves housed in groups of four. This study suggests that sufficient space is necessary for expression of play behavior. However, it still remains unclear if play behavior and increased locomotion is indicative of adequate calf welfare. Jensen and Kyhn (2000) reported that calves housed individually show more locomotor behavior when released into a large arena at 10 weeks of age, so the functionality of locomotor play as an indicator of welfare could be age dependent. It is clear that individual housing is better for calf health. However, group housing may provide more opportunity for the calf to

exhibit her natural behaviors, and provide her with more positive feelings. Therefore, the question of which method is better for the welfare of the calf remains unclear.

3.5 Precision dairy technologies

Another potential advantage to group housing and sophisticated management systems for dairy calves is the availability of automatically captured data for management purposes. Recently, the overwhelming emphasis in the dairy industry has been on promoting health management strategies at the herd level. As dairy farms have grown in size, management has focused on herd and group averages. Precision dairy technologies have the potential to change this approach to management. Precision dairy management (**PDM**) is the use of technologies to measure physiological, behavioral and production indicators on individual animals to improve management strategies and farm performance (Bewley, 2010). Perhaps the simplest and most widely used example of PDM is the use of milk meters in the parlor to measure daily milk yield (Lukas et al., 2009). With this parameter, producers can monitor the production of an individual animal over time, and be alerted when the animal suddenly drops in milk production, indicating dysfunction on the individual animal level. Herd level monitoring is also possible (e.g. average milk production per cow per day).

Automated calf milk feeders are an example of a precision dairy technology that can aid calf management programs. Briefly, an individual calf is uniquely identified by her ear tag when she enters a feeding stall in the group pen. If the calf is entitled (has a

milk meal available) a milk meal (either milk replacer or pasteurized whole milk) is automatically dispensed. These systems are completely customizable by the user. For example, the dairy farmer can determine how much milk is allotted per meal and per day, and how often they want to feed their calves (ie a feeding allotment every two hours). The automation of these systems is one reason that they are attractive to producers, as the robot can be relied upon to feed the calves. As a further advantage, these robots can be programmed to feed more milk, which would require an extra feeding and more labor hours in an individually housed calves. The average calf, if allowed, will drink between 9 and 11L (3 – 4 gal) of milk per day (Jasper and Weary, 2002; Berberich and Grimm, 2013), which would require additional feedings in individual housing.

Sophisticated robotic machines are linked to software programs that can monitor feeding behaviors of individual animals including the number of visits to the feeder per day with and without a milk meal, the speed that the calf drinks her meal (ml/min), and the amount of milk she drinks per day (L/day). The feeder then relays this information to a computer software program that keeps track of each individual calf's running tally for the day, as well as a log of daily averages for the time that the calf is on the feeder. It is advantageous to the producer to be able to monitor individual animals in this way, as the software can alert the producer if the calf has a change in feeding behavior. Current software algorithms in the Förster-Technik® system (Kalb-Manager, Förster-Technik®, Engen, Germany) (provides machines and software for US companies including GEA,

Lely, and DeLaval) base this alert on a 25% deviation in the three day daily average of an individual animal.

Indwelling Rumen Temperature Boluses (**RTB**) are another example of a precision dairy technology specific to an individual animal. Briefly, a temperature sensing bolus is orally administered to the calf soon after birth, and then automatically transmits her temperature to a software program in real time. The producer can then use the software to monitor temperature, without having to take a rectal temperature, which can be difficult or unfeasible in a group housing setting (Bewley et al., 2008). The software then gives the producer an alert when the calf is ± 1.1ºC from her 7d average, thus aiding in the identification of biological dysfunction. While these RTB systems are validated in the adult lactating cow, (Bewley et al, 2008; Timsit et al, 2011; Rose-Dye et al, 2011), there is still a question of whether or not they are useful and economically feasible in the pre-weaned calf.

3.6 Behavior of sick animals

Typically, sick calves are identified by visual observation of trained observers. In individual housing, extra attention is typically paid to a calf that does not finish her milk meal, fails to get up when it is feeding time, or remains standing for longer than normal after drinking. These calves can then be identified and investigated further, for example through a visual calf check list as proposed by McGuirk (2008). In group housing this becomes more difficult, as it is more difficult to find a sick calf in a group of animals

(Steenkamer, 1982). Additionally, it can be more difficult to take rectal temperatures and perform physical exams in a group. It can also be challenging to detect individuals with diarrhea, as they are free to move about the group pen and wet tails are not a sensitive screening tool (Knauer, unpublished data).

The investigation into the behavioral changes that occur in ill animals is an emerging area of work among dairy well-being researchers. Infectious disease increases the circulation of inflammatory cytokines and can induce fever, all of which contribute to the feelings of malaise that accompany morbidity. Anorexia and depression are the two most common behavioral signs of illness. (Hart, 1988) Millman (2007) argues that a group housed animal represents an especially vulnerable population when illness occurs, especially when the animals are a prey species. Questions remain as to what to do with sick animals that are group housed. Removal from the group pen may be appropriate for very young animals, or animals that are very sick so that they can be monitored more closely. Conversely, removal from the group could be socially stressful for a herd animal, and may hinder recovery. Ultimately this depends on factors such as risk of spread to other animals, the disease diagnosed, the level of debilitation of the sick animal, and ease of treatment while in the group.

Several studies have found behavioral changes in morbid calves. After injection with a very low dose of lipopolysaccharide (**LPS**), calves of varying ages (3wks and 20wks of age) showed decreased rumination time and spent a greater amount of time

lying inactive as compared to calves injected with a saline placebo in the 4hrs surrounding peak rectal temperature. However, there was no difference between groups in concentrate or milk intake. (Borderas et al, 2008) Calves experiencing a sickness event (fever, respiratory disease, and/or diarrhea) have a lower probability of approaching a novel object or human as compared to healthy pen mates (Cramer and Stanton, 2015), yet the sensitivity and specificity of using these behaviors as a test for morbidity was poor (Cramer et al., 2016). Finally, a reduction in time at the feed bunk in adult cattle (Sowell et al, 1999; Quimby et al, 2001; Urton et al., 2005) and changes in individual feeding behaviors in calves (Svensson and Jensen, 2007; Borderas et al, 2009) have been shown to be associated with morbidity. The behavioral changes that occur with even very mild disease may be useful in disease prediction and detection.

3.7 Using precision technologies to detect illness

Precision technologies may be useful in the detection and diagnosis of morbidity in dairy calves. Feeding behavior of the calf has been shown to change during a morbidity event (Svensson and Jensen, 2007; Borderas et al, 2009). Svensson and Jensen explored the relationship between a natural illness event and feeding behaviors during the pre-weaning period [rewarded visits (visits with a milk meal), unrewarded visits (visits without a milk meal), drinking speed (ml/min) and total consumption (L/day)]. These authors reported the only behavior that changed in these calves during illness were unrewarded visits to the feeder. The relationship between day of sickness diagnosis and feeding behavior was not explored in this study. Additionally, the group sizes were small

(5-10 calves per group) and the age of introduction was between 10-14 days, both of which do not reflect group pen management in the US (Jorgensen et al., 2017).

Borderas et al (2009) explored the association between feeding level (high vs. low level of milk) and morbidity events on feeding behaviors. Calves that were on a low daily allotment of milk (4L/d) did not change their feeding behaviors during a sickness event. However, calves that were on a higher daily allowance (ad libitum or ≥ 12L/d) had fewer visits to the feeder and drank less per day during a sickness event. This work also indicated that a calf offered a high milk allowance had a change in feeding behavior on the same day that she was detected as being sick by a trained human observer. However, when changes in feeding behavior on the days prior to being detected by a human were explored, there was no difference found between sick and healthy calves. These findings suggest that the feeding behavior measures currently reported by Automatic Feeder software may not be timely in detecting sick calves, as compared to a human observer (in the case of calves fed a high milk allowance) and may not be sensitive in detecting sick calves, as compared to a human observer (in the case of calves fed a restricted milk allowance). However, this study was based on daily and meal averages by group, not on changes in individual animals. With a more sensitive monitoring technique that is specific to an individual calf, subtle changes to individual calf feeding behavior may be more evident.

In addition to feeding behavior, calf body temperature can also be automatically captured. Body temperature frequently increases as part of the immune system's response to bacterial (Godinho et al, 2009; AlZahal et al, 2011) and viral infections (Timsit et al, 2011), and can often be increased before clinical signs of disease are evident, making temperature a sensitive predictor of disease (Rose-Dye et al, 2011). In older cattle, it has been shown to precede clinical signs of respiratory disease by up to four days (Schaefer et al, 2004; Rose-Dye et al, 2011). Therefore, an automated system, such as an indwelling temperature sensing bolus could be useful in calf health management programs. This relationship between rectal temperature and rumino-reticular temperature has not been well described in the literature in dairy calves, but different systems have been shown to have a utility in lactating cattle (Bewley et al, 2008; Timet et al, 2008; Rose-Dye et al, 2011).

3.8 Statistical process control

Monitoring averages and daily changes to detect morbidity in calves has been shown to not be a sensitive technique as compared to trained farm personnel or veterinarians. Therefore, more sensitive and timely monitoring techniques may be useful to monitor the feeding behavior or temperature of an individual animal over time. Statistical Process Control (**SPC**) is an analytical approach, traditionally used in manufacturing process monitoring that utilizes control charts to differentiate common cause from special cause variation in a process over time (Hawkins and Olwell, 1998). Special cause variation in livestock production data can be the result of human error,

technical failures, contamination of feed or water, or health (Mertens et al, 2011). Control charts (i.e. Shewhart, CUSUM) are the major SPC tool that is used to monitor these process changes, combining time series analysis with a graphical presentation of the data. Statistical limits and charting technique influence the sensitivity of SPC to find an out of control state. For example, Shewhart SPC charts target changes > 3 SD away from the process mean. Subtler changes in variation can be observed with CUSUM charts, which are more sensitive (detect shifts < 1.5 SD) (Hawkins and Olwell, 1998). A disadvantage of a more sensitive chart is a greater chance of a false positive alert. False positive alarms can be costly, lead to producer discouragement and/or abandonment of technology, and should be minimized (Steeneveld et al, 2010; Ruttan et al, 2013). SPC can be used to monitor livestock health data at the herd (Lukas et al, 2008b), group (Madsen and Kristensen, 2005), and individual level (Quimby et al, 2001; Lukas et al, 2009).

Traditionally used in manufacturing, SPC techniques have been applied to livestock health data to monitor parameters such as bulk tank somatic cell count, disease prevalence, milk production, and feed intake (Quimby et al 2001; Reneau et al, 2006; Lukas et al, 2008a; Lukas et al, 2008b). For example, Quimby et al (2001) used CUSUM control charts to monitor time at the feed bunk in newly arrived feedlot steers. They found that this monitoring technique provided a more sensitive and timely alert to find an animal with respiratory disease than a trained pen rider with many years of experience. Similarly, Madsen and Kristensen (2005) used CUSUM control charts to monitor weaned piglet health, based on water consumption. Pen level water consumption from piglets

aged 4 to 11 weeks was continuously monitored. In addition to interesting diurnal patterns in drinking behavior, the authors found that an outbreak of diarrhea can be detected using SPC techniques approximately one day prior to disease detection based on clinical signs. This study further supports the hypothesis that SPC can be a useful tool to detect disease in a timely manner, and is an example of this strategy applied at the group level.

3.9 Justification

Increasingly, dairy producers are housing calves in groups and are utilizing automatic feeding systems. There are advantages to this management change, including the reallocation of labor, earlier socialization of the calf as well as the ability to feed more milk more easily. Unfortunately, group housing can also lead to an increased incidence of morbidity and mortality and delays in disease detection. The use of precision dairy technologies, namely automatically captured feeding behavior and body temperature data, may aid producers in earlier disease detection and intervention. However, research to date suggests that changes in feeding behavior, as currently calculated and reported by automatic feeders, are not a timely or sensitive screening tool to detect morbidity in group-housed calves. Similarly, studies are lacking to investigate the utility of using rumen temperature boluses as a method of detecting morbidity in pre-weaned calves. We know that calves change their behaviors when they are sick, and hypothesize that subtle changes in feeding behavior or body temperature, that may occur prior to clinical signs of illness, may be detected through the use of SPC. To our knowledge, SPC has not been

applied in this way previously, and represents a novel approach to the problem of detecting morbidity in group-housed preweaned dairy calves. If this work is successful, it could inform the robotic feeder manufacturers to modify their current algorithms, thus helping producers find sick animals in a more sensitive and timely manner, allowing for earlier diagnosis and intervention and a more successful outcome when treating sick calves.

The overall aim of this thesis research is to understand how individual animal data collected from precision technologies could aid in the prediction and/or detection of disease in group housed automatically fed preweaned dairy calves. If successful, this work could change the way calves are monitored in group housing systems, resulting in a healthier weaned animal. To achieved this, the following objectives were defined.

3.10 Objectives

Objective 1: To describe the diagnostic utility of daily average feeding behaviors to predict and detect morbidity in automatically fed group-housed preweaned dairy calves.

1A. To describe the association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves.

1B. To apply statistical process control techniques to daily average feeding behavior to detect disease in automatically fed group-housed preweaned dairy calves.

Objective 2: To describe the diagnostic utility of visit average feeding behaviors to predict and detect morbidity in automatically fed group-housed preweaned dairy calves.

2A. To describe the association between visit average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves.

2B: To apply statistical process control techniques to visit average feeding behaviors to detect disease in automatically fed group-housed preweaned dairy calves.

Objective 3: To describe the diagnostic utility of an indwelling rumen temperature bolus system to predict and detect morbidity in automatically fed group-housed preweaned dairy calves.

3A: To validate an indwelling rumen temperature bolus system for use in dairy calves.

3B: To describe the utility of an indwelling rumen temperature bolus system to predict and detect disease in automatically fed group-housed preweaned dairy calves.

4 CHAPTER TWO

The association between daily average feeding behaviors and morbidity in automatically fed group-housed pre-weaned dairy calves.

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4.1 SUMMARY

Group housing and computerized feeding of pre-weaned dairy calves is gaining popularity among dairy producers worldwide, yet disease incidence and detection remain a challenge in these systems. The aim of this prospective observational cohort study was to describe the relationship between morbidity and feeding behavior around the period of illness detection. Calves were enrolled upon entrance to the group pen on ten farms in MN (n=4) and Virginia (n=6) utilizing group housing and computerized feeding from February until October 2014. Morbidity and mortality events were recorded by the calf caregiver. Farms were visited either every week (MN) or every other week (VA) to collect calf enrollment data, feeding behavior data, and health records. Daily average feeding behaviors (drinking speed (**DS**) ml/min; daily consumption (**CON**) L/d; rewarded visits (**RV**) to the feeder; and unrewarded visits (**URV**) to the feeder) were described both overall and for sick and healthy calf days. Multivariable mixed models were built to assess the differences in daily average feeding behaviors (DS, CON, RV, URV) between matched sick and healthy calves around the time of an illness event (-10 to +10d). Final models controlled for calf age, region (MN/VA), group size, disease diagnosis, the

random effect of farm, and repeated measurements on calf. A stratified analysis was performed by both day from treatment event and disease diagnosis. One thousand and fifty two calves representing 43,607 calf days were enrolled over 9 months. From these, 176 sick calves had a matched control and were carried forward to the matched pair analysis. Fifty five percent of sick calves (97/176) were treated for diarrhea (**DIA**), 30% (53/176) were treated for pneumonia (**RESP**) and 15% (26/176) were treated for ill thrift (**ILL**). Sick calves drank 183 ± 27 ml/min more slowly, drank 1.2 ± 0.6 L/d less, and had 3.1 ± 0.7 fewer URV than control calves on the first day of treatment. These differences began up to 4 days before the calf was detected as sick, and persisted for 7 to 10 days after treatment. However, changes in feeding behaviors varied by disease diagnosed. Rewarded visits were not associated with morbidity status. The results of this study indicate that sick calves change their feeding behavior before and during an illness event, suggesting that feeding behavior may be a useful tool to detect disease onset.

4.2 INTRODUCTION

Group housing of pre-weaned dairy calves has recently increased in popularity among dairy producers, with an estimated 15% of farms in the U.S. housing calves in groups (USDA NAHMS-Dairy, 2014). Automatic, or computerized feeding, is one method of delivering milk to group-housed calves. This management strategy has the benefits of facilitating the ability to feed more milk per day (Huuskonen and Khalili, 2008; Roth et al, 2008), reallocation of calf labor (Kung et al, 1997), and social benefits to the calf (Jensen et al, 1999; Vieira et al, 2012). However, calves housed in large groups

(≥ 7 calves) had an increased incidence of respiratory disease (Svensson et al, 2003; Svensson and Liberg, 2006) and mortality (Losinger and Heinrichs, 1997) compared to calves housed in small groups. In addition, it can be more difficult to detect diseased calves in group housing systems (Steenkamer, 1982). It has been well established that calf morbidity is associated with future impaired performance including reduced rate of gain, increased culling risk, increased age at first calving and reduced milk yield (Waltner-Toews et al, 1986; Correa et al, 1988; Virtala et al, 1996; Heinrichs and Heinrichs, 2011). Therefore, understanding how sick calves behave in a group is an important first step to determine management strategies and tools that can improve disease detection and intervention.

Regardless of housing strategy, the two most prevalent causes of morbidity and mortality during the pre-weaning period are diarrhea and respiratory disease, accounting for 21.3% and 12% of morbidity and 55.9% and 26.2% of mortality respectively (USDA NAHMS-Dairy, 2014). Infectious and inflammatory processes induce physiological and behavioral changes (Johnson, 2002) including fever, anorexia, lethargy, depression, social isolation, and a reduction in grooming behavior (Hart, 1988), and these adaptive responses have been shown to be important for survival (Kluger and Vaughn, 1978; Murray and Murray, 1979). Infectious disease induction models have been shown to decrease TMR intake in heifers (Steiger et al, 1999) and decrease rumination time, hay intake, self-grooming behavior, and increase the duration of lying behavior in dairy calves (Borderas et al, 2008). Recent work has shown that group-housed calves that have

respiratory disease, a fever, or are recovering from diarrheal disease are half as likely to approach a novel object or human as compared to healthy pen mates (Cramer and Stanton, 2015). These studies indicate that calves change their behavior when ill, and suggest that feeding behavior may be a useful predictor and indicator of disease onset.

One potential advantage of sophisticated computerized milk delivery systems over other (manual) milk delivery systems is that computer software can record and report individual calf feeding behaviors that may be useful for disease monitoring purposes. For example, a calf may be flagged by computer software as being a suspect for illness if it shows a deviation (reduction) in daily milk intake as compared to a rolling average. However, there is reason to believe that current algorithms used by computer feeding software lack in timeliness and/or sensitivity to detect disease events in some calves. For example, Borderas et al (2009) reported that calves fed a high level ($\geq$ 12L/d) of milk drank significantly less milk the day they were detected as sick by an observer as compared to healthy calves, but this behavior change only occurred on the same day as illness detection. Svensson and Jensen (2007) reported that sick calves had a reduction in visits to the feeder without milk (unrewarded), but there was no difference in speed of milk consumption or visits to the feeder with a milk meal (rewarded). That work explored associations between health status and some but not all measureable feeding behaviors (i.e. drinking speed (ml/min), visits to the feeder with a milk meal, visits to the feeder without a milk meal, and total consumption (L/d)). However, these studies had a small sample size and smaller group sizes than are frequently observed in the U.S. (8 to 13 vs.

20 to 25 calves per group). To better understand how sick calves behave in a group pen, it is important to do large observational field studies, to capture a better understanding of both variation and the dynamic nature of these systems. The objective of the current study was to describe the relationship between feeding behaviors and morbidity around the time of an illness event. We hypothesized that calves experiencing a morbidity event would exhibit changes in feeding behaviors on the days leading up to and during the sickness event as compared to a matched healthy calf.

4.3 METHODS

4.3.1 Herd Selection

This prospective observational cohort study was conducted in a convenience sample of 10 commercial dairy herds; 4 herds in Minnesota and 6 herds in Virginia. Herds were selected based on their use of a sophisticated calf feeding system (Forster-Technik®, Engen, Germany) and must have had the system in place for greater than one year. Herds also must have provided a peak daily milk allowance of $\geq$ 7L per day at a total solids level >125g/L (g milk powder added to 1L of water).

4.3.2 Calf Management and Data Collection.

The use of animals in the study was approved by the University of Minnesota Institutional Animal Care and Use Committee #1308-30844A. Data collection occurred from February 2014 to October 2014. An initial questionnaire was used to describe calf facilities and general calf management. Heifer and bull calves were enrolled into the

study when they entered the group pen, and exited the study when they were weaned from the automatic feeder. For each calf entering a group pen, the calf manager recorded the calf id, breed, gender, birth date and pen entry date. Sick calves were identified based on daily subjective evaluations by the calf manager, and the date, time, treatment, and disease treated was recorded for each morbidity event. Mortality events were recorded similarly. We attempted to standardize case definitions across farms through training and use of a visual scoring system that evaluates ocular and nasal discharge, cough, head tilt, fecal score, and general attitude (McGuirk, 2008).

A study technician visited the farm each week (MN) or biweekly (VA), to collect calf enrollment data, treatment records and mortality data. An 8ml venous blood sample was collected from the jugular vein of a convenience sample of calves between 24hr to 7d of age for serum total protein measurement (g/dL) with a digital serum refractometer (MISCO Palm Abbe Model PA203X, MISCO, Cleveland, OH). Approximate birth (d1-d7 of life) and weaning (d50-d60 of life) weights were estimated in kg from a convenience sample of calves using a weight tape (Nasco, Fort Atkinson, WI). Day-level average feeding behavior data was collected through the automatic feeding software program (Kalb Manager, Förster-Technik®, Engen, Germany) each week. Specific feeding behaviors recorded included: total milk intake (**CON**) (L/d), average drinking speed (**DS**) (mL/min), and total number of rewarded (**RV**) (visits to the feeder with a milk meal) and unrewarded (**URV**) (visits to the feeder without a milk meal) visits to the feeder. Farm personnel were blinded to sensor-derived feeding behavior data throughout

the study, beyond what was already being reported and used for animal monitoring by the on-farm software. The Kalb Manager software provides drinking speed, daily consumption, and deviation data to the producer. The majority of calf caregivers used milk consumption (n=6) to screen calves, but none used the data from Kalb Manager as the sole trigger for calf diagnosis or treatment.

4.3.3 Statistical Analysis

Sample Size. 1052 calves were enrolled on 10 farms in MN and VA. Sixty three percent of calves had a first treatment event, resulting in 660 treated calves and 392 healthy calves respectively. This sample size (>250 calves per group) provided in excess of 80% power and 95% confidence to detect a 1L difference in daily milk intake (i.e. 8L/d vs. 7L/d) between sick and healthy calves. (Assumed a SD=4L/d, one tailed test).

Case Definitions. Disease diagnosis was based on visual assessment by the on-farm calf caregiver and attempts were made to standardize across farms based on use of a modified scoring system as defined by McGuirk (2008). A case of diarrhea (**DIA**) was defined as visible diarrhea (very loose or watery feces; fecal score of 2 or 3 on a 0 to 3 scale) and treatment with antibiotics, electrolytes, or IV fluids, or a combination of the three. A case of respiratory (**RESP**) disease was defined as a calf with an increased respiratory rate or effort, cough, and treatment with antibiotics. A case of ill thrift (**ILL**) was defined as either 1) a calf that had a rectal temperature > 39.5˚C; 2) a calf that was depressed but for which the caregiver did not have a clear diagnosis or; 3), a calf with

other miscellaneous illnesses such as umbilical infection, joint infection and injury and treatment with antibiotics and/or non-steroidal anti-inflammatories. Treatment events known to be prophylactic (i.e. antibiotic administered at pen entry) which occurred on one farm only were excluded from analysis. Duration of treatment was defined as the period between the first and last treatment. A new treatment event was defined as; an event that occurred for the first time or; an event that occurred at least 5 d after conclusion of treatment for a previous event or; an event that occurred within 5 d of the conclusion of a previous event but represented a separate disease diagnosis.

Producer-Reported Treatment Validation. To address concerns over the use of producer reported treatment data to represent true disease diagnosis, a validation study was performed on four MN farms in August, 2015. All farms were visited once and a trained veterinarian walked all pens and identified calves to be screened for illness detection using the modified calf health scoring system (McGuirk, 2008). This list was then compared to the calves that the herdsman had identified to screen or treat on the same day. The veterinarian was the reference standard. A positive test was a calf that was screened or treated on the test day. A negative test was a calf that was in the group pen but was not identified as needing an intervention. Diagnostic test characteristics were calculated from a 2 x 2 table (Dohoo et al, 2009).

Descriptive Statistics. All statistical analyses and modeling were performed in SAS (v.9.4 SAS Institute, Cary, NC). Calves already in the pen when the study began,

calves with fewer than 10 days of feeder data (excluding mortalities), and calves that had substantial missing data (> 10d in the pen) were excluded from analysis. Descriptive statistics (mean, median, SD, range) were generated to describe general calf management overall, and to describe for calves overall; I) General calf characteristics (e.g. age at entry and exit from pen, group size, days in pen) II) Calf health data according to farm personnel (e.g. disease treated, days treated, days of age at treatment, days in pen at treatment, number of treatments, proportion treated by disease and treatment event) and III) Feeding behavior data (e.g. DS, CON, URV, RV) both overall, by feeding period (ramp up, (**RU**) phase where calves are gradually increased in milk; hold, (**HOLD**) phase where calves are held at a peak allowance; and ramp down, (**RD**) where the daily milk allowance is reduced until calves are completely weaned from the auto-feeder), and for sick (treated) and healthy calf (not treated) days. Histograms for outcome variables were generated to check for normality. Correlations between linear predictors of interest were assessed to check for collinearly.

Matched Pair analysis. A matched pair design was used to determine the difference in feeding behaviors (DS, CON, RV, URV) between sick and healthy calves. Feeding behaviors during the entire milk fed period were evaluated, excluding the weaned period. Cases were defined as a calf that had a first treatment event during the time that they were in the group pen. Control calves were matched by age (+/- 7 days), pen, breed, and sex, and were defined as a calf that did not have a treatment event from birth until weaning. The first day of sickness diagnosis (by farm personnel) was

designated as day "0", and the 10 days before and after diagnosis were matched to the age-matched healthy control. A 10 day window (-10d to + 10d) around the treatment event was chosen as the period of interest based on work by Quimby (2001) and Schaeffer (2004) reporting that behavioral signs of illness can occur 4 to 7 days before clinical signs are noticed. Case and control calves were matched on a 1:1 ratio. Treated calves that did not have a pen and age matched untreated calf available to serve as the control were excluded from this analysis. Only first treatment events were considered in the analysis.

Univariable analyses were first performed to determine the relationship between feeding behavior (DS, CON, RV, URV; outcome variables) and the occurrence of a sickness event (treated; Y/N), as well as additional predictors of interest including region (MN/VA), farm, machine, pen, month of year, group size, age at pen entry, disease status before pen entry (treated before entrance to the group pen; Y/N), disease diagnosis (DIA, RESP, ILL), days in pen at disease diagnosis, calf breed, day relative to diagnosis of illness (-10 to +10), feeding period (RU, HOLD, RD), and stocking density (m^2 per calf). Predictors that were significant at the $P < 0.20$ level in the univariable analysis were carried forward to offer to the multivariable analysis.

Multivariable generalized linear regression was used to describe the difference in each feeding behavior (DS, CON, RV, and URV; outcome variables) for matched healthy or sick calves (predictor) on the days before and during illness (-10 to +10). All other calf

days in the group pen were excluded from analysis. Predictors with $P < 0.20$ in the univariable models were initially offered to the full main effects model. A backwards stepwise variable selection was used, with the least-significant variables being removed one by one until all predictors remaining had $P < 0.05$. Farm was controlled for as a random effect, repeated measures by calf were accounted for. Final models were chosen based on lowest Akaike's Information Criteria. Interactions between biologically plausible main effects were explored. The effect of time was explored by evaluation of an interaction term that described the association between day relative to diagnosis of illness and feeding behavior (DS, CON, RV, and URV). Models were then stratified by both day from illness (-10 to +10) and disease status (DIA, RESP, ILL) to explore the association between feeding behaviors and day from illness detection for different diseases treated. Final significance was determined at $P < 0.05$.

4.4 RESULTS

4.4.1 General Farm and Feeder Management Description

Ten farms were enrolled in the study, four in MN and six in VA. Dairies had between 110 and 850 lactating dairy cows, and one enrolled farm was a custom heifer grower. All automatic calf feeders were Forster-Technik® machines representing six Lely calf feeders (Lely Calm n=3; Lely Calm Combi n=3) (Lely North America, Pella, IA), and four DeLaval calf feeders (DeLaval CF1000) (DeLaval, Tumba, Sweden). Four farms had two feeders with four pens of calves, and six farms had one feeder and two pens of calves. Each pen of calves had one nipple feeding station. All farms managed

calves in individual housing prior to introduction to the feeder, and introduced calves to the group pen between 1 and 12 days of age. The majority of farms managed their calf pens as dynamic groups (n=8) with two farms practicing all in/all out management. Pens were bedded with straw (n=2), a combination of straw and sawdust (n=6), wood chips (n=1), or corn stalks and sand (n=1). Estimated average space per calf per pen across all farms was 7.4 ± 3.3 m^2.

Two farms fed pasteurized whole milk with balancer (Land O'Lakes Milk Balancer, Land O'Lakes Animal Milk Products, Shoreview, MN) and the remaining farms fed milk replacer only (Blueprint 22-20 Dx, Form-A-Feed Inc. Stewart, MN; Cow's Match ColdFront®/WarmFront®, Land O'Lakes Animal Milk Products, Shoreview, MN; Maxi Balance® Plus BVT BM, Purina Animal Nutrition, Shoreview, MN; 20/22WPL BOV-MOS DFB, Renaissance Nutrition, Roaring Spring, PA). The average ramp up period lasted 9.7 days (range: 3 – 18), calves were held at their maximum feeding level for 29.9 days (range: 18 – 41) and calves were ramped down and weaned over an average of 13 days (range: 2 – 42). Average full feeding level offered was 9.4 L/d (range: 7 – 16), fed at a total solids concentration of 155 g/L (range: 143 – 165).

4.4.2 General Calf Level Description

1304 calves were enrolled between February 2 and October 16, 2014. Of these, 153 had greater than 10d of missing data, and 99 had less than 10d on the feeder, and

were therefore excluded, leaving 1052 calves (n=987 heifers; n=65 bulls) to consider for analysis representing 43,607 calf days on the automatic milk feeder. From the 1052 eligible calves, 176 pairs (n=352) of calves were carried forward to the matched pair analysis, representing 5,984 calf days.

General calf level descriptive statistics are reported as mean ± SD. For all enrolled calves (n=1052) the age at calf entry to the pen was 9.1 ± 5 d with a group size of 17 ± 5 calves per group. Not all calves had a weaning date reported (n=656), but those that did were weaned from milk at 55.4 ± 8.2 days of age. Of the calves sampled, enrolled calves had an average serum total protein of 5.7 ± 0.8 g/dL, a birth weight of 42.2 ± 4.7 kg and a weight at weaning of 79.0 ± 11.3 kg. Calves in this study gained an average of 0.72 kg/day. Matched pair calves were lighter than the total population at both birth and weaning, had a lower ADG, and were approximately similar between treated and control calves (Table 1).

4.4.3 Morbidity and Mortality

Of the 1052 enrolled calves, 63% (660/1052) experienced a first treatment event, 22% (232/1052) a second treatment event, and 7% (77/1052) were treated for a third time while in the group pen. Treatments were distributed evenly among days of the week (Sunday through Saturday). The most common first treatments were for diarrheal disease (50%; 331/660), respiratory disease (19%; 127/660), fever (21%; 137/660) and ill thrift (8%; 54/660). First treatments occurred at an average ± SD of 9.3 ± 8.5 days after

introduction to the group pen, and calves diagnosed with illness were treated an average of 3.8 ± 3.9 days duration. One hundred and fifty-one enrolled calves were treated before they entered the group pen during the period in individual pens or hutches. The mortality rate of enrolled calves during the group housing period was 1.14%.

Of the 176 sick calves in the matched pair analysis, 55% (97/176) were treated for diarrheal disease, 30% (53/176) were treated for respiratory disease, and 15% (26/176) were treated for ill thrift including calves with a fever. Treatment for scours occurred at 7 ± 5.1 days in the group pen and lasted for 3.1 ± 3.7 days. Treatment for respiratory disease occurred at 14 ± 10 days in the group pen and lasted for 2.6 ± 3.3 days, and treatment for ill-thrift occurred at 12 ± 10 days in the group pen and lasted for 3.2 ± 2 days.

4.4.4 Validation of using Producer-Identified Treatment Events

Two hundred and thirty five calves were evaluated on four MN farms. As compared to an experienced veterinarian (reference standard), the overall sensitivity, specificity and accuracy of using the producer to determine the health status of a calf (sick or healthy) in a pen of group-housed pre-weaned calves was 26%, 97%, and 84% respectively. The kappa statistic was 0.31, indicating fair agreement.

4.4.5 Feeding Behaviors for All Calves

Feeding behaviors (DS, CON, RV, and URV) were analyzed for 1,052 calves representing 43,607 calf days on the automatic feeder during the milk fed period. Over the entire feeding period, all calves in this study drank milk at an average (± SD) of 877 ± 344 ml/min, averaged 6.6 ± 2.2L of milk consumed per day, visited the feeder an average of 4.3 ± 2.9 times with a milk meal, and visited the feeder an average of 7.2 ± 7.7 times without a milk meal. All calves in this study increased their daily average DS and URV over the course of the feeding period. Daily average CON and RV increased from the ramp up to the hold period, and then decreased during the ramp down period. On the days they were treated, sick calves (n=3,230 calf days) had a numerically lower daily average drinking speed as compared to healthy calves (n=40,377 calf days) as well as fewer URV per day. Daily average CON and RV were not numerically different (Table 2).

4.4.6 Matched Pair Analysis

Drinking Speed. The final mixed model describing the association between DS and disease status over the 10 days surrounding a treatment event controlled for the effect of region, day from treatment event, month, calf age, diagnosis, the random effect of farm, and repeated measurements by calf. Over the 20 days surrounding a treatment event, sick calves drank an average of 88 ± 20 ml/min ($P < 0.001$) more slowly than matched healthy control calves. On the day of illness detection by a calf caregiver, sick calves drank an average of 183 ± 27 ml/min ($P < 0.001$) more slowly than matched healthy control calves. When considering all diseases, results of the day stratified model

showed a significant decrease in drinking speed two days before a treatment event, which persisted through the end of ten days (Figure 1). However, after stratifying by disease type, it was noted that calves treated for RESP only drank significantly more slowly than their healthy controls on the day of illness detection. In contrast, calves treated for DIA drank significantly more slowly from day -3 to day +10. ILL calves drank more slowly on the day of treatment, and this difference persisted until day +6. (Figure 2)

Unrewarded Visits. During the time surrounding a treatment event, treated calves had 2.3 ± 0.4 fewer ($P < 0.001$) URV to the automatic feeder after controlling for the effect of calf age, group size, month, pair diagnosis, the random effect of farm and repeated measurements by calf. When considering all diseases, results of the day stratified model showed a significant difference in URV between treated and healthy calves on days -10, -9, -6 and then from day -4 through the end of ten days post treatment (Figure 3). On the day of treatment, sick calves had 3.1 ± 0.7 ($P < 0.001$) fewer unrewarded visits to the feeder than matched healthy calves. However, after stratifying by disease type calves treated for RESP only showed a significant difference on the day of treatment, ILL calves differed on day -6, -3 and then from -1 day to +1 day, and calves with DIA had significantly fewer visits to the feeder without a milk meal from day -2 until the end of the observation period. (Results not shown)

Milk Consumption. Over the 20 days surrounding a treatment event, treated calves drank an average of 0.6 ± 0.1L ($P < 0.001$) less milk per day than healthy calves,

after controlling for the effect of calf age, group size, diagnosis, month, repeated measurements on calf and the random effect of farm. When considering all diseases, the results of the daily stratified model showed that sick calves drank significantly less milk day 7, 5 and 2 days before a treatment event, and this effect persisted for 10 days after treatment (Figure 4). On the day of illness detection, sick calves drank 1.2 ± 0.2 L/d ($P <$ 0.0001) less than matched healthy calves. Similarly, after stratifying by disease type, calves treated for DIA drank significantly less milk 8 and 2 d before a treatment event which persisted through the end of the observation period. However, calves treated for RESP or ILL did not drink significantly different volumes than their matched healthy controls. (Results not shown)

Rewarded Visits. Sick calves visited the feeder for a milk meal 0.2 ± 0.1 fewer times per day than a matched healthy calf over the 20 days surrounding a treatment event after controlling for the effect of calf age, group size, diagnosis, month and region, and this was not statistically different ($P = 0.12$). Stratification by disease revealed identical results.

4.5 DISCUSSION

Increased morbidity and delays in disease detection are significant challenges for dairy producers who house calves in groups during the pre-weaning period. Current software algorithms used by automated computer feeder systems may not be timely and/or sensitive in helping producers to detect sick calves. Management strategies and

tools that improve screening and detection of disease are of great importance for the welfare of the calf and the sustainability of these housing and calf feeding systems. This prospective observational cohort is the largest field study to date to investigate the association between morbidity and automatically captured feeding behaviors in dairy calves, and gives insight into feeding behavior parameters that will be useful to investigate further as potential predictors and/or indicators of disease in group-housed pre-weaned dairy calves.

Calf Management

Little work to date has been focused on describing the management of computer feeders, and these systems can vary greatly based on software settings and producer and technician preference. The average feeding plan in this study included a 10d background period where calves were manually fed their milk, then, upon entrance to the group pen, an average ramp up of 10d, a hold period of 30d, and a ramp down of 13d, with average milk offered during the hold period of 9.4L/d. This is a high level of milk intake, as other studies have reported that the average calf that is allowed ad libitum milk intake will drink between 9 and 11L/d on average (Jasper and Weary, 2002; Bierbich and Grimm, 2013). This also represents a longer period of total average milk intake (63d), which is becoming more common as producers understand the benefits of feeding milk to calves for a longer time period.

Individual Calf Characteristics

Calves in this study were managed in individual hutches prior to entrance to the group pen at 9.1 ± 5 d of age. This is later than the 5.1 ± 3.9 d reported for calf facilities in the upper Midwest in the United States (Jorgensen, PhD Thesis, University of Minnesota, 2016) but within the range of 1-2 weeks described in Sweden (Svensson et al, 2003). Therefore our results may not be generalizable to calves that are introduced to the group pen very early as calves that are introduced early in life have been shown to need more time and assistance to adapt to the feeder (Jensen, 2007), which may affect feeding behaviors. On average, calves in this study gained 0.72kg/d, which is comparable to the performance reported in other studies feeding comparable amounts of milk (Appleby et al, 2001; Jasper et al, 2002), and which is sufficient for the doubling of birth weight by 60d of life, as is recommended by the industry (Soberon and Van Amburgh, 2013). Treated calves had reduced growth compared to control calves in the matched pair analysis, which is expected for calves experiencing a morbidity event during the preweaning period (Virtala et al, 1996).

Calf Health

Calves housed in large groups (≥ 7 calves per group) are at an increased risk for morbidity (Svensson and Liberg, 2006). In this study, 63% of calves had a first treatment event, similar to what has been reported in the literature for other group housing studies, with reported disease incidence ranging from 49 to 78% (Svensson and Jensen, 2007; Roth et al, 2009). Diarrheal disease remains the most common cause of pre-weaning morbidity, and represented 50% of cases treated in this study. Diarrhea incidence

reported in the literature in group-housed calves ranges from 19 to 96% (Svensson and Liberg, 2006; Borderas et al, 2009). The incidence of respiratory disease in this study was 18%, within the range of previously reported incidences of 4 to 31% (Svensson and Liberg, 2006; Svensson and Jenson, 2007). Of the calves that entered the group feeding pens, 1.14% died on our 10 study farms. A 16 month longitudinal study of Midwestern calf operations utilizing automatic calf feeders found a higher mortality at 3.85% per year (Jorgensen, PhD Thesis, University of Minnesota, 2016). Our study also represents a lower mortality rate than has been previously reported in the U.S. (USDA-NAHMS-Dairy, 2014), but it is difficult to compare across studies because of seasonal, regional, and housing differences. We also only selected farms for enrollment if they kept good treatment records, so we may have introduced a selection bias as herdsmen on our farms may have been more skilled (than average) at finding and treating sick animals in a timely manner.

Feeding Behaviors

Individual calf feeding behaviors are influenced by many factors, and these influences lead to the large variation that was seen in daily average feeding behaviors in this study. The automatic feeder itself could mechanically influence drinking speed through hose diameter and/or the size of the nipple opening or nipple type. Though unreported, descriptive analysis of DS by farm supports this hypothesis though there are many other variables that could influence this difference. We accounted for this by controlling for the random effect of farm in our models. Both rewarded and unrewarded

visits to the feeder can be influenced by meal size, total volume offered, and day in the group pen (Jensen, 2009). Total milk offered can also affect feeding behaviors, as limit fed calves have been shown to visit the feeder 40% more than calves fed an ad libitum amount (Bierbich and Grimm, 2013). Other management factors can also influence feeding behaviors. For example, large group size can cause an increase in URV as calf displacements increase as a function of calf competition for resources (Jensen, 2004). Additionally, mixing calves has been shown to affect CON and total visits to the feeder (O'Driscoll et al, 2006), which could affect these parameters in dynamic pens. Calf level factors can also influence feeding behaviors, including breed and calf age (Jensen and Holm, 2003). These factors, in combination, contribute to the large variation that we saw in this study in daily average individual calf feeding behaviors. The large numeric difference we observed in daily average DS between sick and healthy calf days has not been described in the literature. Svensson and Jensen (2007) found a non-significant difference in drinking speed between sick and health calves, however these calves were housed in smaller groups (range: 5-10 per pen) than the calves in the present study. The same study found no difference between RV in sick and healthy calves, suggesting that calves will continue to visit the feeder when they are ill as was observed in this study.

Feeding Behaviors and Disease

The results of the matched pair analysis show that calves have reduced daily average DS, URV, and CON starting as early as four days before and during a producer identified illness event, but these changes varied by feeding behavior and disease

diagnosed. Rewarded visits were not found to be associated with morbidity, suggesting that calves will continue to visit the feeder during an illness event, though they may drink more slowly, drink smaller volumes, and they will not be motivated to visit the feeder more frequently than they have to. Calves change their behavior during an illness event. Cramer and Stanton (2015) showed that calves were less likely to rise when approached by a human when ill. This reluctance to get up could be regulated by infectious and inflammatory mediators, and could explain the decrease in URV to the feeder observed by this study and others (Jensen and Holm, 2003; Svensson and Jensen 2007; Borderas et al, 2009). In our study, calves had significantly fewer URV to the feeder up to four days before a treatment event, though the stratified analysis suggests that sick calves had numerically fewer visits than healthy calves over the entire observation period (-10 to +10d). This could be explained by the large proportion of calves with diarrhea, that these calves may be different from healthy calves upon entrance to the group pen, or that a decrease in URV is a stronger indicator of disease than has previously been thought. Drinking speed has not been evaluated previously in a matched pair design in the literature, despite producers' reported use of this parameter as a screening tool to find sick calves in the field. Our work shows that sick calves change their drinking speed up to three days before a diarrheal episode, indicating its utility as a potential indicator of disease onset.

It is possible that differences in disease pathophysiology cause calves to behave differently. This is important to note, as there may be some diseases (i.e. diarrheal

disease) that are more easily identified by a change in daily average feeding behaviors than others (i.e. respiratory disease). In this study, diarrheal disease was consistently associated with a change in CON, DS, and URV up to 3 days before treatment. This same pattern was not observed for calves treated for either RESP or ILL. Though the objective of this study was not to investigate recovery from disease, it is interesting to note that changes in feeding behaviors persisted from between 7 and 10 days after disease diagnosis. There are several possibilities for this observation. First, it could simply take calves a significant amount of time to recover from illness. Second, treatments may not be delivered in a timely fashion or are inappropriate for the diagnosis, though review of treatment records does not indicate this. Third, treatment dosage, administration, or duration may not be appropriate to support a rapid recovery by the animal. These questions require further investigation.

Now that we have a better understanding of which feeding behaviors are more likely to change in advance of a disease event, studies are needed to investigate the utility of using feeding behavior to predict, detect, and treat disease. Maatje et al (1993) examined DS, CON, RV and URV and deviations from average to identify sick calves and found DS to have the highest sensitivity (46%) to screen a sick calf, and that when combinations of behaviors were explored that sensitivity increased to 77%. However, this work only used a mean deviation percent, which does not take variation into consideration. The results of the present study suggest that CON, DS, and URV are

promising early indicators, and may help predict or detect disease in these systems. Future work is needed to investigate these hypotheses.

4.6 CONCLUSIONS

The results of this prospective observational cohort study indicate that sick calves change their feeding behavior during the time leading up to and during an illness event. In a matched pair analysis, treated calves drank more slowly, drank smaller volumes, and visited the feeder fewer times without a milk meal (unrewarded visits) up to four days before they were identified as sick by a herdsman, and this difference persisted for up to 10 days after diagnosis and treatment. However, these behavioral changes differed by disease; compared to health control calves, calves with DIA had the earliest and most consistent changes in feeding behaviors, followed by ILL calves, and finally RESP calves. More work is needed to investigate the utility of using feeding behavior to identify, diagnose, and treat calves in a group-housed setting.

Table 1. Descriptive calf level statistics of all enrolled calves, both overall and by matched pair analysis.

	All Enrolled Calves (n=1052)		Matched Pair Calves (n=352)			
Variable			Treated Calves		Control Calves	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Age at Pen Entry	1052	9.1 ± 5	176	9.8 ± 4.4	176	9.9 ± 4.4
Wean Age	656	55.4 ± 8.2	115	57.2 ± 14.5	126	53.8 ± 6.9
Serum Total Protein (g/dL)	367	5.7 ± 0.8	63	5.6 ± 0.9	71	5.7 ± 0.8
Birth Weight (kg)[1]	182	42.2 ± 4.7	35	41.6 ± 5.0	32	42.1 ± 3.7
Wean Weight (kg)[2]	72	79.0 ± 11.3	8	78.1 ± 8.5	16	81.8 ± 11.0
ADG (kg/d)		0.72		0.72		0.76

[1] Birth Weight: defined as a weight tape measurement taken from 1-7 days of life.
[2]Wean Weight: defined as a weight tape measurement taken from 50 – 60 days of life.

Table 2. Daily average feeding behaviors, both overall and for sick and healthy days for calves housed in groups and fed automatically. Reported are mean ± SD.

Variable[1]	Healthy Calf Days[2] n=40,377	Sick Calf Days[3] n=3,230	Feeding Stage[4]	n	All Calf Days
DS (ml/min)	896 ± 337	645 ± 345	RU	5,904	680 ± 310
			HOLD	29,620	859 ± 318
			RD	8,083	1089 ± 350
CON (L/d)	6.7 ± 2.2	6.2 ± 2.5	RU	5,904	5.2 ± 2.0
			HOLD	29,620	7.4 ± 1.9
			RD	8,083	4.8 ± 2.1
RV (Count)	4.3 ± 3.0	4.8 ± 3.1	RU	5,904	4.4 ± 3.8
			HOLD	29,620	4.7 ± 2.8
			RD	8,083	3.0 ± 2.6
URV (Count)	7.5 ± 7.7	4.3 ± 6.2	RU	5,904	4.6 ± 6.9
			HOLD	29,620	6.3 ± 6.7
			RD	8,083	12.7 ± 9.0

[1] DS = Drinking Speed (ml/min); CON = Daily milk consumption (L/d); RV= Rewarded visits to the milk feeder; URV=Unrewarded visits to the feeder.
[2] Healthy Calf Days = days in the group pen when the calf was not being treated for illness
[3]Sick Calf Days = days in the group pen when the calf was being actively treated for illness
[4]RU = Ramp up on milk feeding level immediately after entrance to the group pen; HOLD = the maximum milk feeding level where the calf is held for 2 to 3 weeks; RD = ramp down on milk feeding level immediately prior to weaning.

Figure 1. Results of a stratified mixed model explaining the relationship between morbidity (all diseases) and daily average drinking speed (ml/min) for matched pairs of sick (case; n=176) and healthy (control; n=176) calves. This model controlled for the effect of region, month, calf age, and disease treated and controlled for the random effect of farm. Reported are adjusted means and standard error bars. (* = $P < 0.05$)

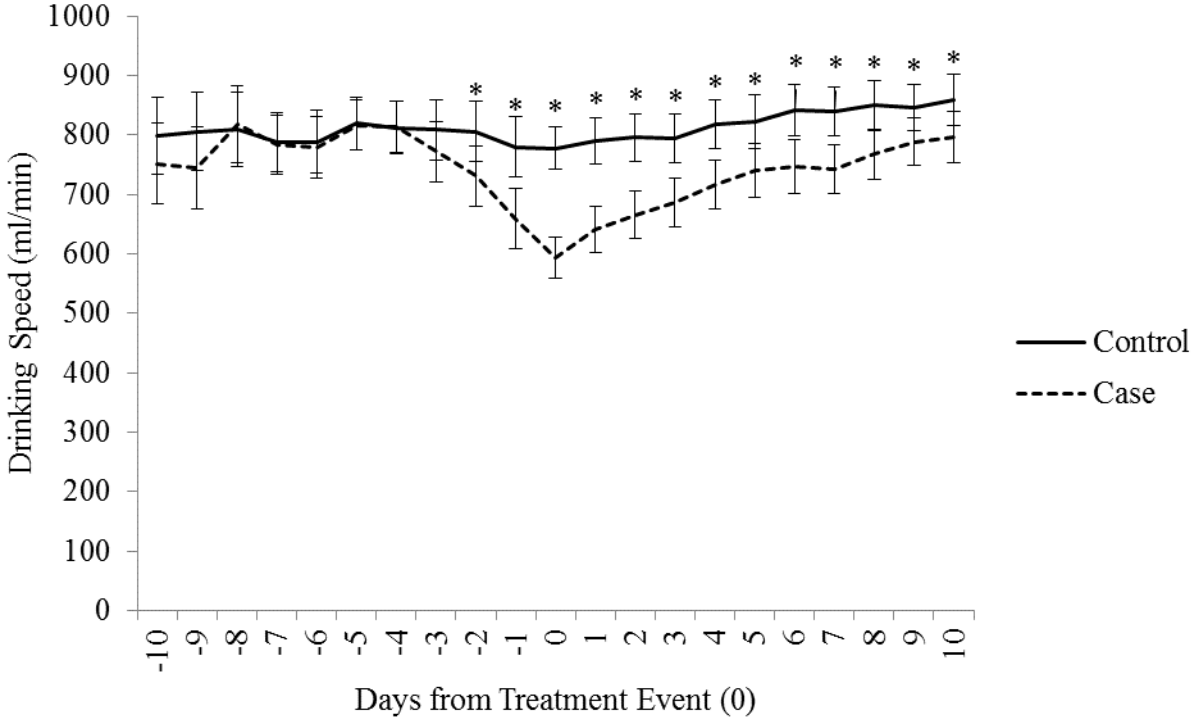
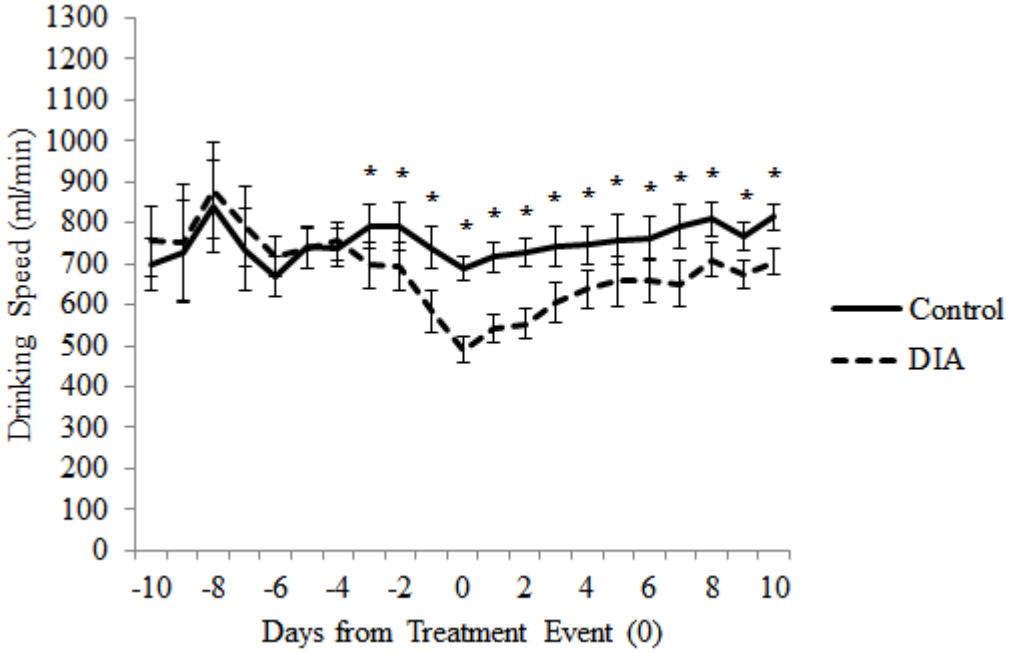
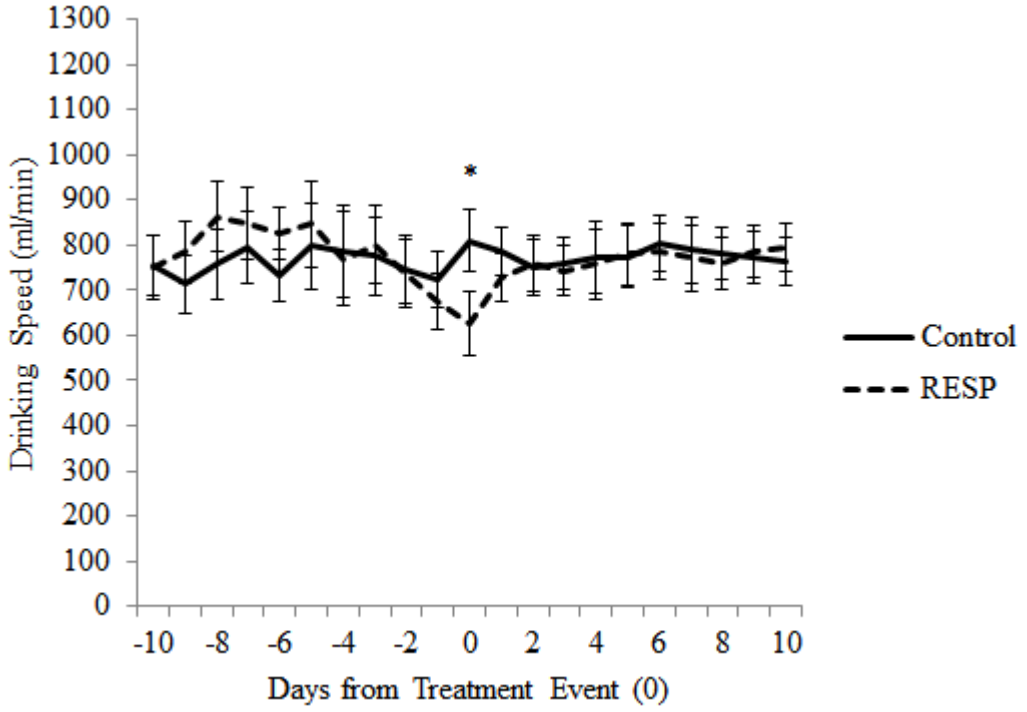


Figure 2. Results from a disease stratified mixed model exploring the relationship between disease status and drinking speed (ml/min) over the 20 days surrounding a treatment event for matched pairs of sick (case) and healthy (control) calves. Reported are adjusted means and standard error bars for; A) Diarrheal (DIA) disease (n=97 pairs); B) Respiratory (RESP) disease (n=53 pairs) and; C) Ill thrift (ILL) (n=26 pairs). (* = $P < 0.05$)

A.



B.


1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Drinking Speed (ml/min)
*
-10 -8 -6 -4 -2 0 2 4 6 8 10
Days from Treatment Event (0)
Control
RESP

C.

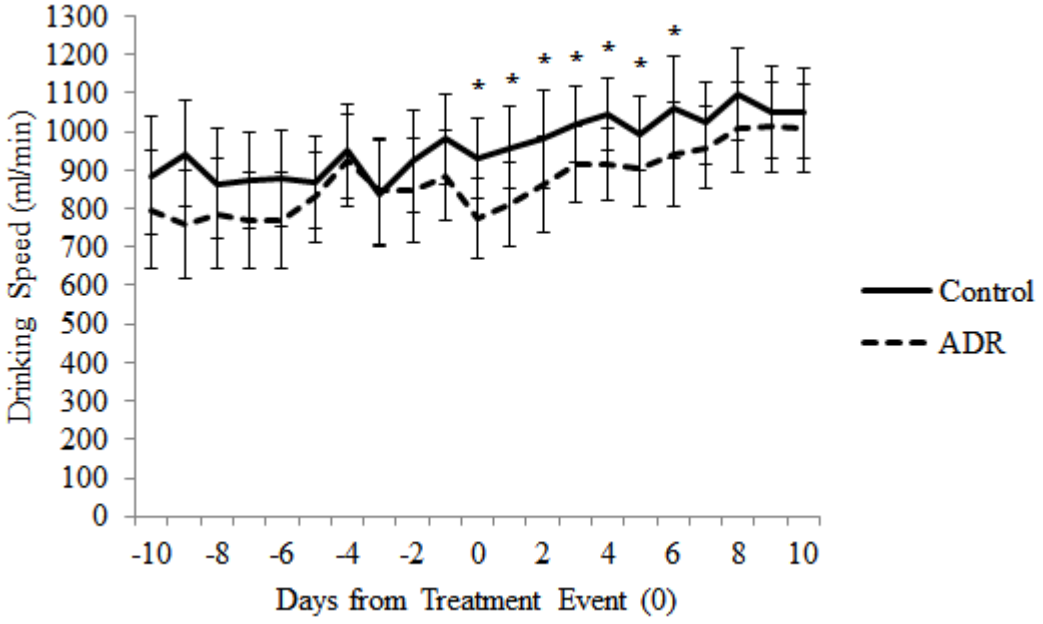

1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Drinking Speed (ml/min)
* * * * * * *
-10 -8 -6 -4 -2 0 2 4 6 8 10
Days from Treatment Event (0)
Control
ADR

Figure 3. Results of a stratified mixed model describing the relationship between daily average unrewarded visits to the automatic calf feeder and morbidity (all diseases) over the 20 days surrounding a treatment event for matched pairs of sick (case; n=176) and healthy (control; n=176) group-housed calves. This model controlled for calf age, group size, disease diagnosis, month, and the random effect of farm. Reported are adjusted means with standard error bars. (* = $P < 0.05$)

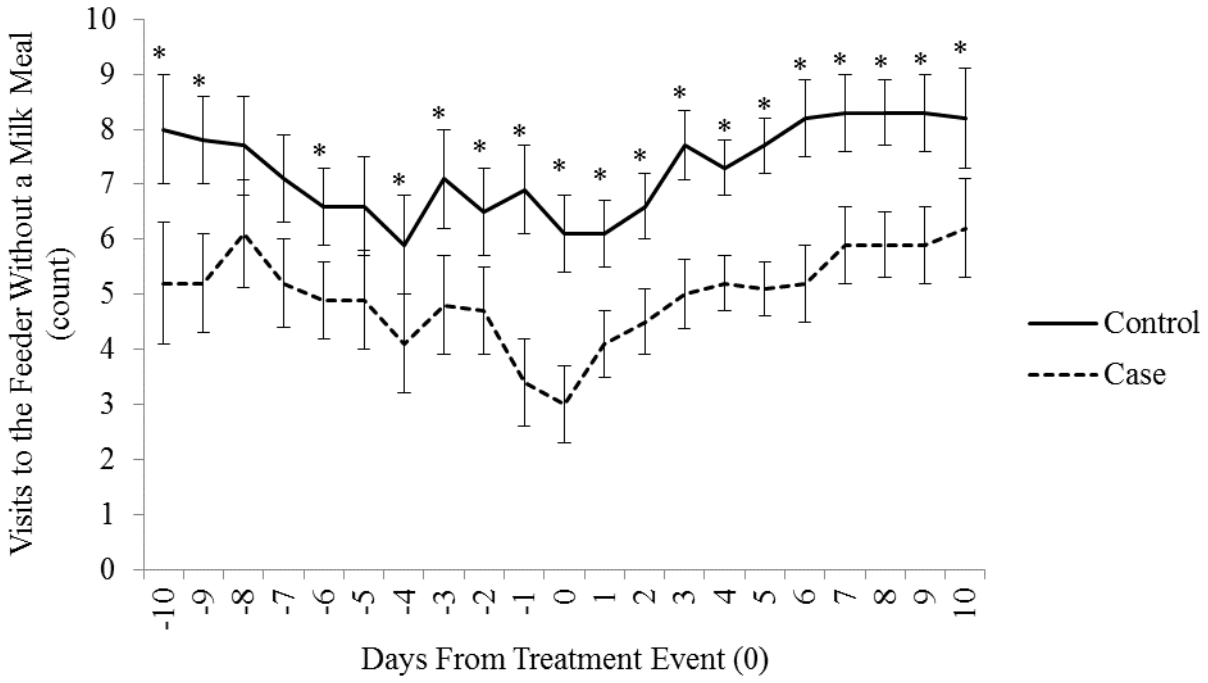
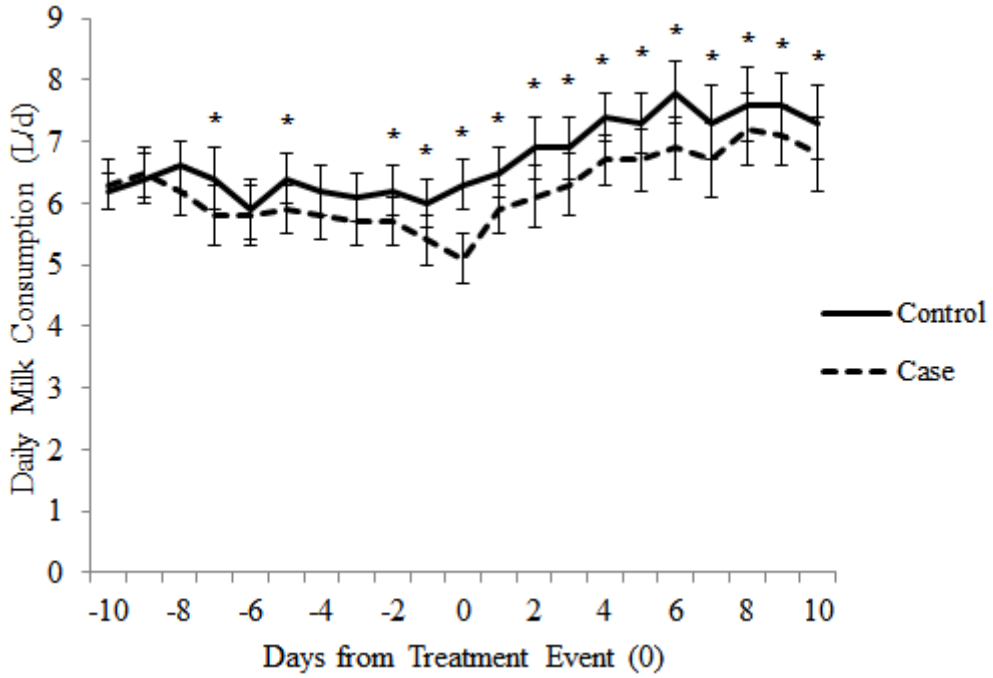


Figure 4. Results of a stratified mixed model describing the relationship between daily average milk consumption (L/d) and morbidity (all diseases) over the 20 days surrounding a treatment event for matched pairs of sick (case; n=176) and healthy (control; n=176) calves. This model controlled for calf age, group size, disease diagnosis, month and the random effect of farm. Reported are adjusted means with standard error bars. (* = $P > 0.05$)



5 CHAPTER THREE

The application of statistical process control techniques to daily average feeding behaviors to detect disease in automatically fed group-housed pre-weaned dairy calves.

W.A. Knauer, A. Dietrich, R.E. James, D.M. Hawkins, and S.M. Godden

5.1 SUMMARY

Group housing and computerized feeding of pre-weaned dairy calves is gaining in popularity among dairy producers, yet disease detection remains a challenge for this management system. The aim of this study was to investigate the application of statistical process control (**SPC**) techniques to daily average feeding behavior to predict and detect illness, and to describe the diagnostic test characteristics of using this technique to find a sick calf, as compared to a trained human observer. This prospective cross-sectional study was conducted on ten farms in MN (n=4) and Virginia (n=6) utilizing group housing and computerized feeding from February until October 2014. Calves were enrolled upon entrance to the group pen. Farm personnel recorded morbidity and mortality events. Farms were visited either every week (MN) or every other week (VA) to collect calf enrollment data, computer-derived feeding behavior data, and caregiver-derived health records. Self-starting cumulative sum (**CUSUM**) charts were generated for each calf for each daily average feeding behavior including drinking speed (**DS**; ml/min), milk consumption (**CON**; L/d), and visits to the feeder without a milk meal (**URV**;

count). A testing subset of 352 calves (176 treated, 176 healthy) was first used to develop and fine tune the SPC control charts to optimize sensitivity and timing of a negative mean signal. The parameters with the optimal sensitivity and timing were then applied to all calves (n=1052). Generalized estimating equations were then used to estimate the diagnostic test characteristics of a negative mean SPC signal (index test) to detect a sick calf (reference test) for a single feeding behavior. Combinations of feeding behavior signals were explored using parallel and series interpretation. Drinking speed and milk consumption in combination with a parallel interpretation provided the most sensitive (70.9 (62.1, 78.5)), and the timeliest test, finding a sick calf an average of 3.1 ± 8.8 d before a trained observer. However, there was no clear advantage to any one feeding behavior or combination of feeding behaviors when predictive values were examined. The results of this study suggest that the use of SPC self-starting CUSUM control charts do not provide sufficient sensitivity or specificity to detect a sick calf in a timely manner as compared to a trained observer, and that this approach to examining daily average feeding behaviors cannot take the place of a trained human observer.

5.2 INTRODUCTION

Group housing and computerized feeding of dairy calves during the pre-weaning period is gaining in popularity among dairy producers worldwide, with an estimated 15% of preweaned dairy calves in the US housed in groups (USDA NAHMS, 2014). Computerized milk feeding systems offer an easy method to deliver more milk

(Huuskonen and Khalili 2008; Roth et al, 2008), reductions in labor needed per calf (Kung et al, 1997), and social benefits for the calf (Jensen et al, 1999; Vieira et al, 2012). However, morbidity (Svensson et al, 2003) and mortality risks (Losinger and Heinrichs, 1997) are significantly increased in large groups (≥ 7 calves) as compared to small groups. Additionally, timely disease detection can be a challenge for calves housed in groups (Steenkamer, 1982). Therefore, management tools and strategies that can help dairy producers predict and detect disease are of great interest for producers who utilize this calf rearing strategy.

One potential solution to the challenge of disease detection is that computer software can record and report individual calf feeding behaviors that may be associated with illness. For example, the Kalb Manager feeding software program (Forster-Technik®, Engen, Germany) provides the producer with an alert when an individual calf deviates in its three day average drinking speed or milk consumption by 25% (Jan Zimerick, personal email communication, July 3, 2017) However, the accuracy and timeliness of current algorithms to detect disease are suspect. For example, in one observational study Borderas et al. (2009) found that calves fed a high level of milk (>8L/d) decreased their daily average milk intake on same the day that they were detected as sick by a trained human observer, but due to a lack of timeliness, this approach offered no advantage over a trained observer. Furthermore, in the same study, calves offered a restricted level of milk (4L/d) did not reduce their daily average milk intake at all during a period of illness. In another study, Svensson and Jensen (2007) reported that sick calves

had a reduction in the number of visits to the feeder without a milk meal, but there was no difference in the speed of milk consumption or rewarded visits to the feeder between sick and healthy calves. These studies suggest that current computer feeder software algorithms that use daily averages may have limited utility as an indicator of disease onset in that they lack in either timeliness or sensitivity or both, as compared to a trained human observer. However, a different approach to evaluate changes in feeding behavior in individual animals could result in a more sensitive and timely monitoring technique to detect morbidity in group housed calves.

Statistical Process Control (**SPC**) is an analytical approach, traditionally used in manufacturing process monitoring, that utilizes control charts to differentiate common cause from special cause variation in a process over time (Hawkins and Olwell, 1998). Special cause variation in livestock production data can be the result of human error, technical failures, contamination of feed or water, or health (Mertens et al, 2011). With the advent of precision livestock farming and the abundance of sensor derived information, efforts have been made in the past decade to apply these analytical techniques to production data to monitor health (De Vries and Reneau, 2010). By applying cumulative sum control charts to steer feeding time, Quimby et al (2001) reported that they could detect a sick feeder calf up to 4.5 days earlier than an experienced pen rider. Madsen and Kristensen (2005) applied similar methodologies to pen level water intake in piglets and found they could predict a diarrheal event one day before clinical signs were observed.

Traditionally, control charting has been used in manufacturing where historical data was available and common cause variation was minimal. However, certain challenges are introduced when we consider applying this approach to biological systems. For example, when a calf enters a group pen, we have no historical data on her behavior, yet want to begin monitoring as soon as possible because disease can occur early in the feeding period. Self-starting cumulative sum (**CUSUM**) control charts may be useful to address this problem (Hawkins and Olwell, 1998). In this procedure, the first several observations are used to establish the mean and variation, and the mean is then updated with each new observation. CUSUM charts also have an advantage over other charting methods in that they are sensitive to small shifts ($< 1.5\sigma$), which are particularly of interest in biological processes (Mertens et al, 2011). The sensitivity of the chart to signal is determined by the upper and lower control limit, defined as $+/- \sigma$ by which the mean is allowed to vary. When the mean falls outside the upper or lower limits, as pre-determined by the user, the process is said to be out of control and corrective actions are taken.

The objective of this study was to evaluate the sensitivity and timeliness of applying SPC charting techniques to computer feeder sensor derived daily averages of dairy calf feeding behaviors to find a sick calf as compared to a trained human observer. We hypothesized that SPC techniques applied to daily average individual calf feeding behavior data, alone or in combination, would be useful to detect a sick calf in a timely and sensitive manner as compared to a trained observer. If successful, we anticipated that

this technique could be applied to calf feeding behaviors in computer feeder software algorithms as a screening test to aid producers in the detection of sick calves in the field.

5.3 METHODS

The use of animals in the study was approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol #1308-30844A). This manuscript was prepared according to the Standards for Reporting Diagnostic accuracy studies (STARD Group, 2015).

5.3.1 Herd Selection

This prospective cross-sectional study was conducted in a convenience sample of ten commercial dairy herds; four herds in Minnesota and six herds in Virginia. Herds were selected based on their use of an automated calf feeding system (Forster-Technik®, Engen, Germany) that must have been in use for greater than one year's duration. Herds also had to provided a high peak daily milk allowance of $\geq$ 7L per day at a total solids level of >125g/L (g milk powder added to 1L of water).

5.3.2 Calf Management and Data Collection.

Data collection occurred from February 2014 - October 2014. An initial questionnaire was used to describe calf facilities and general calf management. Heifer and bull calves were enrolled into the study when they entered the group pen, and exited the study when they were weaned from the automatic feeder. For each calf entering a

group pen, the calf manager recorded the calf id, breed, gender, birth date and pen entry date. Sick calves were identified based on daily subjective evaluations by the calf manager, and the date, time, treatment, and disease treated was recorded for each morbidity event. Mortality events were recorded similarly. Case definitions were standardized across farms through training and use of a visual scoring system that evaluates ocular and nasal discharge, cough, head tilt, fecal score, and general attitude (McGuirk, 2008).

A study technician visited the farm each week (MN) or biweekly (VA), to collect calf enrollment data, treatment records and mortality data. An 8ml venous blood sample was collected from the jugular vein of a convenience sample of calves (n=367) between 24h to 7d of age for serum total protein measurement (g/dL) with a digital serum refractometer (MISCO Palm Abbe Model PA203X, MISCO, Cleveland, OH). Approximate birth (1d – 7d of age) and weaning weights (50d – 60d of age) were estimated in kg from a convenience sample of calves (n=182 birth weights; n=72 wean weights) using a weight tape (Nasco, Fort Atkinson, WI). Day level average feeding behavior data was collected through the automatic feeding software program (Kalb Manager, Förster-Technik®, Germany) at each farm visit by the study technician. Specific daily average feeding behaviors recorded included total milk intake (**CON**) (L/day), average drinking speed (**DS**) (mL/min), and total number of rewarded (**RV**) (visits to the feeder with a milk meal) and unrewarded (**URV**) (visits to the feeder without a milk meal) visits to the feeder. Farm personnel were blinded to sensor derived

feeding behavior data throughout the study, beyond what was already being reported and used for animal monitoring by the on-farm software. The Kalb Manager software provides drinking speed, daily consumption, and deviation data to the user. Calf caregivers on 6 of the 10 study farms used daily average milk consumption to screen calves, but none used the data for the sole purpose of calf diagnosis and/or treatment.

5.3.3 Statistical Analysis

Sample Size. 1052 calves were enrolled on 10 farms in MN and VA. Sixty three percent of calves had a first treatment event, resulting in 660 treated calves and 392 healthy calves respectively. This sample size (>250 calves per group) provided in excess of 80% power and 95% confidence to detect a 1L difference in daily milk intake (i.e. 8L/day vs. 7L/day) between sick and healthy calves (Assumed a SD=4L/day, one tailed test).

Case Definitions. Disease diagnosis was based on visual assessment by the on-farm calf caregiver and attempts were made to standardize across farms based on use of a modified scoring system as defined by McGuirk (2008). A case of diarrhea (**DIA**) was defined as visible diarrhea (very loose or watery feces; fecal score of 2 or 3 on a 0-3 scale) as well as treatment with antibiotics, electrolytes, or IV fluids, or a combination of the three. A case of respiratory (**RESP**) disease was defined as a calf with an increased respiratory rate or effort, cough, plus treatment with antibiotics. A case of ill thrift (**ILL**) was defined as either 1) a calf that had a rectal temperature > 39.5˚C; 2) a calf that was

depressed but for which the caregiver did not have a clear diagnosis or; 3), a calf with other miscellaneous illnesses such as umbilical infection, joint infection, or injury, and treatment with antibiotics and/or non-steroidal anti-inflammatories. Treatment events known to be prophylactic (i.e. antibiotic administered to all calves at pen entry) occurred on one farm and were excluded from analysis. Duration of treatment was defined as the period between the first and last treatment. A new treatment event was defined as; an event that occurred for the first time or; an event that occurred at least 5 days after conclusion of treatment for a previous event or; an event that occurred within 5 days of the conclusion of a previous event but represented a different disease diagnosis.

Investigation of SPC model parameters. A matched pair analysis was first performed to determine the difference in feeding behaviors in a matched pair of sick and healthy calves (n=176 pairs) around the time of a treatment event which is described elsewhere (Knauer et al., 2017). The results of that analysis showed that a reduction in daily average DS, CON and URV were all significantly associated with a treatment event both before and during the event, and were subsequently investigated as potential feeding behaviors of interest for SPC analysis. Self-starting CUSUM charts were generated for DS, CON and URV for all calves during the milk feeding phase as described by Hawkins and Olwell (1998) using R Statistical Software (V3.3.0, R Core Team, Vienna, Austria). The CON and URV feeding behavior data had many "0's" or repetitive numbers (i.e. Day 1 = 5L; Day 2 = 5L) at the beginning of the feeding period which is problematic as the CUSUM chart cannot function properly with a variance of "0". Therefore, a random number

between 0 and 0.01 was added to all of the CON and URV daily average measurements for all calves in order to add sufficient variation. A training data set (n=352) was used to tune the CUSUM chart, including exploring different upper (**UCL**) and lower control limits (**LCL**). Control limits are typically chosen based on the cost of type I and type II errors, which should be limited (DeVries and Reneau, 2010). In a self-starting CUSUM chart, the time to a positive signal (average run length (**ARL**)) is defined by three parameters: Δ, k and h. Δ is defined at the smallest shift in the process mean to be detected. "k" is the magnitude of the deviation of an observation from the target, and is often referred to as the reference value. In self-starting CUSUMs, "k" is typically set to 0.5 (Hawkins and Olwell, 1998). "h" is the magnitude required to conclude that the process is out of control and defines the UCL and LCL. "h" is typically referred to as the decision interval, and "h" values of 4.5, 4.0, 3.5, 3.0, 2.5, 2.0 and 1.5 were tested. Exploratory data analysis revealed that individual calf DS and URV increased over the time the calf was in the group pen, whereas CON followed a curve similar to the feeding plan of the calf; increasing in the first days in the pen, then holding at a maximum feeding level, then decreasing as the calf was weaned from milk. Both of these characteristics of the data are problematic and can lead to an increase in Type 1 error. Therefore, individual calf daily averages were standardized to the daily average of all of the calves in the data set. Then, self-starting CUSUM charts were created for each calf at each decision interval for both standardized and raw daily average feeding behavior data.

A data base was then created that contained the date and type of SPC signal (**PM** = positive mean; **NM** = negative mean; **PV** = positive variance; **NV** = negative variance) at each of the decision intervals for both raw and standardized feeding behaviors. General descriptive statistics were generated to describe the direction and type of signal generated by the control chart, as well as to describe the SPC signal date as compared to the date that a first treatment event was recorded for treated calves. Based on sick calf behavior around the time of illness, a positive NM signal was chosen as the index test and was defined as any NM signal that occurred during the time the calf was in the group pen. The reference standard was defined as a producer reported first treatment event. Treatments were defined as DIA, RESP and ILL.

Diagnostic test characteristics for an NM SPC signal to detect a sick calf as compared to an observer were calculated for each of the feeding behaviors, at each of the decision intervals from 2 x 2 tables. Diagnostic test characteristics included sensitivity (**Se**), specificity (**Sp**), accuracy (**Ac**), positive predictive value (**PPV**) and negative predictive value (**NPV**). Se was defined as the proportion of truly sick calves (as identified by the producer) that had a positive SPC test. Sp was defined as the proportion of healthy calves (never diagnosed by the producer as sick) that were negative on SPC (no NM signal). Ac was defined as the total proportion of tests that correctly identified a sick or healthy calf. PPV was defined as the proportion of positive NM tests that correctly identified a sick calf, and NPV was defined as the proportion of negative NM tests that correctly identified a healthy calf (Dohoo et al., 2009). The test characteristics for each

decision interval for each feeding behavior were determined. For those calves that achieved a positive NM SPC test and were truly sick (true positives), the timeliness of the signal was assessed based on subtracting the date of the SPC signal from the date that the producer identified the calf as sick. After completing the SPC analysis for all disease events, this analysis was repeated for each individual disease diagnosed (RESP, ILL or DIA). The decision interval and data method (raw or standardized) with the optimal sensitivity and signal timing for each feeding behavior to detect a sick calf was identified and applied to the entire data set.

SPC Diagnostic Test Characteristics. After completing the previously described analysis using the training data set (n=352 calves), the optimal SPC data form and decision interval identified for each feeding behavior (DS = RAW, 1.5; CON = STD, 1.5; URV = STD, 1.5) were applied to daily average feeding behaviors of the entire data set consisting of 1052 calves. All self-starting CUSUM charts were created using R (v. 3.3.0, R Core Team, Vienna, Austria). Descriptive statistics calculated included timing of signal relative to day on the automatic feeder, time between signals for combination signals, and for treated calves, the timing of the signal relative to the first day of treatment. Diagnostic test characteristics (Se, Sp, Ac, PPV and NPV)) of using a single positive NM signal to detect a sick calf were estimated using generalized estimating equations, accounting for the random effect of farm. First, univariable models were generated to describe the relationship between NM Sig (Y/N) and predictors of interest including calf treatment (Y/N; main predictor of interest), breed, age at pen exit, age at

signal, feeding day, sick before entrance to the feeder (Y/N), pen, farm, and region. Variables that were significant $P < 0.20$ in a univariable model were then offered to the full model and backwards stepwise elimination was used until all variables remaining were significant at $P < 0.05$. The main predictor and outcome were then switched in the model to determine predictive values. Test characteristics and 95% confidence intervals were calculated from model output as described by Dohoo (2009). Finally, the utility of using feeding behaviors in combination was investigated. For the latter, the diagnostic test characteristics of using two and three way combinations of DS, CON and URV were modeled similarly using both parallel and series interpretation. With series interpretation, only calves that test positive to both tests are considered test positive. With parallel interpretation, animals that test positive to one test, the other test, or both are considered test positive. (Dohoo, 2009). All statistical modeling was performed in SAS (V 9.4, SAS Institute Inc, Cary, NC).

5.4 RESULTS

5.4.1 General Calf Characteristics.

Descriptive statistics describing farm and calf characteristics as well as a validation of producer reported treatment events are described in detail elsewhere (Knauer et al., 2017). Briefly, herd size ranged from 110 to 850 lactating cows, and one farm was a custom heifer grower. Four farms had two feeders with four pens of calves, and six farms had one feeder and two pens of calves, with each pen having one nipple feeding station. Average full feeding level offered was 9.4L/d (range: 7-16), fed at a total

solids concentration of 155g/L (range: 143 – 165). All farms managed calves in individual housing prior to introduction with average age at introduction to the group pen at 9.1 ± 5d with a group size of 17 ± 5 calves per group. One thousand fifty two calves were enrolled into the study, with 62.7% (660/1052) experiencing a first treatment event for DIA (50%; 331/660), RESP (19.2%; 127/660), or ILL (30.6%; 202/660). First treatments occurred at an average ± SD of 9.3 ± 8.5 days after introduction to the group pen, and calves diagnosed with illness were treated an average of 3.8 ± 3.9 days duration.

5.4.2 Signal Timing

The following reports the total number of NM signals, then average day of signal relative to day of treatment ± SD. When feeding behaviors were considered alone, the NM signal occurred earliest in the feeding period when using DS (n=533; 5.4 ± 2.6d), then CON (n=428; 10.6 ± 6.8d) and URV (n=211; 10.8 ± 7.3d). Drinking speed also resulted in the most timely signal as compared to day of treatment in treated calves (n=347; -2.8 ± 8.5d), then CON (n=288; 0.7 ± 10.3d) and URV (n=131; 1 ± 11.3d). When considering two NM signals and using either parallel or series interpretation, parallel interpretation resulted in the earliest and most timely signal as compared to series interpretation. The earliest parallel interpretation combination NM signal during the feeding period occurred when DS and URV were considered (n=619; 6.9 ± 4.2d), then DS and CON (n= 719; 7.1 ± 5.5d), then URV and CON (n=532; 10.2 ± 6.8d). The most timely signal relative to day of treatment was DS and CON (n=463; -3.1 ± 8.8) followed by DS and URV (n=409; -2.3 ± 8.8), and CON and URV (n=346; -0.5 ± 10.4). When

considering all three feeding behaviors in combination, parallel interpretation resulted in the earliest (n=765; 7.8 ± 5.5d) and most timely (n=487; 0 ± 10.1d) signal (Table 1). The proportion of calves that signaled relative to when they were treated is reported in table 2. For a DS and CON signal in parallel combination, 16.6% of signals occurred 8 or more days before the calf was found as sick, 37.7% of signals occurred in the week leading up to illness detection. Fourteen percent of NM signals occurred on the day the calf was found to be sick by farm personnel, and 31.5% of signals occurred after the calf was treated for illness.

5.4.3 Diagnostic Test Characteristics

Reported are point estimates and 95% confidence intervals of the diagnostic test characteristics estimated from the generalized estimating equations (Table 3). When considering the ability of a signal on a single feeding behavior to detect a sick calf, a NM signal on DS data had the highest Se at 56.4 (47.1, 65.5) and a PPV of 66.6 (54.4, 76.9). However, the Sp and NPV were only 49.5 (41.1, 57.6) and 38.9 (29.1, 47.4), respectively. When considering two parameters in combination, a NM positive signal on DS and CON interpreted in parallel had the highest Se to detect a sick calf with a Se of 70.9 (62.1, 78.5) and a PPV of 65.3 (53.5, 75.4). However, the Sp and NPV were only 32.9 (26.7, 40.3) and 38.7 (28.0, 50.6), respectively. Parallel interpretation of the three feeding behavior parameters in combination resulted in a Se of 74.9 (65.5, 82.6), a Sp of 27.1 (21.7, 33.2), a PPV of 64.6 (52.5, 75.1), and a NPV of 37.4 (27.1, 48.9). The remaining diagnostic test characteristic results are reported in Table 3.

5.5 DISCUSSION

Group housing of preweaned dairy calves is gaining in popularity worldwide due to perceived labor benefits. However, challenges with this management system include increased risk for morbidity and mortality as compared to individually housed calves as well as increased difficulty detecting sick calves in a group setting (Steenkamer, 1982; Svensson et al, 2003). One potential solution to the latter problem may lay in the fact that computerized feeding systems automatically capture individual animal feeding behavior data that could be used to predict and detect disease, as changes to some feeding behaviors have been shown to occur during an illness event. However, algorithms used by current computer feeder software have not been shown to be any more timely or sensitive than a trained human observer to detect sick calves. Statistical process control and the use of control charts are a powerful statistical tool to monitor a process mean over time. This is the first study to investigate the application of statistical process control techniques to daily average feeding behaviors in pre-weaned dairy calves to detect disease. As part of our investigation, we considered not only the diagnostic test characteristics of using SPC signals to detect disease, but also the timeliness of the signal, relative to disease detection by a trained human observer.

Signal Timing Relative to Day of Treatment

The results of our analysis showed that using DS and CON in combination in a parallel interpretation provides the most timely signal relative to a treatment event, with positive

signals occurring an average of 3.1 days prior to disease detection by a trained human observer. Very few studies have investigated the timing of control chart signaling relative to disease onset in individual animals (Quimby et al, 2001; Madsen and Kristensen 2005; Lukas et al, 2009). Lukas et al described the application of CUSUM charting to milk yield and milk conductivity to detect health events in lactating dairy cows. These authors found that SPC could alarm up to 9 days prior to a diagnosis event, but there was also a very high type 1 error rate (98%) which makes this detection technique less useful in the field setting. Quimby et al (2001) explored the application of self-starting CUSUM charts to time at the feed bunk in young steers and its association with when the calf was detected as sick by a pen rider. These authors found that an SPC signal could detect an animal with bovine respiratory disease up to 4 days before an experienced animal observer. Madsen and Kristensen (2005) also found a timing advantage when applying similar charting techniques to piglet water drinking behavior, and were able to predict diarrheal onset one day prior to the start of clinical signs. While it is difficult to compare across studies because of age, species, SPC charting, and differences in the data that these studies analyzed, our results also show an advantage to using SPC to detect disease, though this difference varies depending on which feeding behavior is used, and in what combination. Of additional interest, though we did find an advantage to DS and CON in combination, it is important to note the large range of day of signal relative to day of treatment (-46 to + 16d). Many signals occurred after the calf was sick, on the day of illness detection, and more than 2 weeks post treatment. Only 37.7% of signals occurred during the week before a treatment event, which one can argue might be the most useful

period for a producer as they can then take the necessary steps of giving the calf a thorough physical exam, making a diagnosis (if appropriate) and applying an appropriate treatment. Signals that occur on or after the day of illness detection and treatment will be less useful to producers, though we could imagine that this might still be useful to farms where staff lack the relative training and experience in detecting illness as compared to herds people involved with this study.

Diagnostic Test Characteristics

Good performance of the control chart, defined as detecting as many problems as possible with as few false alarms as possible, is necessary for SPC to become an integrated livestock management tool (Mertens et al, 2011). To this end, it is important to report diagnostic test characteristics, so the potential user has the opportunity to make an informed decision about the utility of the test in the field. Emphasis on which test characteristics are important will depend on the use of the new index test and the goals of the user. We anticipate the use of an SPC signal as a screening test, and therefore are mostly interested in maximizing test sensitivity. Users of the test should be mostly interested in predictive values, because these values describe the performance of the test in the field. The results of our analysis suggest that using DS and CON in combination with parallel interpretation provides the highest Se, though there is no clear advantage to any of the single, double or triple combination interpretation when predictive values are considered. When using DS and CON in combination with parallel interpretation, the PPV and NPV were estimated to be 65.3% and 38.7%, respectively, meaning that 65.3%

of calves with a positive signal will truly be diagnosed as sick at some time during their stay in the pen, while only 38.7% of calves that never display a positive signal will never be diagnosed as sick during their stay in the pen. Obviously, when considering both these imperfect test characteristics as well as limitations to timeliness of signals, we must conclude that applying SPC techniques to daily average computer-derived feeding behaviors will have limited utility for use in detecting disease in group housed preweaned dairy calves. Even if it is possible that SPC techniques may be useful as an additional management tool for disease screening, producers should not rely on this approach, and daily examination of calves by trained human observers will still be necessary.

Very few investigators have described the diagnostic test characteristics of SPC charting techniques to detect sick livestock. Quimby et al (2001) describes an overall Se, PPV and Accuracy of 90%, 91% and 87% respectively for the ability of a CUSUM chart to detect a sick steer. Lucas et al (2009) found low sensitivities when using milk yield and milk electrical conductivity CUSUM control charts to detect mastitis and metabolic disease in lactating dairy cattle (range: 25.0 to 58.3%), however these sensitivities were higher than the algorithm the farm was currently using when the two were compared. When Cornou et al (2008) applied CUSUM control charts to individual animal feeding rank in three electronic sow feeder herds, they found that the Se of the analysis to detect a sick sow ranged from 0 to 75%, and this technique had a very high type I error rate (range: 99.6 – 100%). Those authors suggested that there may be utility in adding other monitoring parameters to the charting to enhance the Se of detection.

It is very difficult to compare our results to those of other studies as differences exist in species, animal age, study design and analytic technique. When we compare our results to Quimby, perhaps the most similar study in animal age, species and technique, we find our test performance to be relatively poor. Those authors used time at the feeder as their parameter of interest, and it may be that time at the feeder is more sensitive than DS, CON or URV to detect as sick animal. Others have shown that lactating dairy cattle change the amount of time they are at the feed bunk in the days before an illness event (Gonzalez et al, 2008). The hypothesis that time at feeding station might be a useful screening tool for detecting disease requires further investigation in group housed automatically fed pre-weaned calves.

Strengths and Limitations

This is the first study to investigate the application of statistical process control techniques to daily average feeding behaviors in pre-weaned dairy calves to detect disease. The study was conducted on 10 commercial farms in 2 states (VA and MN) using computerized feeding equipment. Calf management (e.g. age at introduction to pen, milk feeding programs, group sizes) and calf morbidity rates are similar to previous reports of producers using similar systems (Svensson and Jensen, 2007; Roth et al., 2009; Jorgensen et al., 2017).

Because we only included farms feeding a high plane of nutrition (≥7L/d) our results may not be generalizable to farms with restricted milk feeding programs. Another potential limitation is that we used producer-reported treatment data to indicate illness rather than clinical diagnosis by a veterinarian or laboratory diagnosis. Unfortunately having a veterinarian perform daily examinations of all calves on 10 farms would have been cost prohibitive. The potential for producer misclassification of calf health status could affect the results of this study in several ways. First, if calves that were truly sick were missed by the calf caregivers, it is possible that there are actually fewer false positives with SPC than it seems, resulting in a higher Sp and PPV for the SPC test. This would be an advantage for a screening test, as the user would have even more confidence in the true meaning of a positive signal. Conversely, if calves were over treated by producers and the true incidence of first treatment events was lower, then the Se and NPV might be underestimated in the current study. A higher NPV would be advantageous when using SPC as a screening tool, as the user would be more confident in a negative result and less concerned about missing a sick calf.

One challenge lays in the attempt to apply SPC techniques to a biological system. Statistical process control techniques are commonly used for monitoring industrial production processes. The proper use of control charting requires that three assumptions are met, the data have to be stationary, independent, and follow the distribution function associated with the control chart used (Mertens et al., 2011). Livestock process data often violate the assumptions of stationary data and independence, which may explain the poor

performance of control charts to detect illness in this study, as well as the poor adoption of control chart techniques to monitor livestock processes. Hawkins and Orwell (1998) demonstrate that even small autocorrelation in the data can have dramatic effects on the ARL, thus increasing the false alarm rate. These authors suggest using a Box-Jenkins autoregressive moving average model, which could be another approach to use with this data. Feeding behavior data is also not stationary, and depending on the behavior, increases or decreases over the feeding period. We attempted to deal with this problem through standardization of the data.

Future studies should reevaluate the SPC analysis approach developed in this study when using trained veterinarians or other accepted gold standard as the referent test. Furthermore, other charting techniques or feeding behavior data types should be explored before real time validation on the farm is performed.

5.6 CONCLUSIONS

The results of this study suggest that the application of SPC control charting techniques to daily average feeding behaviors in group housed pre-weaned dairy calves may not be a useful stand alone test to predict or detect disease. The combination of signals on DS and CON in parallel interpretation provided the most sensitive and timely test, however no single or combination of feeding behavior signals provided enough of an advantage to abandon daily observation of calves. As such, it will still be necessary to have well trained calf managers with good observational skills observing calves daily for

clinical signs of disease.

Table 3. The timing of the negative mean (NM) SPC signal relative to days on the calf feeder (n=1052 calves) and days from treatment for treated calves (n=660 calves).). For parallel interpretation, calculations are from the date of the first signal. For series interpretation, calculations are from the date of the last signal. The table presents mean ± SD (range).

			Signal Timing		
Signal Type		N	Days on Feeder	N	Days from Treatment
One Signal					
DS[1]		533	5.4 ± 2.8 (3, 26)	347	-2.8 ± 8.5 (-46, 16)
CON[2]		428	10.6 ± 6.8 (3, 38)	288	0.7 ± 10.3 (-40, 33)
URV[3]		211	10.8 ± 7.3 (3, 44)	131	1 ± 11.3 (-34, 41)
Two Signals					
DS and CON	Parallel[4]	719	7.1 ± 5.5 (3, 38)	463	-3.1 ± 8.8 (-46, 16)
	Series[5]	242	10.1 ± 5.8 (3, 35)	172	0.03 ±9.9 (-33, 27)
DS and URV	Parallel	619	6.9 ± 4.2 (3, 42)	409	-2.3 ± 8.8 (-46, 35)
	Series	128	10.9 ± 6.8 (4, 44)	82	0.1 ± 11.5 (-35, 40)
CON and URV	Parallel	532	10.2 ± 6.8 (3, 42)	346	-0.5 ± 10.4 (-41, 35)
	Series	107	13 ± 7.4 (4, 44)	73	1.5 ± 11.5 (-33, 40)
Three Signals					
DS, CON, URV	Parallel	765	7.8 ± 5.5 (3, 42)	487	0 ± 10.1 (-46, 40)
	Series	70	13.4 ± 7.9 (4, 44)	48	2.1 ± 12.9 (-33, 40)

[1] DS = daily average drinking speed (ml/min)
[2]CON=daily average milk consumption (L/d)
[3]URV = daily average unrewarded visits to the feeder (count)
[4]Parallel interpretation = The NM SPC signal had to be positive for DS, CON or both for the test to be considered positive
[5]Series interpretation = The NM SPC signal had to be positive for DS and CON for the test to be considered positive

Table 4. Timing of negative mean (NM) signals relative to day of treatment for all treated calves. Reported is frequency of signal (Proportion of total treated calves) at different intervals before, on the day of, or after the day of treatment.

				Timing of Signal Relative to Calf Treatment				
Signal type		N[1]	N[2]	Signal 8 days or more before treatment	Signal 4 to 7 days before treatment	Signal 1 to 3 days before treatment	Signal on the same day of treatment	Signal after the calf received treatment
One Signal								
DS[3]		533	347	54 (15.6)	39 (11.2)	80 (23.1)	60 (17.3)	114 (32.9)
CON[4]		428	288	38 (13.2)	24 (8.3)	47 (16.3)	43 (14.9)	136 (47.2)
URV[5]		211	131	19 14.5)	9 (6.9)	20 (15.3)	17 (12.9)	66 (50.4)
Two Signals								
DS and CON	Parallel[6]	719	463	77 (16.6)	64 (13.8)	111 (23.9)	65 (14.0)	146 (31.5)
	Series[7]	242	172	23 (13.4)	9 (5.2)	21 (12.2)	28 (16.3)	91 (52.9)
DS and URV	Parallel	616	396	62 (15.7)	44 (11.1)	95 (23.9)	61 (15.4)	134 (33.8)
	Series	128	82	11 (13.4)	4 (4.9)	5 (6.1)	16 (19.5)	46 (56.1)
CON and URV	Parallel	532	346	4813.9)	30 (8.6)	58 (16.8)	54 (15.6)	156 (45.1)
	Series	107	73	9 (12.3)	3 (4.1)	9 (12.3)	6 (8.2)	46 (63)
Three Signals								
DS, CON, URV	Parallel	765	487	63 (12.9)	41 (8.4)	72 (14.8)	69 (14.2)	242 (49.7)
	Series	70	48	7 (14.6)	0 (0)	3 (6.3)	5 (10.4)	33 (68.8)

[1] N= total number of SPC positive calves out of total calves (n=1052)

[2] N = total number of SPC positive signal calves out of total calves treated (n=660)

[3] DS = daily average drinking speed (ml/min)

[4]CON=daily average milk consumption (L/d)

[5]URV = daily average unrewarded visits to the feeder (count)

[6]Parallel interpretation = The NM SPC signal had to be positive for DS, CON or both for the test to be considered positive

[7]Series interpretation = The NM SPC signal had to be positive for DS and CON for the test to be considered positive

Table 5. Diagnostic test characteristics of the ability of a negative mean CUSUM signal to detect a sick calf using daily average individual animal feeding behaviors, alone or in combination. Point estimates are the result of generalized estimating equations controlling for the random effect of farm. Presented are mean (95% CI).

		Diagnostic Test Characteristics[1]				
Signal Type		Se	Sp	PPV[2]	NPV	Ac
One Signal						
DS[3]		56.4 (47.1,65.5)	49.5 (41.4, 57.6)	66.6 (54.4, 76.9)	38.9 (29.1, 47.4)	53.8 (49.6, 57.9)
CON[4]		42.8 (36.3,49.6)	63.7 (52.2, 73.8)	67.7 (54.9, 78.3)	38.4 (27.6, 50.5)	51.6 (47.7, 55.5)
URV[5]		19.9 (16.7, 23.5)	79.8 (73.5, 84.9)	63.8 (52.8, 73.5)	35.9 (25.6, 47.7)	42.2 (35.2, 49.5)
Two Signals						
DS and CON	Parallel[6]	70.9 (62.1, 78.5)	32.9 (26.7, 40.3)	65.3 (53.5, 75.4)	38.7 (28.0, 50.6)	57.9 (52.2, 63.4)
	Series[7]	27.2 (20.7, 34.8)	81.4 (77.1, 85.1)	72.0 (58.7, 82.4)	38.5 (28.2, 49.9)	48.3 (41.3, 55.3)
DS and URV	Parallel	63.6 (53.2, 72.8)	40.7 (33,1, 48.7)	65.7 (53.7, 75.9)	38.4 (27.9, 50.2)	56.2 (50.4, 61.8)
	Series	12.7 (7.3, 17.5)	88.5 (82.4, 92.7)	66.5 (54.0, 76.9)	36.3 (26.1, 47.8)	40.2 (32.1, 48.8)
CON and URV	Parallel	51.9 (45.7, 58.0)	51.9 (44.5, 59.4)	65.6 (53.2, 76.2)	37.5 (27.3, 48.9)	52.6 (49.3, 55.9)
	Series	10.5 (7.8, 14.1)	91.8 (87.3, 94.8)	69.8 (60.7, 77.5)	36.5 (25.8, 48.6)	39.9 (30.0, 50.9)
Three Signals						
DS, CON, URV	Parallel	74.9 (65.5, 82.6)	27.1 (21.7, 33.2)	64.6 (52.5, 75.1)	37.4 (27.1, 48.9)	59.1 (51.2, 66.6)
	Series	7.3 (5.9, 8.9)	94.4 (91.6, 96.3)	71.0 (60.7, 79.5)	36.4 (26.2, 48.1)	38.7 (29.3, 49.1)

[1] Diagnostic Test Characteristics: Se = sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; Ac = accuracy
[2]PPV and NPV were calculated under the conditions of a first treatment prevalence of 63%
[3] DS = daily average drinking speed (ml/min)
[4]CON=daily average milk consumption (L/d)
[5]URV = daily average unrewarded visits to the feeder (count)
[6]Parallel interpretation = The NM SPC signal had to be positive for DS, CON or both for the test to be considered positive
[7]Series interpretation = The NM SPC signal had to be positive for DS and CON for the test to be considered positive

6 CHAPTER FOUR

The association between visit level feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves.

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6.1 SUMMARY

Group housing and computerized feeding of dairy calves during the preweaning period is gaining in popularity worldwide, yet disease incidence and detection remain a challenge in these systems. The objective of this prospective observational cohort was to investigate the association between visit level feeding behaviors and morbidity in group housed pre-weaned dairy calves. Calves were enrolled upon entrance to the group pen on eight dairy farms, 3 in MN and 5 in VA from March to October 2014. Morbidity and mortality were recorded by farm personnel. Herds were visited either weekly (MN) or biweekly (VA) to collect calf enrollment, treatment, and feeding behavior data. Visit average feeding behaviors (milk consumption, ml; drinking speed, ml/min; total visit time, s; and total drinking time, s) were described both overall, and by healthy and sick calf days. Visit level behavior was also averaged and described by quarter day (**QDAY**, visit average per 6 hr time period). Multivariable generalized mixed models were built to describe the difference in QDAY visit average feeding behaviors (milk consumption, drinking speed, total visit time, total drinking time) for matched pairs of sick and healthy calves in the 20 QDAYs surrounding a treatment event (-5 to 5 d). Final models

controlled for farm, age at treatment, month, QDAY from treatment, the interaction of treatment (Y/N), and repeated measurements by calf. Models were also stratified by disease diagnosed. A total of 653 calves were enrolled representing 305,083 total visits and 92,621 QDAY visits to the feeder. Three hundred and eighty one (58.4%) calves had a first treatment event. Diarrheal disease, respiratory disease, and ill thrift represented 42.2%, 16.5%, and 36.6% of first treatments, respectively. Of these, 117 pairs of calves were available for matched pair analysis. Sick calves drank 139 ± 77 ml less, at 125 ± 29 ml/min slower, and stayed at the feeder drinking for 78 ± 19 s longer on the QDAY of illness detection as compared to healthy calves. The differences in drinking speed and total drinking time began 24h prior to detection by farm personnel, and persisted for up to 36h post treatment. The results of this study suggest that QDAY visit average drinking speed and QDAY total drinking time may be useful in the prediction or detection of disease in automatically fed group housed, preweaned dairy calves.

6.2 INTRODUCTION

The period from birth to weaning represents a high risk time for the dairy calf, with approximately 38% of calves becoming ill and 8% dying during this rearing phase (USDA, 2007). One management strategy utilized to reduce morbidity and mortality in the pre-weaned calf is to house them individually, as calves housed in this manner have a lower incidence of diarrhea (Waltner-Towes et al, 1986) and respiratory disease (Svensson et al, 2003). This housing method is also labor intensive (Kung et al., 1997), and for this reason, dairy producers are becoming more interested in alternative housing

systems for pre-weaned dairy calves. One such method is to house calves in groups and feed them automatically with a robotic calf feeder. Advantages of this system are reductions in labor (Kung et al., 1997), the ability to feed increased quantities of milk more easily (Roth et al., 2008), and social benefits for the calf (Jensen et al., 1999; Vieira et al., 2012). However, calves housed in large groups (≥ 7 calves per group), typical for group housing systems in the United States (Jorgensen et al., 2017), have an increased risk for morbidity (Svensson et al., 2003) and mortality (Lonsinger and Heinrichs, 1997) as compared to calves housed in small groups or housed individually. Of additional concern for calf caregivers is that it is simply more difficult to find a sick calf in a group (Steenkamer, 1982), as ruminants are a prey species, and are more likely to hide illness to avoid predation (Weary et al., 2009). Therefore, alternative strategies to visual detection to predict and detect calf-hood morbidities are needed.

One advantage of computerized milk delivery systems over manual milk delivery systems is that computer software can record and report individual calf feeding behaviors that may be useful for disease monitoring purposes. Physiological changes that accompany acute infectious or inflammatory processes and can manifest as behavioral changes in individual animals (Hart, 1988). In both natural and induced disease models, calves have been shown to change their feeding behaviors. In a small dose lipopolysaccharide induction model, treated calves were shown to have a reduction in time eating hay and rumination, and an increase in time spent lying inactive when comparing behaviors in the 4hrs surrounding peak rectal temperature. However, calves did not change their milk intake during the same time period. (Borderas et al, 2008) In

observational studies, sick calves have been reported to decrease their milk consumption (Borderas et al, 2009), visits to the feeder without a milk meal (Svensson and Jenson, 2007), and speed of milk consumption (Knauer et al., 2017), though these results are not consistent across studies. Additionally, these studies all used daily average feeding behaviors, and only one (Knauer et al, 2017) reported that sick calves changed their feeding behaviors prior to detection.

Methods that utilize more information per calf (i.e. 4 measurements per day, averaged per 6 hours (QDAY)) may offer advantages as a tool to detect morbidity in calves as compared to the aforementioned studies that evaluated daily average feeding behaviors. Such meal-level information can be captured with existing technology: The Institute Function (Förster-Technik, Engen, Germany) is a software program that integrates into the auto-feeder hand-held device and continuously records what happens at the automatic feeder. The output is a running tally of every calf that enters the feeder, how long she stays (min), and how much she drinks (L/meal). Advantages to using a meal or visit level variable may be two-fold. First, considering more time points per day might be more sensitive, as using daily averages may mask small changes in behavior that could be meaningful. Second, more observations per day may improve timeliness of detection, as the lag between observations could represent less than one full day. However, as a first step in exploring this hypothesis we must first understand the overall behavior of calves on a visit level, and how these behaviors may change when a calf experiences an illness event. The objective of this study was to describe the association between illness and visit average feeding behaviors. We hypothesized that calves will

change their visit level feeding behaviors before and during a producer reported illness event.

6.3 METHODS

The use of animals in this study was approved by the University of Minnesota Institutional Care and Use Committee (Protocol #1308-30844A).

6.3.1 Herd Selection

This prospective observational cohort study was conducted using a convenience sample of eight commercial dairy farms, three herds in Minnesota and five herds in Virginia representing 11 automatic calf feeders and 22 pens of calves. Herds were selected based on their use of an automated calf feeding system (Förster-Technik®, Engen, Germany) that must have been in use for greater than one year's duration. Additionally, herds must have had the capability to record visit level feeding behaviors (Institute Function software, Förster-Technik, Engen, Germany), and must have provided a peak daily milk allowance of ≥ 7 L/d fed at a total solids level of >125g/L (g milk powder added to 1L of water).

6.3.2 Calf Management and Data Collection

Data collection occurred from March 2014 - October 2014. An initial questionnaire was used to describe general calf facilities and calf management on each farm. For each calf entering the group pen, farm personnel recorded calf id, birth date, and pen entry date. Farm personnel recorded calf morbidity and mortality events,

including calf id, date, and time of treatment or death event. Case definitions were standardized across farms through the use of a modified visual scoring system that evaluates ocular and nasal discharge, coughing, head tilt, fecal score and calf attitude (https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring _chart.pdf).

Calves were enrolled into the study when they entered the group pen, and exited the study when they were weaned from milk. A study technician visited the farm each week (Minnesota) or biweekly (Virginia), to collect calf enrollment data, treatment records and mortality data. Visit level feeding behavior data for each feeder (n=11) was collected directly from the automatic feeder hand held (Institute Function, Forster-Technik, Engen, Germany) and then transferred to an SD card (SanDisk, 2GB, Milpitas, CA) and stored there until analysis. An 8 ml jugular blood sample was collected from a convenience sample of calves aged 1 – 7d (n = 170) to test for serum total protein level (g/dL) (MISCO Palm ABBE model PA203X, MISCO, Cleveland, OH). Approximate birth (d 1 – 7 of life) and weaning weights (d 50 – 60 of life) were estimated in kg using a weight tape (NASCO, Fort Atkinson, WI). Farm personnel were blinded to sensor derived visit level feeding behavior data throughout the study.

Case Definitions. Disease diagnosis was based on visual assessment by farm personnel. A case of diarrhea (**DIA**) was defined as visible diarrhea (very loose or watery feces; fecal score of 2 or 3 on a 0-3 scale) as well as treatment with antibiotics,

electrolytes, or IV fluids, or a combination of the three. A case of respiratory (**RESP**) disease was defined as a calf with an increased respiratory rate or effort, cough, plus treatment with antibiotics. A case of ill thrift (**ILL**) was defined as either: 1) a calf that had a rectal temperature ≥ 39.5˚C; 2) a calf that was depressed but for which the caregiver did not have a clear diagnosis or; 3), a calf with other miscellaneous illnesses such as umbilical infection, joint infection, or injury, and treatment with antibiotics and/or non-steroidal anti-inflammatories. Treatment events known to be prophylactic (i.e. antibiotic administered to all calves at pen entry) occurred on one farm and were excluded from analysis. Duration of treatment was defined as the period between the first and last treatment. A new treatment event was defined as; an event that occurred for the first time or; an event that occurred at least 5 days after conclusion of treatment for a previous event or; an event that occurred within 5 days of the conclusion of a previous event but represented a different disease diagnosis.

6.3.3 Statistical Analysis

Sample Size. A total of 653 calves were enrolled on the 8 farms. Of these, 381 (58.4%) had a first treatment event, leaving 272 (41.6%) healthy/untreated calves, respectively. This sample size (>250 calves per group) provided in excess of 85% power and 95% confidence to detect a 0.5 L difference in milk intake per visit to the automatic feeder (i.e. 1L/visit vs. 0.5L/visit) between sick and healthy calves (Assumed a SD=2L/visit, one tailed test).

Descriptive Statistics for All Calves. All statistical analysis was performed in SAS (v 9.4; SAS Institute, Cary, NC) except where noted. Calves that were already on the feeder at the start of data collection were excluded from analysis (n= 78). Descriptive statistics (mean, median, SD, range) were generated to describe: (1) general calf management (age at entry to pen, group size, days in pen, age at weaning); and (2) calf health according to farm personnel (diagnosis, day of week at treatment, time of treatment, days of treatment, day of age at treatment, proportion treated both overall and by disease treated). R (v. 3.3.0, R Core Team, Vienna, Austria) was used to assemble the visit level (**VIS**) feeding behavior data base. First, data was averaged across individual calf visits. The Institute Function differentiated between visits with and without entitlement, so each time that the machine recognized a new visit (i.e. the visit number increased by 1) was considered a new visit. A running tally of milk consumption was available, so visit level milk intake (**CON**; ml/visit) was calculated by subtracting the new visit total milk consumption from the previous (i.e. 2500 (new) – 1000 (old) = 1500ml milk consumed at the visit). Total visit time at the feeder (**TVT**; sec) was calculated by subtracting the time the calf exited the feeder from the time the calf entered the feeder (11:33:30AM – 11:30:00AM = 3min * 60 + 30 sec = 210sec), regardless of whether the calf drank milk during the visit. Total drinking time (**TDT**; sec) was calculated in the same way, but defined as the time the calf was in the feeder actively consuming milk. This differentiation between TVT and TDT was necessary because 10.7% percent of drinking events began after the calf had stood in the feeder and in 78.4% of visits, calves stood at the feeder after they were done drinking. Drinking speed

(**DS**; ml/min) was calculated by dividing the amount the calf consumed (ml) at a visit by the number of minutes the calf was drinking, similarly to what has been described by Jensen (2004). Visit level feeding behaviors (CON, TVT, TDT and DS) were then averaged over a 6-hour interval (**QDAY**; 4 data points/day; (12AM – 6AM, 6AM – 12PM, 12PM – 6PM, 6PM – 12AM). For example, if two visits to the feeder occurred between the hours of 12AM and 6AM, the average of those two visits was the new QDAY average for that time period. This was done to try to reduce the impact of variation from visit to visit, and perceived utility from a management standpoint. Descriptive statistics (mean, median, SD, range) were then generated for visit level feeding behaviors at the QDAY and VIS level for all calf visits to the feeder in the following ways: (1) overall; (2) on sick and healthy calf days; (3) by feeding period (ramp up phase = when calves are gradually increased in milk allowance; hold phase = when calves are held at a peak milk allowance; and ramp down phase = where calves are gradually reduced in milk allowed until they are completely weaned from milk); (4) by hour of the day; and (5) by QDAY. Plots were generated and visually assessed to check for normality of continuous variables.

Matched Pair Analysis. A matched pair design was used to determine the relationship between QDAY visit level feeding behaviors (CON, TVT, TDT, and DS) and morbidity around the time of a treatment event. Cases were defined as a calf that had a first treatment event during the time they were in the group pen. Control calves were matched by age (± 7d) and pen, and defined as a calf that did not have a treatment event

during the time it was in the group pen. The first QDAY of illness diagnosis (based on farm personnel) was designated as time 0, then the 20 QDAYs before and after time 0 were matched to the age-matched healthy control (-5d - +5d). The five days surrounding a treatment event was chosen as the window of interest based on our results when examining day level averages where sick calves deviated from healthy calves in DS and CON up to 3 days prior to a treatment event (Knauer et al., 2017). Cases were matched to healthy controls on a 1:1 ratio. Treated calves that did not have a healthy matched control were excluded from analysis.

Univariable analyses were first performed to determine the relationship between QDAY average feeding behaviors (CON, DS, TVT and TDT) and the occurrence of treatment (treated; yes or no) and other predictors of interest including: age at treatment, QDAY from treatment (-20 - +20), group size, machine, pen, farm, diagnosis (DIA, ILL, RESP), month of illness, QDAY , and region (MN/VA). Predictors significant at the $P < 0.2$ level were carried forward to offer to the multivariable analysis.

Multivariable generalized mixed models were used to describe the difference in each QDAY level feeding behaviors (CON, DS, TVT and TDT) between matched pairs of sick and healthy calves. Predictors that were significant at $P < 0.2$ were offered to the full main effects model and backwards variable selection was used until all remaining predictor were significant at $P < 0.05$. An interaction term (QDAY from treatment*treatment Y/N) was added to the model to describe the association between illness and visit level feeding behaviors in the 10 days surrounding a treatment event. Farm was controlled for in all models as a fixed effect, and repeated measurements by

calf were accounted for with an exchangeable correlation structure which was chosen based on the lowest Akaike's Information Criteria. Models were also stratified by diagnosis (DIA, ILL, RESP) to explore the relationship between illness and visit level feeding behaviors in the 10 days surrounding a treatment event by disease treated. Adjusted means were plotted and final significance was determined at $P < 0.05$.

6.4 RESULTS

6.4.1 General Farm and Feeder Management

The calves in this study represent a subset of calves from those that are described in Knauer et al, 2017. Eight herds were enrolled in this study, three herds in Minnesota and five herds in Virginia, representing 11 automatic calf feeders and 22 pens of calves. Herd size ranged from 160 to 850 milking cows, and one study site was a contract heifer grower receiving calves from four farms. Six farms managed the group pens as dynamic groups, and two were all in all out. Average group size over the course of the study was 16 ± 6 (range: 4 – 25) calves per pen. Pens were bedded with a combination of sawdust and straw (n = 7 farms), with one farm using a combination of corn stalks and sand. Two farms fed pasteurized whole milk with balancer added (Land O'Lakes Milk Balancer, Land O'Lakes Animal Milk Products, Shoreview, MN) and the remaining six farms fed milk replacer only (Blueprint 22-20 Dx, Form-A-Feed Inc. Stewart, MN; Cow's Match ColdFront®/WarmFront®, Land O'Lakes Animal Milk Products, Shoreview, MN; Maxi Balance® Plus BVT BM, Purina Animal Nutrition, Shoreview, MN; 20/22WPL BOV-MOS DFB, Renaissance Nutrition, Roaring Spring, PA).

A total of 15 feeding plans were used throughout the study period. The average ramp up period lasted 10.1 ± 4.3 d (range: 5 – 18) and increased from a total daily allowance of 5.4 ± 0.9L/d (range: 4 – 7L/d) to 8.9 ± 2.3 L/d (range: 6 – 16L/d). The average minimum and maximum meal allowance for a calf during the ramp up period was 1.3 ± 0.4L/visit (range: 0.2 – 2L/visit) and 2.2 ± 0.3 L/visit (range: 2 – 3 L/visit), respectively. The average peak feeding period lasted 27.9 ± 7.9d (range: 10 – 41) and averaged an allotment of 8.8 ± 2.2 L/d (range 7 – 16 L/d). The average minimum and maximum meal allowance for a calf during this feeding phase was 1.7 ± 0.5 L/visit (range: 1 – 2.5 L/visit) and 2.7 ± 0.8 L/visit (range: 2 – 5L/visit), respectively. The average ramp down period lasted 13.3 ± 9.2 d (range: 7 – 42 d), decreasing from an allotment of 8.8 ± 2.2 L/d (range: 7 – 16L/d) to 1.7 ± 1.6 L/d (range: 0 – 4.4 L/d) before discontinuing milk altogether (weaning). The average minimum and maximum meal allowance for a calf during the ramp down phase was 1.8 ± 1.5 L/visit (range: 1 – 2.5 L/visit) to 2.5 ± 0.5 L/visit (2 – 3 L/visit). The average milk feeding solids concentration over the course of the feeding period on all farms was 150g/L (range: 150 – 164).

6.4.2 General Calf Level Description

A total of 653 calves met the inclusion criteria, were enrolled, and had visit level feeding behavior data available between March 6 and November 7, 2014. These 653 calves represented 305,083 total and 92, 621 QDAY visits to the feeder. From the 653 total calves, 117 calf pairs (n = 234 calves) were eligible to be carried forward to the matched pair analysis representing 7,873 visit QDAYs. General calf level descriptive

statistics for all 653 calves are reported as mean ± SD (Table 1). Calves entered the group pen at 10.4 ± 5.4 days of age after being housed individually. Calves were weaned from the feeder at an average age of 55.7 ± 7d. The average STP of enrolled calves sampled was 5.7 ± 0.8 g/dL, with 78% (132/170) sampled calves achieving a STP ≥ 5.2 g/dL. There were 104 birth weights available for analysis; The average birth weight was 42.2 ± 5.1kg while the average weaning weight was 78.7 ± 11.2 kg (n=37). The ADG from birth to weaning was 0.71kg/d. The general descriptive characteristics of the matched pair calves were very similar to the entire cohort of 653 calves, though treated calves had a lower ADG (0.68kg/d) than their healthy controls (0.75kg/d). (Table 1).

6.4.3 Calf Health

Of the 653 calves, 381 (58.4%) had a first treatment event, 127 (19.3%) had a second treatment event, and 36 (5.5%) had a third treatment while in the group pen. Of those first treatments, 42.2% (161/381) of calves were treated for diarrheal disease, 36.6% were treated for ill thrift, and 16.5% (63/381) were treated for respiratory disease. The average first treatment event lasted for 3.8 ± 3.2 days (range: 1 – 31) in duration, and occurred at 10.3 ± 9.2 (range: 1 – 53) days after introduction to the group pen. First, second and third treatments were evenly distributed throughout the days of the week. The mortality rate in the group pen was 0.6% (4/653) and 18.5% of calves (121/653) were treated before they entered the group pen.

Of the 117 sick calves selected for the matched pair analysis, 34.2% (40/117) were treated for diarrheal disease, 52.1% (61/117) were treated for ill thrift, and 13.7%

(16/117) were treated for respiratory disease. Calves treated for diarrhea were treated at an average of 9.6 ± 7.3 days on the feeder for an average of 2.3 ± 2.1 days duration. Calves treated for ill thrift were found at 11 ± 8.1 days on the feeder and were treated for an average of 3.3 ± 1.9 days. Calves treated for respiratory disease were treated at 18.4 ± 9.2d in the group pen for an average of 2.1 ± 1.6 days.

6.4.4 Visit Level Feeding Behaviors

Visit. For all 653 calves, there were 305,083 total visits to the feeder. Visits that were less than 5s (n= 10,660) in duration were considered to be due to ear tag reader dysfunction and visits that were greater than 1500 s (25 min at the feeder) (n = 645) were strong outliers (> 3 interquartile ranges from the third quartile (Tukey, 1977)) and thus were removed from the data set leaving 293,778 total visits to the feeder for analysis. There were 98,740 visits to the feeder where the calf drank a milk meal. Of the 98,740 rewarded visits that resulted in a milk meal, visits that were less than 5 s (n = 376), visits that were greater than 1500s (n=365), visits to the feeder in which the calf drank less than 100ml of milk (n=382), and visits in which a calf had a drinking speed of less than 100ml/min (n=4151) and greater than 3000ml/min (n=366) were omitted from analysis, leaving 93,727 calf visits to the feeder for analysis. Omissions of rewarded visits were considered either as strong outliers as defined by Tukey (1977) (drinking speed > 3000ml/min; visit time > 1500s) or implausible based on biology or computer feeder malfunction (consumption < 100ml; drinking speed < 100ml/min; visit time < 5s). Calf visits to the feeder followed a bimodal distribution with respect to time of day of visit

(Figure 1): Visits to the feeder without a milk meal (unrewarded) peaked from 6 – 8AM and again from 5 – 7PM. Visits to the feeder in which the calf drank (rewarded) peaked from 4 – 6AM and from 7 – 8PM.

Overall, the average visit to the feeder lasted 228 ± 212 s. The average visit where the calf drank lasted 231 ± 124 s and a calf drank 1806 ± 597 ml of milk at a speed of 546 ± 237 ml/min. Ten point seven percent of all visits resulted in the calf standing in the feeder for an average of 17 ± 71 seconds before starting to drink, and 78.4% of visits resulted in the calf standing in the feeder for an average of 131 ± 153 seconds after finishing drinking. Calves drank less, drank slower, and stood in the feeder for a longer period of time during visits when they were undergoing treatment for a disease event, as compared to visits on days when they were not being treated. (Table 2).

Quarter Day Average Descriptive Statistics. From the original 653 calves enrolled, there were 92,620 quarter day average rewarded and unrewarded visits to the feeder. Visits to the feeder that were less than 5 s (n= 152) or greater than 1500 s (n = 234) were removed leaving 92, 234 total visits to the feeder for analysis. There were 72,262 rewarded visits to the feeder in which the calf drank. The same exclusion criteria were used as in the visit level data, leaving 70, 263 total QDAY visits for analysis. Calves tended to drink their largest meal of the day (1,969 ± 443ml) from 12AM – 6AM, with the average meal size then decreasing over the rest of the 24 hr period. Drinking speed (ml/min) was fairly consistent throughout the day. Average total time at the feeder per QDAY decreased almost one minute throughout the day, with the average in the

morning of 336 ± 207s decreasing to 275 ± 174 s from 6PM – 12AM. Total average drinking time decreased throughout the day, though only by an average of 20s. (Table 3)

The same patterns in QDAY average CON, DS, TVT and TDT were observed over the course of a 24 hr period as the feeding period progressed. Of additional interest, QDAY average CON increased from the ramp up to the peak milk period and then decreased during the ramp down period, which correlates to the average feeding plan of calves in this study. Calves increased their QDAY average drinking speed over the course of the feeding period, at the same time decreasing both their total time and total drinking time at the feeder. (Table 4).

6.4.5 Matched Pair Analysis

QDAY Visit Average Milk Consumption. There were 117 pairs of calves available for the matched pair analysis. The final model describing the association between QDAY visit average milk consumption and disease status over the ±5 days surrounding an illness event controlled for the effect of age at treatment, farm, group size, QDAY from treatment (-20 to +20), and repeated measurements on calf. Over the 5 days surrounding a treatment event, sick calves drank 67 ± 78 ml/visit ($P = 0.043$) less than matched healthy calves. On the QDAY of illness detection by farm personnel, sick calves drank 139 ± 77 ml ($P = 0.071$) less than matched healthy calves. Sick calves drank significantly less in visits to the feeder in the QDAY (6 hour time period) prior to illness detection, but this difference did not persist. (Figure 2). Calves treated for ILL showed a similar pattern, with sick calves drinking significantly less in the QDAY prior to disease

detection, but there was no difference in QDAY consumption at any time between matched pairs of DIA and RESP calves. (Results not shown).

QDAY Visit Average Drinking Speed. The final model describing the association between illness and visit average drinking speed controlled for the effect of age at treatment, farm, group size, QDAY from treatment and repeated measurements on calf. Overall, sick calves drank 77 ± 29 ml/min ($P = 0.0052$) slower than healthy matched calves in the ±5 days surrounding a treatment event. On the same QDAY of disease diagnosis, sick calves drank 125 ± 29 ml/min slower ($P < 0.0001$) than matched healthy calves. Sick calves drank more slowly on QDAYs -4, -3, from -1 to +2 and then from +4 to +5 QDAYs as compared to healthy calves. (Figure 3). Calves treated for ILL had significantly slower drinking speeds in QDAY -1 to QDAY +2 and +4 to +5 as compared to matched healthy controls. Calves treated for DIA drank more slowly in the QDAY they were found to be sick and QDAYs +2, +4, and +9. Calves treated for RESP had no difference in their drinking speed at any time as compared to matched healthy calves. (Results not shown)

QDAY Visit Average Total Time at the Feeder. The final model describing the association between an illness event and the QDAY total average visit time at the automatic calf feeder (rewarded and unrewarded visits) controlled for the effect of farm, QDAY from treatment, month of the year, and repeated measurements on calf. Over the ±5 days surrounding a treatment event, sick calves stayed at the feeder for 23 ± 25

seconds longer per visit (P = 0.011) than a matched healthy control calf. On the QDAY of illness detection, sick calves stayed at the feeder an average of 45 ± 25 (P = 0.071) seconds longer than a healthy calf. Sick calves spent significantly longer in the feeding stall in the QDAY prior to treatment, and then from +2 to +4 QDAYs post treatment. (Figure 4) Calves treated for DIA and ILL had no differences between sick and healthy calves in the QDAYS leading up to illness detection, but calves treated for RESP stayed at the feeder longer than matched healthy calves -2 QDAYs prior to disease detection. (Results not shown)

QDAY Visit Average Drinking Time at the Feeder. The final model describing the association between an illness event and visit average drinking time at the feeder controlled for the effect of farm, group size, QDAY from treatment and repeated measurements on calf. Over the entire ±5 day observation period, sick calves stayed at the feeder drinking 23 ± 20 s longer (P = 0.0006) than healthy calves. On the QDAY of illness detection, sick calves had a total drinking visit time that was 78 ± 19 s (P < 0.0001) slower than matched healthy calves. Sick calves were at the feeder drinking for a significantly longer time on QDAY -6, and then from -4 to +5 QDAYs surrounding the time of illness detection. (Figure 5). Calves treated for ILL drank at the feeder for significantly longer -3 and then from -1 to +3 QDAYs from treatment. Calves treated for RESP showed significantly longer drinking visit times -3 QDAYs from illness detection. Calves treated for DIA showed differences on QDAYs -4, 0, +2, and +4 from when they were detected as sick. (Results not shown).

6.5 DISCUSSION

Group housing and computerized feeding of dairy calves is growing in popularity among dairy producers, yet calves are more likely suffer from morbidity and mortality in these systems (Losinger and Heinrichs, 1997; Svensson et al, 2003). Management strategies that help to predict and detect disease are of great interest to personnel who work with group housed calves. Calves do change their feeding behaviors when they are ill (Svensson and Jensen, 2007; Borderas et al, 2009), but these differences have not led to sensitive or timely detection when calculated as daily averages. To the authors' knowledge, this is the first study to describe the association between QDAY visit average feeding behaviors and producer reported illness events. If associations are found to be present, then further study can investigate the potential utility (e.g. diagnostic test characteristics, timeliness) of using QDAY (6-hr) average feeding behaviors as a means of predicting or detecting morbidity in group housed calves. Furthermore, if evaluation of QDAY average feeding behaviors shows improvements in timeliness or sensitivity in predicting or detecting morbidity as compared to day level averages (for the previous 24-hr period), then software in computer feeder systems may potentially be reprogrammed to calculate and report QDAY average feeding behavior measures, instead of 24-hr averages.

To the authors' knowledge, this is the first study to describe the association between visit average feeding behavior and morbidity in group housed preweaned dairy calves. Strengths of this study include that it was performed in multiple commercial herds

in two regions in multiple seasons. We also had a relatively large sample size with many observations per calf. However, the majority of calves in this study represent the Holstein breed and all farms utilized the same automatic feeder type with a relatively high milk allowance. As such, these results may not be generalizable to calves of other breeds or farms that provide less milk per day with alternative feeding systems.

Matched Pair Analysis - Association between morbidity and feeding behaviors

The results of our matched pair analysis showed that calves with illness drink less per visit, drink more slowly, and spend more time in the feeding stall, both overall and during a drinking bout in the six hour time period prior to illness detection as compared to healthy matched controls, with some behaviors changing up to 36hrs prior to detection. Acute illnesses such as calf-hood diarrheal or respiratory disease, is accompanied by generalized malaise, often characterized by behavioral changes such as lethargy and anorexia (Hart, 1988). Visit average milk consumption was only significantly different in the QDAY prior to illness detection by farm personnel (12AM – 6AM (difference); 6AM – 12PM (detection by farm personnel). This is similar to what was found by Borderas et al (2009) when daily average milk consumption was considered, but in contrast to what we found when exploring the same relationship with daily average milk consumption, where a sick calf had a decreased milk consumption two days prior to illness detection (Knauer et al, 2017). We speculate that this difference may be due to a decreased error when feeding behaviors are calculated as daily averages as compared to the QDAY visit averages described in this study. Additionally, the matched pair analysis only contained

234 calves, so some of the signal to noise problem might be teased out with a larger sample size.

Visit average drinking speed was a predictor of morbidity, with calves deviating from healthy controls one full day (4 QDAYs) prior to illness detection by farm personnel. This is not as timely as when drinking speed is considered as a daily average, as we found that sick calves have significantly lower daily average drinking speed two days prior to illness detection by farm personnel (Knauer et al, 2017). Total average drinking time at a visit to the feeder was a predictor of morbidity with calves spending more time at the feeder one day prior to illness detection by farm personnel. Differences in feeding time have been described as an indicator of morbidity in feedlot calves (Sowell et al, 1999) as well as in transition cows (Urton et al, 2005). However, in contrast to young calves that increase their time at the feeding stall, older animals decrease their time at the feeding bunk. Rewarded visits to the automatic feeder have not been shown to be a good indicator of morbidity in calves (Svensson and Jensen, 2007), as calves have a high motivation to drink milk. Early disease may just cause sluggishness, explaining why calves have an increased time at the feeder, while still maintaining their allotted milk intake.

Calves treated for ILL had similar patterns in CON and TDT as compared to all calves, but there was no real advantage for any of the visit level feeding behaviors for detection of a specific disease. ILL calves also represented over 50% of the matched pair cohort, so it is possible that we may see different results with more DIA or RESP calves in the data set if a larger sample size were available for the latter two disease conditions.

Because many calves were treated, we were limited on the number of control calves available for matching. However, the goal of using feeding parameters for disease prediction or detection would be to screen a pen of calves for signs of illness, regardless of the eventual disease diagnosis. To that end, DS and TDT show the most promise as parameters that may be useful for further consideration as a predictor of morbidity. Additionally, all calves did not have data for all time points, so some time points had more calves represented than others. It's possible that a sick calf did not go to the feeder when she was feeling sick, and therefore did not get a chance to have a decrease in DS or CON or stand at the feeder for a long time (increased TDT or TVT) biasing the results towards the null hypothesis.

To summarize; when visit level feeding behaviors were summarized in 6 hour intervals, calves with illness drink less per visit, drink more slowly, and spend more time in the feeding stall, both overall and during a drinking bout in the six hour time period before they were found as ill as compared to healthy matched controls. However, just because associations were found to exist between feeding behaviors and morbidity, readers should be cautious inferring that monitoring of these feeding behaviors will be good diagnostic tests to detect impending or current morbidity on farms. Further study is required to investigate the utility (e.g. diagnostic test characteristics, timeliness) of using feeding behavior changes to detect disease in group-housed computer-fed preweaned dairy calves. Furthermore, an evaluation will need to investigate whether day-level versus meal-level average feeding behaviors may be a better monitoring tool.

Though not a major objective, completion of this observational study allowed us to discover other new information surrounding the care and natural behaviors of group-housed computer-fed preweaned dairy calves. The following sections will discuss these observations including milk feeding management schemes used on farms, general calf health and general feeding behaviors.

Milk Feeding Management

Very few studies exist that describe the general management of group housed, pre-weaned dairy calves. However, calf management on our 8 farms was similar to that which has recently been reported to describe management in group feeder herds in the Upper Midwest US (Jorgensen et al, 2017). The diversity in feeding plans and ranges of milk offered during the feeding period in this study highlights the flexibility of automatic feeders to deliver milk to calves. Peak feeding level in this study was 8.8 ± 2.2L/d, which represents a fairly high milk allowance in the US (USDA, 2011). Calves allowed to drink ad libitum will drink an average of 9-11L/d (Jasper and Weary, 2002; Berberich and Grimm, 2013), suggesting that calves on these study farms were having their natural milk consumption behavioral needs met. Of additional interest are the large ranges of milk meal allowances throughout the milk feeding phases. Individual milk meals could range anywhere from 0.2L/meal up to 5L/meal, depending on the phase of the feeding period. This is important because it can influence visit level consumption, as well as visits to the feeder. Jensen (2004) reported that calves that were restricted to a smaller milk allowance per meal visited the feeder more frequently than calves allowed a larger milk allowance

per visit, despite the fact that both groups were allowed the same milk consumption on a per day basis.

General Calf Health

In our study, enrolled calves were managed individually for an average of 10 days before entrance to the group pen. Later entrance to the group feeder (> 12 d of age) has been associated with a reduction in the risk for respiratory disease (Svensson et al, 2006) and ease of adaptation to use of the feeder for milk delivery (Jensen, 2007). However, age of the calf at introduction is influenced by many things including producer preference, calving pressure, and space allotment, both in the group pens and in individual housing. Although weight measures were available for only 16% of calves in our study, then average daily gain of 0.71kg/d observed is comparable to what others have reported in calves being fed similar amounts of milk (Appleby et al., 2001; Jasper and Weary 2002). As expected, calves that were treated for illness during the time in the group pen had poorer growth as compared to calves that remained healthy (Virtala et al, 1996).

In our study, 58.4% of calves had a first treatment event while they were in the group pen. This rate is within the range of disease incidence rates that has been previously reported for group-housed calves in the literature, ranging from 42 to 78% (Svensson and Jensen, 2007; Roth et al, 2009). Important to note, first treatments that occurred before entrance to the group pen (n=121 calves) were not included in the first treatment rate. Forty two of those calves went on to be healthy for the remainder of the

preweaning period, while the remainder had at least one treatment event while they were housed in the group pen.

General Feeding Behaviors

Many management and calf factors can influence feeder utilization and feeding behaviors. Jensen (2004) reported that group and meal allowance size can influence time spent in the feeding stall. Jensen and Holm (2003) reported that milk flow rate can also influence feeding behaviors. In another study, Herskin et al (2010) reported that hunger levels and tubing diameter can influence behaviors: the larger the diameter the tube and the hungrier the calf, the faster the calves drank. Another factor that can influence calf behavior are the settings of the auto feeder itself. Automatic feeders are designed so that the calf has an allocation that builds over the course of the day in the form of meal credits. In the Forster-Technik operating system, a meal credit is calculated as the total allotment / 20 hours, the other four hours of the day allowing for things like feeder cleaning and maintenance. The average peak feeding level for all calves in the present study was 8.8 ± 2.2 L/day with an average minimum allotment of 1.7L/meal. In the peak feeding period of calves in this study, meal credits are accumulated at the rate of 8.8 L / 20hrs or 0.44L/hr. If we further assume that the minimum meal size is 1.7L/meal and that the clock starts at 12AM, the average calf in this study can have her first milk meal at approximately 3:45AM, and then approximately every four hours thereafter until the maximum daily allotment of 8.8 L is reached. Figure 1 shows the first peak in calf drinking activity to occur around this time; calves start to increase their rewarded

(drinking) visits to the feeder between the hours of 3 to 4AM. The feeder usage for drinking is then mostly steady throughout the course of the day with another peak occurring between 7 and 8PM. It is possible that calves learn when their allotment is forthcoming and are more likely to enter the feeder and drink during that time, but this hypothesis requires investigation. Calves enter the feeder without an entitlement in a higher proportion after the peak in drinking from 7 to 8AM, and then have another peak in unrewarded visits from 5 to 7PM. These two times of calf activity may correspond to the usual times that farm personnel would be in the barn performing management activities, and so it is possible that human activity in the barn has the potential to influence this calf activity. The overall pattern in feeder usage in the present study is very similar to the one reported by Jensen (2004) where the highest proportion of total visits to the feeder occurred between 6 – 8AM and then again from 5 – 7PM.

The average visit to the feeder over the entire feeding period lasted 228s or 3.8 minutes. The average visit to the feeder where the calf drank milk was slightly longer, lasting approximately 231s or 3.85 minutes. Very few studies have reported visit level behaviors, but several Danish studies have reported feeder usage on a min/24h period basis. Jensen (2004) reported that calves fed a similar amount of milk (8L/d) averaged 16.2 min/24hr period drinking, with a total time at the feeder of 36.7 min/24hr period. Calves in the present study had an average of 4.3 and 7.2 rewarded and unrewarded visits to the feeder, respectively (reported in Knauer et al., 2017). This corresponds to a total drinking time of 16.5 min/24hr period drinking and 43.9 min/24hr total time at the feeder, which is consistent with Jensen (2004). Jensen (2004) also reported a mean calculated

drinking speed of 432 ml/min for heifer calves housed in a group of 24, which is comparable to the overall average of 546 ml/min that was found in the present study. It is important to note that the DS reported in this manuscript represents a calculated number, whereas the drinking speed reported by others typically represents a parameter that is automatically measured directly by a meter located in the feeding mechanism of the auto-feeder. We chose this approach because visit level drinking speed is not currently reported in the institute function, but daily average DS has been reported to be associated with an illness event (Knauer et al, 2017).

When considering all 653 calves, calves that were undergoing treatment drank less milk per visit, drank more slowly, and were in the feeding stall for a longer period of time as compared to days when a calf was not undergoing treatment. Borderas et al (2009) reported a decrease in milk consumption per day in the first two days after an illness event, in agreement with our results. Additionally, these same authors reported that calves fed > 8L/d of milk spent an increased amount of time at the feeder (approx. 7 min/visit vs. 4.5 min/visit) during days two and three post illness detection. Sick calves in our study spent an average of 4.5 min/visit as compared to healthy calves (3.75min/visit). However, when DS is considered, Svensson and Jensen (2007) found no difference between sick and healthy calves, in contrast with our results.

When considering averages of meal level feeding behaviors in 6 hour increments (QDAY), calves in our study tended to have their biggest meals during the first visit of the day. Meal size increased from the ramp up to the hold feeding phase, and then decreased from the hold to the ramp down, following the feeding plan of the automatic

feeder. Drinking speed increased over the course of the feeding period. Total time at the feeder, both with and without milk consumption decreased as the calves got older. Jensen and Holm (2003) reported that calves gradually decreased their time at the feeder from approximately 70 min/calf/day down to approximately 30 min/calf/day from weeks 3 to 9 of life. As calves get older and, assuming they remain healthy, they naturally become more robust and able to consume the same or similar amounts of milk in a shorter period of time. This may be important when considering changes due to illness, as older calves may have more subtle changes in feeding behaviors than a younger, more susceptible calf.

6.6 CONCLUSIONS

The results of this prospective observational study indicate that visit average feeding behaviors are associated with morbidity in group housed pre-weaned dairy calves. In the matched pair analysis, an increase in total visit drinking time at the feeder or a decrease in visit drinking speed were associated with a producer reported illness event up to one day before the calf was found by farm personnel. As such, these two parameters have potential utility as tests for the prediction or detection of calf morbidity in group housed pre-weaned dairy calves. However further investigation is warranted to understand the diagnostic test characteristics and timeliness of using visit average feeding behaviors to predict and detect disease in group housed pre-weaned dairy calves.

Table 6. Descriptive calf level statistics of enrolled calves, both overall and by matched pair analysis.

	All Enrolled Calves (n=653)		Matched Pair Calves (n=234)			
Variable				Treated Calves		Control Calves
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Age at Pen Entry	653	10.4 (5.4)	117	11.2 (4.7)	117	11.1 (4.9)
Wean Age	404	55.7 (7)	73	53.6 (5.2)	80	54.5 (5.7)
Age at Pen Exit	511	59.0 (13.9)	89	55.9 (12.6)	89	56.8 (11.3)
Days in Pen	653	50.1 (18.1)	117	45.8 (18.2)	117	46.6 (17.8)
Serum Total Protein (g/dL)	170	5.7 (0.8)	30	5.9 (1.1)	35	5.7 (0.8)
Birth Weight (kg)	104	42.4 (5.1)	21	43.4 (7.0)	18	41.1 (3.5)
Wean Weight (kg)	37	78.7 (11.2)	3	77.3 (8.2)	8	79.2 (8.8)
ADG (kg/d)		0.71		0.68		0.75

Table 7. Visit average feeding behaviors for visits to the feeder on sick and healthy days.

	Healthy Calf Visits[1]			Sick Calf Visits[2]		
Variable	N	Mean ± SD	Range	N	Mean ± SD	Range
Milk Consumption (ml)	86687	1809 ± 593	(100, 5150)	6571	1768 ± 636	(100, 4000)
Drinking Speed (ml/min)	86687	551 ± 235	(100, 3000)	6571	475 ± 247	(100, 3000)
Total Visit Time (sec)	276031	226 ± 212	(5, 1500)	16532	259 ± 223	(5, 1500)
Drinking Visit Time (sec)	86687	228 ± 121	(5, 1431)	4993	271 ± 149	(6, 1407)

[1]Helathy calf Visits = any visit when the calf was in the group pen and was not being treated
[2]Sick Calf Visit = any visit when the calf was being treated

Table 8. Visit average feeding behaviors by time of day in group housed preweaned dairy calves.

	12 AM – 6AM		6AM – 12PM		12PM – 6PM		6PM – 12AM	
Variable	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Milk Consumption (ml)	15007	1969 ± 443	18713	1950 ± 541	18632	1935 ± 522	17911	1659 ± 592
Drinking Speed (ml/min)	15007	558 ± 222	18713	543 ± 217	18632	551 ± 225	17911	512 ± 222
Total time at the feeder (s)	20568	336 ± 207	24294	312 ± 201	24081	298 ± 189	23291	275 ± 174
Total Drinking time at the feeder (s)	15007	246 ± 119	18713	251 ± 125	18632	248 ± 129	17911	224 ± 121

Table 9. Visit average feeding behaviors by time of day and feeding period.

		12 AM – 6AM		6AM – 12PM		12PM – 6PM		6PM – 12AM	
Variable	Feeding Period[1]	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Milk Consumption (ml)	ru	1399	1701 ± 494	2127	1724 ± 520	1860	1681 ± 541	1824	1535 ± 582
	h	12024	2002 ± 434	13732	1983 ± 515	14042	1964 ± 520	13102	1712 ± 575
	rd	1515	1960 ± 382	2783	1961 ± 453	2670	1961 ± 470	2919	1495 ± 631
Drinking Speed (ml/min)	ru	1399	454 ± 201	2127	460 ± 221	1860	443 ± 209	1824	430 ± 215
	h	12024	560 ± 220	13732	546 ± 211	14042	551 ± 220	13102	517 ± 218
	rd	1515	643 ± 219	2783	600 ± 222	2670	632 ± 230	2919	545 ± 235
Total Visit Time (s)	ru	1868	381 ± 237	2862	383 ± 251	2459	367 ± 221	2463	331.7 ± 213
	h	14904	349 ± 199	16764	317 ± 191	16835	307 ± 185	16263	280 ± 170
	rd	3713	263 ± 204	4755	258 ± 189	4693	235 ± 167	4474	227 ± 152
Total Drinking Time (s)	ru	1399	267 ± 138	2127	276 ± 152	1860	279 ± 151	1824	262 ± 150
	h	12024	249 ± 119	13732	253 ± 123	14042	251 ± 127	13102	230 ± 121
	rd	1515	207 ± 90	2783	224.8 ± 103	2670	210 ± 94	2919	180 ± 87

[1]Feeding period = ru; ramp up, the period when calves are increased to their peak milk allowance: h; hold, time period when calves are held at the peak feeding level: rd; ramp down, time period when calves are slowly weaned from milk.

Figure 5. Proportion of visits to the feeder with (rewarded; n = 93,727) and without (unrewarded; n = 195,729) a milk meal for group housed, automatically fed pre-weaned dairy calves over a 24 hour period.

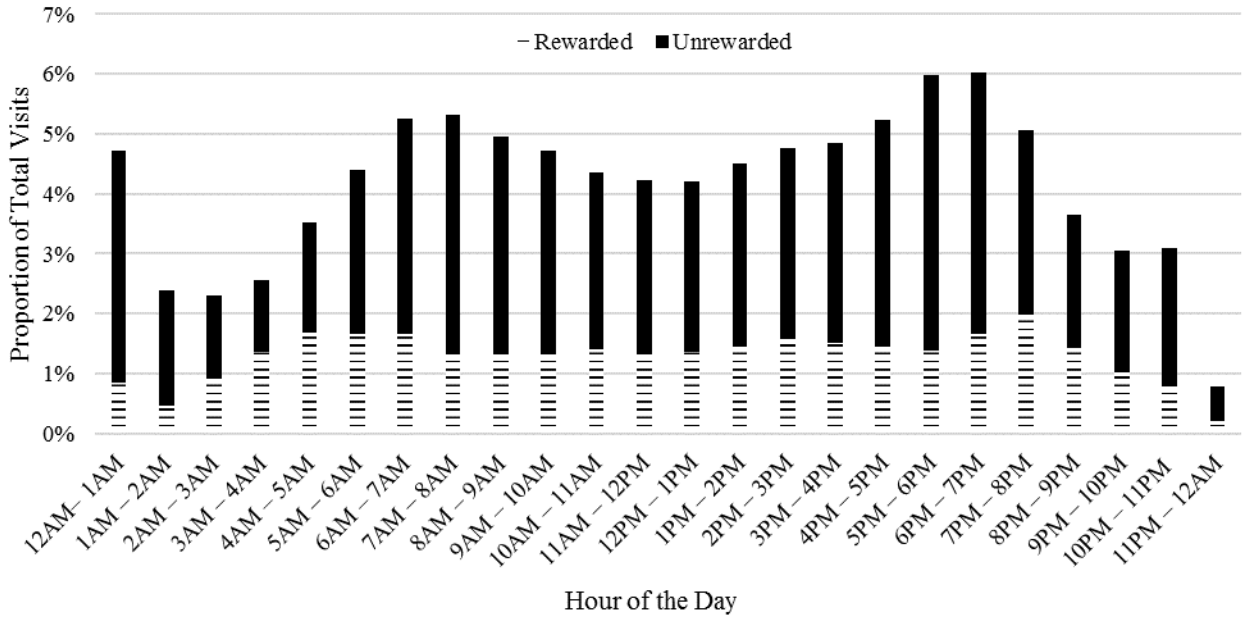


Figure 6. Results of a linear mixed model describing the association between time from a treatment event and average visit level milk consumption (ml) in matched pairs of healthy (n=117) and treated (n=117) calves. The model controlled for the effect of age at treatment, farm, group size and repeated measurements by calf. Reported are adjusted means and SEM. (* = $p < 0.05$)

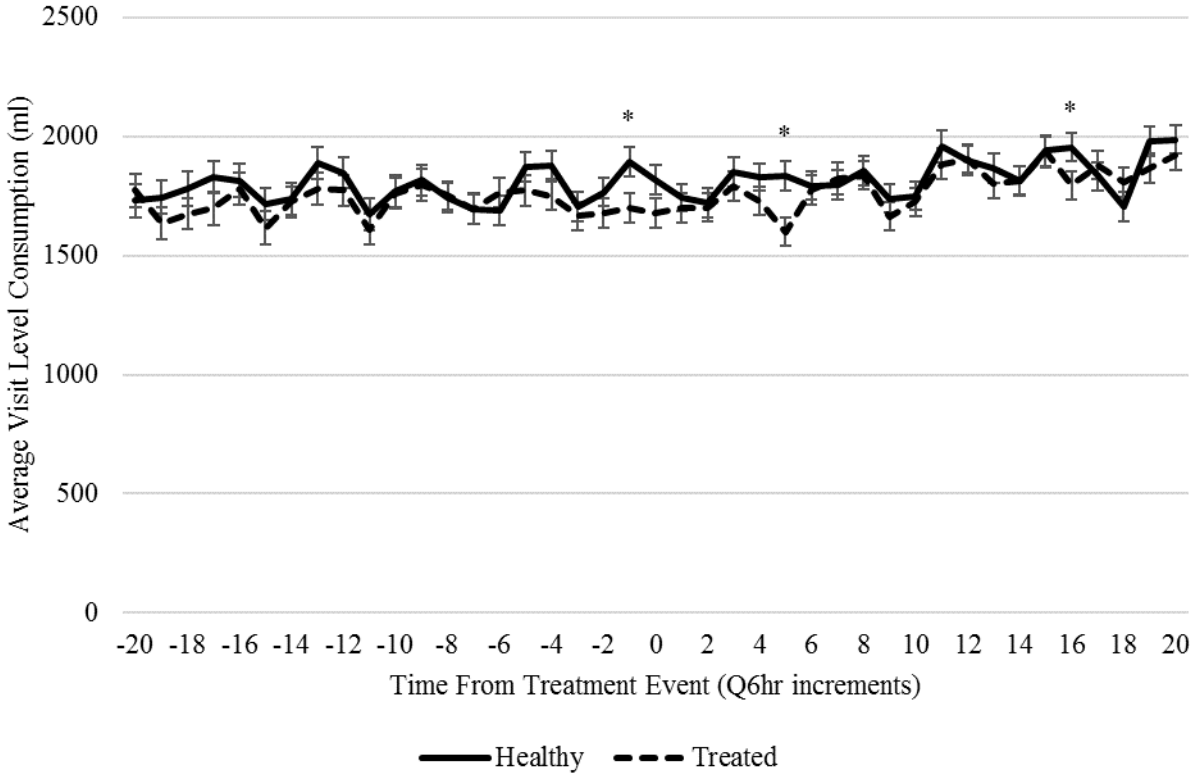


Figure 7. Results of a linear mixed model describing the association between time from a treatment event and visit average drinking speed (ml/min) in matched pairs of healthy (n=117) and treated (n=117) calves. The model controlled for the effect of calf age at treatment, farm, group size and repeated measurements by calf. Reported are adjusted means and SEM. (* = $p < 0.05$)

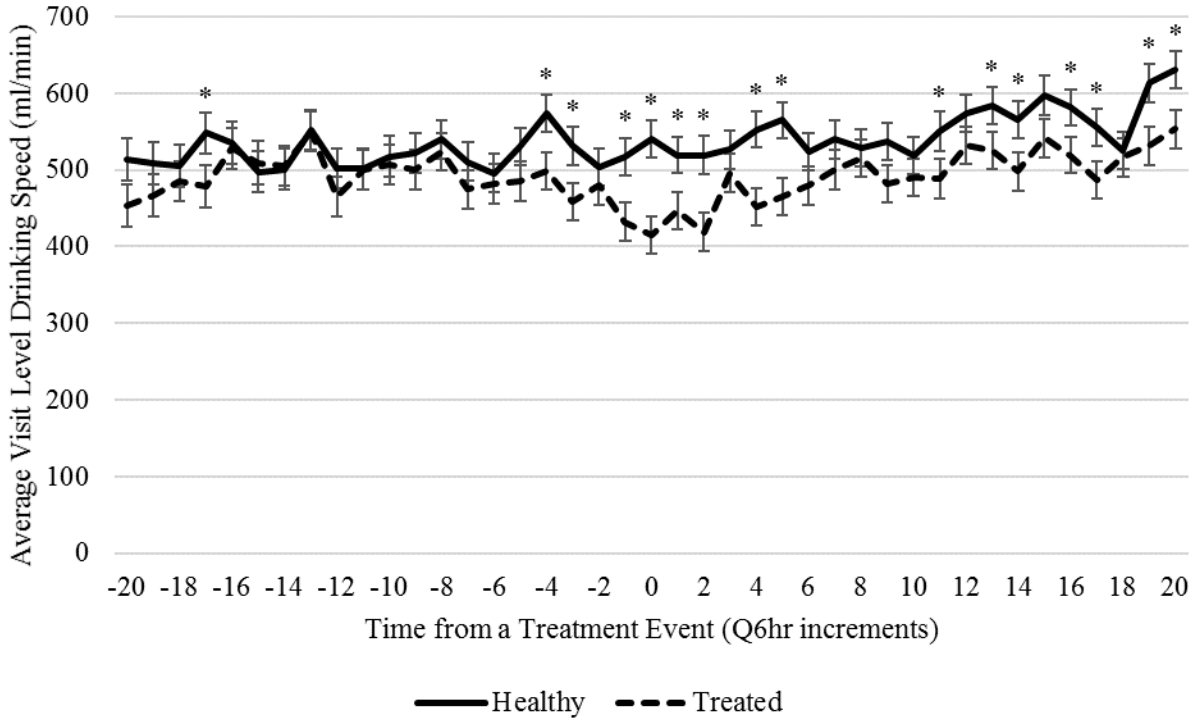


Figure 8. Results of a linear mixed model describing the association between time from a treatment event and visit average total time at the feeding station (sec) in matched pairs of healthy (n=117) and treated (n=117) calves. The model controlled for the effect of farm, month, and repeated measurements by calf. Reported are adjusted means and SEM. (* = $p < 0.05$)

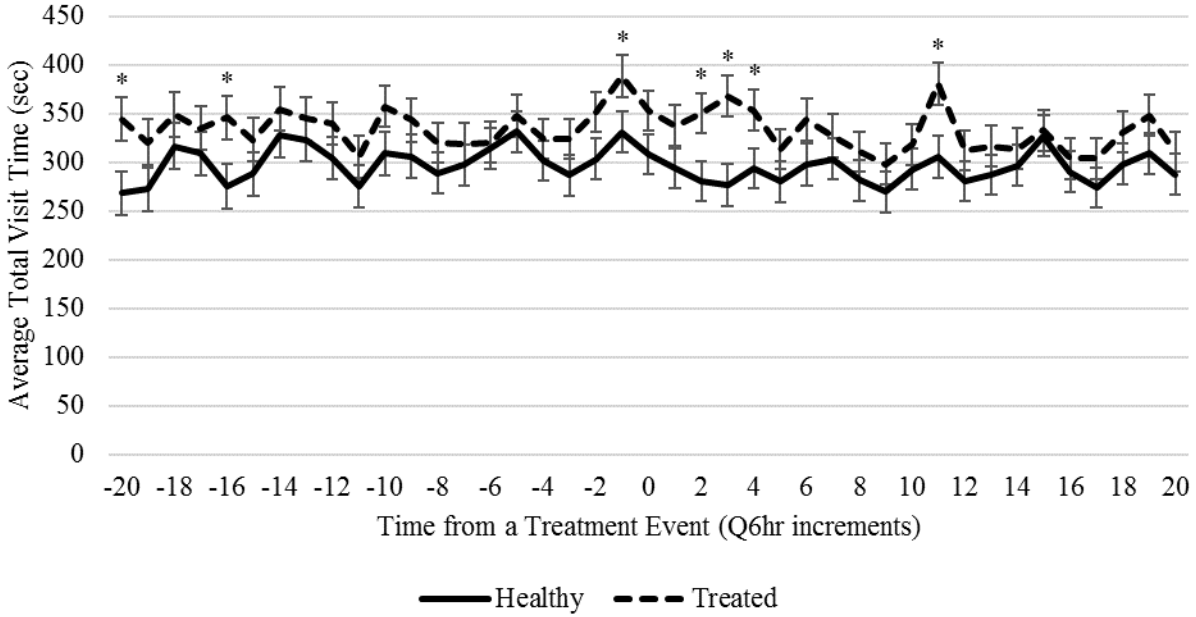
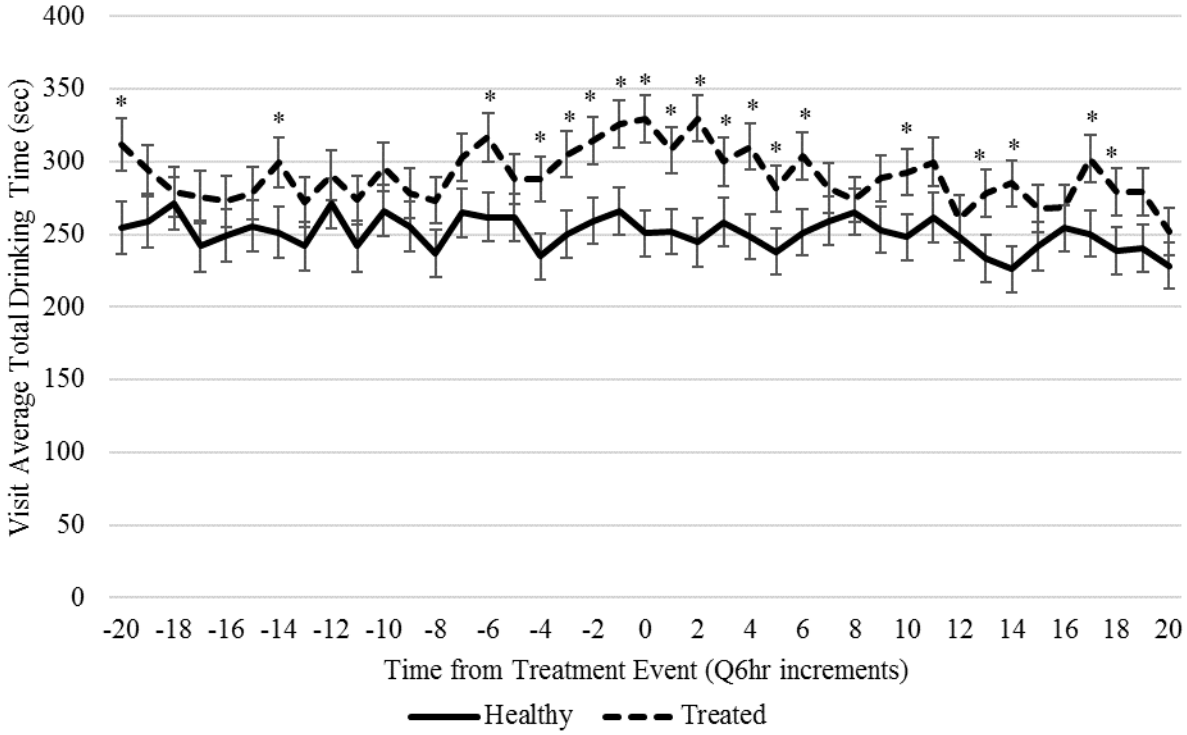


Figure 9. Results of a linear mixed model describing the association between time from a treatment event and visit average drinking time at the feeding station (sec) in matched pairs of healthy (n=117) and treated (n=117) calves. The model controlled for the effect of farm, group size, and repeated measurements by calf. Reported are adjusted means and SEM. (* = $p < 0.05$)



7 CHAPTER FIVE

The application of statistical process control techniques to visit average feeding behaviors to detect disease in automatically fed group-housed pre-weaned dairy calves.

W.A. Knauer, A. Dietrich, R.E. James, D.M. Hawkins, and S.M. Godden

7.1 SUMMARY

The aim of this prospective cross sectional study was to investigate the application of statistical process control techniques to visit average feeding behavior data to predict or detect calf morbidity in automatically fed group-housed preweaned dairy calves. This study was conducted on eight farms in MN (n=3) and VA (n=5) utilizing group housing and computerized feeding from March until August 2014. Calves were enrolled upon entrance to the group pen. Farm personnel recorded morbidity and mortality events. Farms were visited every week (MN) or every other week (VA) to collect enrollment data, computer derived visit level feeding behaviors, and farm personnel derived health records. Self-starting cumulative sum (**CUSUM**) charts were generated for each calf for each six hour average (**QDAY**) visit level feeding behavior including drinking speed (**DS**; ml/min), milk consumption (**CON**; ml/visit), and total drinking time at the feeder (**TDT**; sec). A testing subset of 234 calves (117 treated; 117 healthy) was first used to develop SPC control limits to optimize sensitivity and timing of the SPC signal. The parameters with the optimal sensitivity and timing were then applied

to all calves (n=653). Generalized estimating equations were used to estimate the diagnostic test characteristic of an SPC signal (index test) to detect a sick calf (reference test) for a single QDAY visit level feeding behavior. Combinations of feeding behavior signals were explored using parallel and series interpretation. Drinking speed, TDT, and CON in parallel interpretation provided the most sensitive (89.4 (86.2, 92.0)) and timeliest test finding a sick calf an average (±SD) of 6.5 ± 9.4 d prior to diagnosis by farm personnel. However, the specificity of this same combination was very low (7.7% (4.5, 12.9)), and there was no real advantage to using any of the one, two, or three way combinations of these behaviors when considering predictive values. The results of this study suggest that the use of SPC self-starting CUSUM control charts applied to DS, CON, and TDT QDAY visit level feeding behavior provides high sensitivity to predict or detect a sick calf. However, low specificity and poor predictive values of the test demands that continued frequent observation of calves by skilled calf personnel will be needed to identify sick calves and to differentiate false from true positives.

7.2 INTRODUCTION

Group housing and automatic feeding of dairy calves is growing in popularity (USDA, 2014; Medrano – Galarza et al., 2017) due to labor benefits for the farm (Kung et al, 1997), and nutritional (Jasper and Weary, 2002; Berberich and Grimm, 2013) and social benefits (Jensen et al., 1997) for the calf. However, calves managed in large groups (>7), typical of systems in the US (Jorgensen et al, 2017), are at an increased risk for morbidity (Svensson and Liberg, 2006) and mortality (Lonsinger and Heinrchs, 1997) when compared to small groups or calves housed individually. Because disease detection

can be more challenging when calves are housed in groups, producers using group housing systems must develop systems to detect, diagnose and treat illness in a timely manner.

A potential advantage of delivering milk to calves automatically (i.e. through computer feeders) rather than manually is that computerized feeding system software can record and report feeding behaviors for monitoring purposes at the individual level. Dairy calves change their behavior in response to disease challenge. Malaise, characterized by lethargy, anorexia, and social isolation, is driven by physiological changes that occur during infective and infectious disease (Hart, 1988). Observational studies have reported that morbid pre-weaned calves have a higher risk of lethargy (Cramer and Stanton, 2015), drink less milk (Borderas et al, 2009), drink more slowly (Knauer et al, 2017), and visit the feeder less frequently (Svensson and Jensen, 2007) than healthy calves though these changes are not consistent across studies. An induction model using very low levels of lipopolysaccharide, representative of early and mild infection, reported a reduction in time ruminating and eating hay, and in increase in time spent lying inactive in the 4hrs surrounding peak rectal temperature, demonstrating that even mild disease can result in measurable behavioral changes (Borderas et al, 2008).

As such, computer reports of feeding behavior have the potential to be useful to predict or detect morbidity in calves. However, no study thus far has reported an advantage to disease detection when using feeding behaviors (milk intake (L/d); total visits (visits/d)) calculated as daily averages or visit averages (visit duration (min/visit))

as compared to visual detection by a veterinarian (Borderas et al, 2009). Automatic calf feeders can also record and report individual visit level feeding behaviors including visit level milk consumption (ml), visit level drinking speed (ml/min) and time in the feeding stall (s). Due to more information (more time points per day), visit level feeding behaviors may offer an advantage in sensitivity and/or timing of disease detection, though methods to monitor these behaviors in individual animals for disease detection purposes have not been formally evaluated.

Statistical process control (**SPC**) is a statistical monitoring technique that has traditionally been used in manufacturing to monitor process performance over time (Hawkins and Olwell, 1998). Different methods of SPC have more recently been applied to animal health monitoring, both at the individual (Quimby et al., 2001; Lukas et al, 2008a; Miekley et al., 2013) and group level (Lukas et al, 2008b; Madsen and Kristensen, 2005). The cumulative sum chart offers an advantage over other SPC techniques in that it can detect small shifts in a process mean. Quimby (2001) reported that they could detect morbidity in feeder steers up to 4.5d earlier than pen riders using cumulative sum charting techniques. Similar methods, applied to hourly pen level water consumption, determined that a diarrhea outbreak in a pen of piglets could be predicted up to 1 day prior to onset (Madsen and Kristensen, 2005). Though timing is important, it is also important to understand the diagnostic test characteristics of test use in the field, as producer adoption also depends on accuracy of the given test. In a prior analysis, we evaluated the use of SPC analysis of daily averages of feeding behaviors (i.e. total daily milk intake, L/day; daily average drinking speed, ml/min; unrewarded visits to the feeder,

count) to predict or detect morbidity. We found that daily average consumption and drinking speed, when considered in combination, to have the highest sensitivity (70.9%) and found sick calves an average of 3 days prior to diagnosis by farm personnel. However, there was no advantage to any feeding behavior either alone, or in combination when predictive values were compared (Knauer et al, in preparation). We hypothesized that visit level feeding behaviors may have improved sensitivity and timeliness over daily averages, as subtle changes in behavior may be more apparent with more data available for the same time period. The objective of this study was to describe the diagnostic test characteristics and timing of signal when applying SPC techniques to visit level individual animal feeding behaviors to predict or diagnose disease in automatically fed group housed pre-weaned dairy calves. We hypothesized that SPC, when applied to visit level feeding behaviors, would predict and detect disease in a sensitive and timely manner when compared to detection by trained farm personnel.

7.3 METHODS

The use of animals in the study was approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol #1308-30844A). This manuscript was prepared according to the Standards for Reporting Diagnostic accuracy studies (STARD Group, 2015).

7.3.1 Herd Selection

This prospective cross-sectional study was conducted in a convenience sample of eight commercial dairy herds; three herds in Minnesota and five herds in Virginia. Herds

were selected based on their use of an automated calf feeding system (Forster-Technik®, Engen, Germany) that must have been in use for greater than one year's duration. Herds must also have had the capability to install the Institute Function (Forster Technik, Engen, Germany) in the autofeeder hand-held device so that visit level feeding behaviors could be recorded. Herds all provided a high peak daily milk allowance of $\geq$ 7L per day at a total solids level of >125g/L (g milk powder added to 1L of water).

7.3.2 Calf Management and Data Collection.

Data collection occurred from March - October 2014. An initial questionnaire was used to describe calf facilities and general calf management. Calves were enrolled into the study when they entered the group pen, and exited the study when they were weaned from the automatic feeder. For each calf entering a group pen, the calf manager recorded the calf id, breed, birth date and pen entry date. Sick calves were identified based on daily subjective evaluations by the calf manager, and the date, time, treatment, and disease treated was recorded for each morbidity event. Mortality events were recorded similarly. Case definitions were standardized across farms through training and use of a visual scoring system that evaluates ocular and nasal discharge, cough, head tilt, fecal score, and general attitude (https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf).

A study technician visited the farm each week (MN) or biweekly (VA), to collect calf enrollment data, treatment records and mortality data. Visit average feeding behavior

data was collected at each farm visit from the automatic feeder hand-held (Institute Function, Förster-Technik®, Germany) at each visit and stored in an SD card (SanDisk, 2GB, Milpitas, CA) until analysis. Specific feeding behaviors either recorded or calculated included milk intake (**CON**) (ml/visit), average drinking speed (**DS**) (mL/min), total visit time (**TVT**) (s) and total drinking time (**TDT**) (s). Specific calculations are described elsewhere (Knauer et al, in preparation). An 8ml venous blood sample was collected from the jugular vein of a convenience sample of calves (n=170) between 24h to 7d of age for serum total protein measurement (g/dL) with a digital serum refractometer (MISCO Palm Abbe Model PA203X, MISCO, Cleveland, OH). Approximate birth (1d – 7d of age) and weaning weights (50d – 60d of age) were estimated in kg from a convenience sample of calves (n=104 birth weights; n=37 wean weights) using a weight tape (Nasco, Fort Atkinson, WI). Farm personnel were blinded to sensor derived visit level feeding behavior data throughout the study.

7.3.3 Case Definitions.

Disease diagnosis was based on visual assessment by the on-farm calf caregiver and attempts were made to standardize across farms. A case of diarrhea (**DIA**) was defined as visible diarrhea (very loose or watery feces; fecal score of 2 or 3 on a 0-3 scale) as well as treatment with antibiotics, electrolytes, or IV fluids, or a combination of the three. A case of respiratory (**RESP**) disease was defined as a calf with an increased respiratory rate or effort, cough, plus treatment with antibiotics. A case of ill thrift (**ILL**) was defined as either 1) a calf that had a rectal temperature > 39.5˚C; 2) a calf that was depressed but for which the caregiver did not have a clear diagnosis or; 3), a calf with

other miscellaneous illnesses such as umbilical infection, joint infection, or injury, and treatment with antibiotics and/or non-steroidal anti-inflammatories. Treatment events known to be prophylactic (i.e. antibiotic administered to all calves at pen entry) occurred on one farm and were excluded from analysis. Duration of treatment was defined as the period between the first and last treatment. A new treatment event was defined as; an event that occurred for the first time or; an event that occurred at least 5 days after conclusion of treatment for a previous event or; an event that occurred within 5 days of the conclusion of a previous event but represented a different disease diagnosis.

7.3.4 Statistical Analysis

Initial Investigation of SPC model parameters. In order to identify those feeding behaviors that might be more useful in the SPC analysis, a matched pair analysis was first performed to determine the difference in visit average feeding behaviors (averaged in 6 hour increments (**QDAY**); 12AM-6AM, 6AM – 12PM, 12PM – 6PM, 6PM – 12AM) in a farm-, pen- and age-matched pair of sick and healthy calves (n=117 pairs) around the time of a treatment event (described by Knauer et al., in preparation). The matched pair analysis showed that a reduction in visit average DS and an increase in visit average TDT were significantly associated with a treatment event both before and during the event. Visit average CON was only significantly lower during the QDAY prior to illness detection in sick calves. However, because daily average CON was found to be associated with illness (Knauer et al., 2017), it was still included as a parameter of interest for visit average SPC analysis. Self-starting CUSUM charts were generated for

QDAY visit average DS, TDT, and CON for all calves during the milk feeding phase as described by Hawkins and Olwell (1998) using R Statistical Software (V3.3.0, R Core Team, Vienna, Austria). The CON feeding behavior data had some sequential repetitive numbers (i.e. Visit 1 = 1500ml; Visit 2 = 1500ml) at the beginning of the feeding period which is problematic as the CUSUM chart cannot function properly with a variance of "0". Therefore, a random number between 0 and 0.01 was added to all of the CON visit average measurements for all calves in order to add sufficient variation. A training data set (n=234) was used to tune the CUSUM chart, including exploring different upper (**UCL**) and lower control limits (**LCL**). Control limits are chosen based on the cost of type I and type II errors, which should be limited (DeVries and Reneau, 2010). In a self-starting CUSUM chart, the time to a positive signal (average run length (**ARL**)) is defined by three parameters: Δ, k and h, where Δ is defined at the smallest shift in the process mean to be detected. "k" is the magnitude of the deviation of an observation from the target, and is often referred to as the reference value. In self-starting CUSUMs, "k" is typically set to 0.5 (Hawkins and Olwell, 1998). "h" is the magnitude required to conclude that the process is out of control and defines the UCL and LCL. "h" is typically referred to as the decision interval, and "h" values of 3.0, 2.5, 2.0 and 1.5 were tested with these visit level QDAY averages. Exploratory data analysis revealed a positive association between visit average feeding behaviors and age. Individual calf visit DS increased over the time the calf was in the group pen. Visit level CON followed a curve similar to the feeding plan of the calf; increasing in the first days in the pen, then holding at a maximum feeding level, then decreasing as the calf was weaned from milk. Visit level TDT decreased over the feeding period. These data characteristics have the

potential to be problematic as they can lead to an increase in Type 1 error. Therefore, we standardized individual calf QDAY visit averages to the QDAY visit average of all of the calves in the data set. Then, self-starting CUSUM charts were created for each calf at each decision interval for both standardized and raw visit average feeding behavior data.

A data base was then created that contained the date, QDAY and type of SPC signal (**PM** = positive mean; **NM** = negative mean; **PV** = positive variance; **NV** = negative variance) at each of the decision intervals for both raw and standardized visit average feeding behaviors (CON, DS, TDT). General descriptive statistics were generated to describe the direction and type of signal generated by the control chart, as well as to describe the SPC signal date as compared to the date that a first treatment event was recorded for treated calves. Based on sick calf behavior around the time of illness, a positive NM signal was chosen as the index test for CON and DS and was defined as any NM signal that occurred during the time the calf was in the group pen. A positive PM signal was chosen as the index test for TDT, and was defined as a PM signal that occurred during the time the calf was in the group pen. The reference standard was defined as a producer reported first treatment event. Treatments were defined as DIA, RESP and ILL.

Diagnostic test characteristics for an NM (CON, DS) or PM (TDT) SPC signal to detect a sick calf as compared to an observer were calculated for each of the feeding behaviors, at each of the decision intervals from 2 x 2 tables. Diagnostic test characteristics included sensitivity (**Se**), specificity (**Sp**), accuracy (**Ac**), positive

predictive value (**PPV**) and negative predictive value (**NPV**). The Se was defined as the proportion of truly sick calves (as identified by the producer) that had a positive SPC test. The Sp was defined as the proportion of healthy calves (never diagnosed by the producer as sick) that were negative on SPC (no signal). Accuracy was defined as the total proportion of tests that correctly identified a sick or healthy calf. Positive Predictive Value was defined as the proportion of positive NM/PM tests that correctly identified a sick calf, and NPV was defined as the proportion of negative NM/PM tests that correctly identified a healthy calf (Dohoo et al., 2009). The test characteristics for each decision interval for each feeding behavior were determined. For those calves that achieved a positive SPC test and were diagnosed as sick at some time while in the pen (true positives), the timeliness of the signal was assessed based on subtracting the date of the SPC signal from the date that the producer identified the calf as sick. After completing the SPC analysis for all treatment events, this analysis was repeated for each individual disease diagnosed (RESP, ILL or DIA). The decision interval and data method (raw or standardized) with the optimal sensitivity and signal timing for each feeding behavior to detect a sick calf was identified from the training set and applied to all calves in the data set.

SPC Diagnostic Test Characteristics for Entire Study Population. After completing the previously described analysis using the training data set (n=234 calves), the optimal SPC data form and decision interval identified for each feeding behavior (DS = raw, 1.5; CON = raw, 1.5; TDT = raw, 2.0) were applied to QDAY average visit level feeding behaviors of the entire data set consisting of 653 calves. All self-starting CUSUM charts were

created using R (v. 3.3.0, R Core Team, Vienna, Austria). Descriptive statistics calculated included timing of signal relative to day on the automatic feeder, and for treated calves, the timing of the signal relative to the first day of treatment. For those calves that achieved a positive SPC test and were diagnosed as sick at some time while in the pen (true positives), the timeliness of the signal was assessed based on subtracting the date of the SPC signal from the date that the producer identified the calf as sick. Diagnostic test characteristics (Se, Sp, Ac, PPV and NPV) of using a single positive NM/PM signal to detect a sick calf were estimated using generalized estimating equations, accounting for the random effect of pen. First, univariable models were generated to describe the relationship between NM/PM Sig (Y/N) and predictors of interest including calf treatment (Y/N; main predictor of interest), calf age at signal, group size, age at pen entry, breed, pen, machine, QDAY, month, and farm. Variables that were significant $P < 0.20$ in a univariable model were then offered to the full model and backwards stepwise elimination was used until all variables remaining were significant at $P < 0.05$. The main predictor and outcome were then switched in the model to determine predictive values. Test characteristics and 95% confidence intervals were calculated from model output as described by Dohoo (2009). Finally, the utility of using feeding behaviors in combination was investigated. For the latter, the diagnostic test characteristics of using two and three way combinations of DS, CON and TDT were modeled similarly using both parallel and series interpretation. With series interpretation, only calves that test positive to both tests are considered test positive. With parallel interpretation, animals that test positive to one test, the other test, or both are considered test positive. (Dohoo, 2009). For determination of parallel interpretation signal timing,

calculations are from the date of the first signal. For series interpretation, calculations are from the date of the last signal. All statistical modeling was performed in SAS (V 9.4, SAS Institute Inc, Cary, NC).

7.4 RESULTS

7.4.1 General Calf Characteristics.

Descriptive statistics describing farm and calf characteristics are described in detail elsewhere (Knauer et al., in preparation). Briefly, of 7 herds with lactating cows, herd size ranged from 160 to 850 lactating cows; the 8th farm was a custom heifer grower. The eight herds represented 11 automatic calf feeders and 22 pens of calves enrolled, with each pen having one nipple feeding station. Average full feeding daily milk allowance offered was 8.8 L/d (range: 7 – 16), fed at a total solids concentration of 150g/L (range: 150 – 165). Calves were allowed an average individual meal size of 1.7 L/visit, ranging from 0.2 to 5L/visit depending on feeding stage. All farms managed calves in individual housing prior to introduction to the group, with average (±SD) age at introduction to the group pen at 10.4 (5.4), and with an average group size of 16 (6) calves per group. Six hundred and fifty-three calves were enrolled into the study, with 58.4% (381/653) experiencing a first treatment event for DIA (42.2%; 161/381), RESP (16.5%; 63/381), or ILL (36.6%; 147/381). First treatments occurred at an average of 10.3 (9.2) days after introduction to the group pen, and calves diagnosed with illness were treated an average of 3.8 (3.2) days duration.

7.4.2 Signal Timing

The following reports the total number of NM (DS and CON) and PM (TDT) signals, then average day of signal relative to either day in pen or day of treatment ± SD. When feeding behaviors were considered alone, the signal occurred earliest in the feeding period when using CON (n=434; 3.1 ± 2.1d), then DS (n=357; 3.9 ± 2.4d) and TDT (n=437; 4.5 ± 2.6d). Visit average CON also resulted in the most timely signal as compared to day of treatment in treated calves (n=257; -6.1 ± 9.5d), then DS (n=205; -5.4 ± 9.7d) and TDT (n=250; -4.1 ± 9.4d) (Table 1). When considering two NM signals and using either parallel or series interpretation, parallel interpretation resulted in the earliest and most timely signal as compared to series interpretation. The earliest parallel interpretation combination signal during the feeding period occurred when DS and CON were considered (n=518; 2.7 ± 1.7d), then TDT and CON (n= 578; 2.9 ± 1.8d), then TDT and DS (n=506; 3.5 ± 1.8d) (Table 1). The most timely signal relative to day of treatment was DS and CON (n=301; -6.6 ± 9.7) followed by TDT and CON (n=334; -6.2 ± 9.6), and DS and TDT (n=287; -5.5 ± 9.1). When considering all three feeding behaviors in combination, parallel interpretation resulted in the earliest (n=589; 2.6 ± 1.7d) and most timely (n=339; -6.5 ± 9.4d) signal (Table 1). The proportion of calves that signaled relative to when they were treated is reported in table 2. For a DS and CON signal in parallel combination (the best combination of two signals as it relates to signal timing), 32.2% of signals occurred 8 or more days before the calf was found as sick, 44.8% of signals occurred in the week leading up to illness detection. A total of 5.7% of signals occurred on the day the calf was found to be sick by farm personnel, and 17.3% of signals occurred after the calf was treated for illness.

7.4.3 Diagnostic Test Characteristics

Reported are point estimates and 95% confidence intervals of the diagnostic test characteristics estimated from the generalized estimating equations (Table 3). When considering the ability of a signal on a single feeding behavior to detect a sick calf, a signal on TDT visit data had the highest Se at 66.6% (60.1, 72.5) and a PPV of 52.9% (39.5, 65.8). However, the Sp and NPV were only 31.5% (23.6, 40.6) and 46.8% (34.7, 59.2), respectively. When considering two parameters in combination, a positive signal on CON and TDT interpreted in parallel had the highest Se to detect a sick calf with a Se of 87.9% (84.4, 90.7) and a PPV of 52.6% (39.6, 65.2). However, the Sp and NPV were only 10.1% (6.0, 16.6) and 43.7% (29.3, 52.9), respectively. Parallel interpretation of the three visit average feeding behavior parameters in combination resulted in a Se of 89.4% (86.2, 92), a Sp of 7.7% (4.5, 12.9), a PPV of 52.5% (39.8, 64.9), and a NPV of 41.3% (27.1, 56.7). The remaining diagnostic test characteristic results are reported in Table 3.

7.5 DISCUSSION

Housing calves in groups during the pre-weaning period is gaining in popularity worldwide despite an increase in disease incidence and the increased challenge of disease detection (Steenkamer, 1982; Svensson and Liberg, 2006). An advantage of automatic feeding systems over other manual milk delivery systems is their ability to record and report individual animal feeding behaviors. Thus far, investigators have reported differences between sick and healthy calves when feeding behaviors are reported as daily averages (Svensson and Jensen, 2007; Borderas et al., 2009; Knauer et al., 2017).

However no clear advantage has been reported for monitoring automatically captured day-level average feeding behavior data as a means to predict or detect disease, as compared to detection by farm personnel (Knauer et al., in preparation). We hypothesized that monitoring visit level feeding behaviors may have an advantage over day-level averages, in that more measurements are available per day, potentially increasing the timeliness and/or sensitivity to detect small changes in behavior. This is the first study to investigate the diagnostic test characteristics of individual animal visit level feeding behaviors to predict and detect disease in automatically fed, group housed pre-weaned dairy calves.

Signal timing relative to treatment

In the present study, an SPC signal on DS and CON in parallel combination provided an alert 6.6 days prior to a treatment event. Overall, visit average signal timeliness was improved over what has been reported for daily average signal timeliness. Daily average DS and CON in parallel combination provided a signal an average of 2.8 days prior to clinical diagnosis by farm personnel. (Knauer et al, in preparation). Studies using similar methodologies are uncommon, but report a detection advantage ranging from 1 to 4 days prior to disease onset/detection (Quimby et al, 2001; Madsen and Kristensen 2005). Quimby et al (2001) reported that SPC CUSUM charting could detect a sick calf in a feedlot up to 4.5 days prior to pen rider detection using an alert on decreased time at the feed bunk when time was calculated as 3 hour intervals. An increase in water consumption, calculated in one hour averages, signaled an SPC alert one day prior a diarrheal outbreak in weaned piglets (Madsen and Kristensen 2005).

While these comparisons are useful to describe general patterns, it is difficult to compare across species and studies. For example, pre-weaned dairy calves actually spend more time at the feeder when they are ill on a per visit basis (Knauer et al, in preparation) as compared to older animals that spend less time at the feed bunk during an illness event (Sowell et al, 1999; Urton et al, 2005). These results should be interpreted with caution, however, given the large variation in timing of signal relative to day of treatment.

When considering DS and CON in parallel interpretation, 32% of all signals occurred more than 8 days before a calf was found to be sick. Two possibilities exist for these findings. They could be animals that were showing very subtle signs of mild illness that were not observed by farm personnel. In this case, these signals are useful for the producer, as these calves could be detected and treated earlier, potentially leading to a better treatment outcome. Another possibility is that these are healthy calves very early in the feeding period when calves tend to have greater variation in their visit behavior as they learn to use the automatic feeder, which could then lead to false positive signals. To account for this possibility, we offered to control for calf age at pen entry, both as a continuous variable and as a dichotomous variable (calf $\geq$ 14d: Y/N), but the variable did not remain as significant in the final model. Jensen has reported (2007) that calves introduced at age 6d take longer to adapt to the group feeder than calves introduced at age 14d. We also explored the use of a standardized visit level feeding behavior in preliminary analysis, but this data processing technique did not perform any better than when the data underwent SPC analysis in its raw form. It is also possible that neither of

these techniques adequately controlled for this variation, and that other methods of data pre-processing should be explored.

Diagnostic Test Characteristics

Good test performance, defined as detecting as many problems as possible with as few false alarms as possible, is necessary for any sensor derived information to become an integrated livestock tool (Mertens et al, 2011). A general complaint among dairy farmers who utilize sensor derived information for health monitoring is the relatively large number of false positive alerts (Steeneveld et al, 2010). Over time, a high proportion of false positive results could lead to producer abandonment of the health monitoring system. We anticipate the use of this test as a screening tool in the field. Producers would be alerted to a signal, and the calf would then be checked by farm personnel for signs of morbidity. The highest sensitivity to detect a sick calf in the present study was when all three signals (DS, CON, TDT) were interpreted in parallel combination, though there was no clear advantage to any single, double or triple combination when considering both negative and positive predictive values. When considering three signals in parallel combination, the PPV and NPV were 52.2 and 41.3% respectively, meaning that 52.2% of the time, a positive test was a sick calf, and that only 41.3% of the time, a negative calf on SPC remained truly healthy. When comparing the test characteristics for day and visit level CON and DS, there is an improvement in Se when considering feeding behaviors as visit averages, but no other advantages in diagnostic test performance (Knauer et al, in preparation). We speculate that this increased test sensitivity is due to more data being available per time period in the visit

level data, but that this aspect of the data is also what is responsible for the reduction in Sp, and predictive values. As such, we conclude that SPC analysis of automatically captured meal-level feeding behavior data is not good enough to use as a stand-alone test to predict or detect morbidity in group housed calves. Steeneveld (2010) reported an improvement in test performance to detect clinical mastitis when sensor derived signals (conductivity, milk color) were combined with cow level indicators (visual inspection of milk, udder), suggesting that a combination of calf and robot factors should be explored as a possible means of improving disease prediction or detection.

It is difficult to find comparable work that describes the utility of using this automatically captured data to identify problems in pre-weaned dairy calves. In one of the only other examples to describe the test characteristics of applying SPC to the feeding behavior of cattle, Quimby (2001) reported a Se and Sp of 90% and 86%, respectively, when using feeding behavior (time in close proximity to the feed bunk) to detect morbidity in feedlot steers. While Se of the best combination of parameters in the present study (89%) is similar to the Se reported by Quimby, the specificity of SPC to detect morbidity in pre-weaned dairy calves in our study is very low (7.7%). One reason for this could be that feeding behaviors in older animal are more consistent than in younger animals. Behavioral synchronization is a risk reduction strategy for most social species (Estevez et al, 2013). When enough resources are available (i.e. feed bunk space, resting space), heifers and calves tend to engage in allelomimetic behavior (Keys et al., 1978; Faerevik et al., 2008). Social dynamics in adult dairy cattle are not altered if adequate space is available (DeVries et al, 2004). However, group housed calves do not have the

opportunity to engage in behavioral synchronization in computer-fed group-housed systems, as only one calf has the opportunity to drink at a time. If a calf has to wait too long, especially a young calf, she may go lay down and then not drink for a long time. Or, more aggressive older animals may displace younger more timid animals, leading to discouragement and timid feeding behavior. Both of these situations could lead to visit level feeding behavior changes in a healthy calf that could lead to an SPC alert. To try to alleviate some of this problem, starting the SPC monitoring later in the feeding period was considered. However, because the majority of calves in the present study were treated early in the feeding period, this strategy was ultimately abandoned as it would not be relevant to the needs of the producer.

Strengths and Limitations

This is the first study to investigate the use of visit level feeding behaviors to predict and detect disease in group housed preweaned dairy calves. Strengths include the fact that the study was conducted on 8 commercial dairy farms in 2 states (VA and MN), using computerized feeding equipment that is commercially available to producers, and using records from a sample of 653 calves. Furthermore, calf management (e.g. age at introduction to pen, milk feeding programs, group sizes) and calf morbidity rates are similar to previous reports of producers using similar systems (Svensson and Jensen, 2007; Roth et al., 2009; Jorgensen et al., 2017; Medrano-Galarza et al., 2017), thereby enhancing the generalizability of study findings.

The choice of a gold standard or accepted reference test is important when evaluating the performance of a sensor system to detect disease (Rutten et al., 2013). One

limitation of the current study may be that we used producers to detect disease as the reference method. Dairy producers are not very good at pre-mortem diagnosis of calf disease with reported Se of diagnosis of enteritis and pneumonia of 58 and 56%, respectively (Sivula et al., 1996). Unfortunately having a veterinarian perform daily examinations of all calves on 8 farms in the present study was unfeasible. Misclassification of calves in this study could result in two possible outcomes. First, if calves that were truly sick were missed by farm personnel, there are actually fewer false positives with SPC than it seems, resulting in a higher Sp and PPV for the SPC alert. A previously conducted cross-sectional validation on a subset of study farms supports this hypothesis, with a reported Se of producer detection of 26% as compared to an experienced veterinarian (Knauer et al., 2017). However, these results should be interpreted with caution, as the aforementioned study only represents a one day snapshot of producer performance in 4 herds. If calves were over-treated by farm personnel and the true incidence of first treatment events was lower, then the Se and NPV might be underestimated in the current study. If this methodology were used as a screening tool, in this situation, the user would be more confident in a negative result and less concerned about missing a sick calf.

The application of process control techniques to biological data is another challenge. The proper use of CUSUM charts requires that three assumptions are met: data are stationary, data are independent, and data are normally distributed (Mertens et al., 2011). Visit level calf feeding behaviors violate two of these assumptions. First, feeding behaviors are not stationary. CON changes with the feeding plan of the calf, DS increases

over the feeding period, and TDT decreases as the calf gets older. Non-stationarity can lead to an unstable process where the mean wanders about (Montgomery, 2013). Typically this is addressed though pre-processing of raw data, which we explored through standardization. However, this strategy did not improve test characteristics and in fact, made them worse when tested. Secondly, visit level feeding behaviors are not independent. When autocorrelation exists in the data, it can lead to too many false alarms (Montgomery, 2013), which may explain the poor Sp in the present study. Other process control techniques such as the Box-Jenkins autoregressive moving average model may be more appropriately applied to these data (Hawkins and Orwell, 1998), but this hypothesis requires further investigation.

Future studies should reevaluate the process control approach developed in this study when using trained veterinarians or other accepted gold standards as the referent test. Furthermore, other data analysis techniques such as machine learning should be explored before real time validation on the farm is performed.

7.6 CONCLUSIONS

The results of this study suggest that the application of CUSUM SPC control charting techniques to visit average feeding behaviors in group housed pre-weaned dairy calves may not be a useful test to predict or detect disease when used alone. The combination of signals TDT, CON and DS in parallel interpretation provided the most sensitive and timely test. However, when considering the very poor Sp and low PPV and NPV estimates, no single or combination of feeding behavior signals provided enough of

an advantage to abandon daily observation of calves. As such, it is still necessary to have well trained farm personnel with good observational skills observing calves daily for clinical signs of disease.

Table 10. The timing of the SPC signal (Negative Mean for DS[1] and CON[2]; Positive Mean for TDT[3]) relative to days on the calf feeder (n = 653 calves) and days from treatment for treated calves (n = 381 calves). The table presents mean ± SD (range).

				Signal Timing	
		N	Days on Feeder	N	Days from Treatment
One Signal					
DS		357	3.9 ± 2.4 (1, 22)	205	-5.4 ± 9.7 (-50, 18)
CON		434	3.1 ± 2.1 (1, 23)	257	-6.1 ± 9.5 (-50, 13)
TDT		437	4.5 ± 2.6 (1, 23)	250	-4.1 ± 9.1 (-46, 19)
Two Signals					
DS and CON	Parallel[4]	518	2.7 ± 1.7 (1, 20)	301	-6.6 ± 9.7 (-50, 16)
	Series[5]	273	4.1 ± 2.5 (1, 23)	161	-4.9 ±9.2 (-50, 9)
DS and TDT	Parallel	506	3.5 ± 1.8 (1, 22)	287	-5.5 ± 9.1 (-50, 18)
	Series	288	4.2 ± 2.7 (1, 23)	168	-4.7 ± 9.7 (-48, 19)
CON and TDT	Parallel	578	2.9 ± 1.8 (1, 20)	334	-6.2 ± 9.6 (-50, 16)
	Series	293	4.8 ± 2.8 (1, 23)	161	-3.5 ± 8.7 (-33, 17)
Three Signals					
DS, CON, TDT	Parallel	589	2.6 ± 1.7 (1, 20)	339	-6.5 ± 9.4 (-50, 16)
	Series	215	4.9 ± 2.9 (1, 23)	129	-3.5 ± 8.8 (-32, 17)

[1] DS = visit average drinking speed (ml/min)
[2]CON=visit average milk consumption (L/d)
[3]TDT = visit average time at the feeder while consuming milk (sec)
[4]Parallel interpretation = The SPC signal had to be positive for DS, CON or both for the test to be considered positive
[5]Series interpretation = The SPC signal had to be positive for DS and CON for the test to be considered positive

Table 11. Timing of SPC signal, relative to treatment date. Reported are number of signals (proportion of treated calves that signaled) at different intervals before, on the day of, or after the day of treatment.

		N[1]	N[2]	Signal 8 Days or more before Treatment	Signal 4 to 7 days before Treatment	Signal 1 to 3 days before Treatment	Signal on the Day of Treatment	Signal after the Calf was Treated
One Signal								
CON[3]		434	257	79 (30.7)	45 (17.5)	69 (26.9)	12 (4.7)	52 (20.2)
DS[4]		357	205	62 (30.2)	26 (12.7)	56 (27.3)	19 (9.3)	42 (20.5)
TDT[5]		437	250	60 (24.0)	32 (12.8)	67 (26.8)	21 (8.4)	70 (28.0)
Two Signals								
DS and CON	Parallel[6]	518	301	97 (32.2)	62 (20.6)	73 (24.2)	17 (5.7)	52 (17.3)
	Series[7]	273	161	47 (29.2)	15 (9.3)	49 (30.4)	11 (6.8)	39 (24.2)
DS and TDT	Parallel	506	287	82 (28.6)	46 (16.0)	83 (28.9)	25 (8.7)	61 (21.3)
	Series	288	168	45 (26.8)	23 (13.7)	47 (27.9)	13 (7.7)	40 (23.8)
CON and TDT	Parallel	578	334	102 (30.5)	68 (20.4)	81 (24.3)	21 (6.3)	62 (18.6)
		293	173	41 (23.7)	20 (11.6)	43 (24.9)	11 (6.4)	58 (33.5)
Three Signals								
ALL	Parallel	589	339	107 (31.6)	71 (20.9)	85 (25.1)	21 (6.2)	55 (16.2)
	Series	215	129	32 (24.8)	12 (9.3)	33(25.6)	9 (6.7)	43 (33.3)

[1] N= total number of SPC positive calves out of total calves (n=653)
[2] N = total number of SPC positive signal calves out of total calves treated (n=381)
[3]CON=daily average milk consumption (L/d)
[4]DS = daily average drinking speed (ml/min)
[5]TDT = daily average unrewarded visits to the feeder (count)
[6]Parallel interpretation = The negative mean SPC signal had to be positive for DS, CON or both for the test to be considered positive
[7]Series interpretation = The negative mean SPC signal had to be positive for DS and CON for the test to be considered positive

Table 12. Diagnostic test characteristics of SPC analysis of visit level feeding behaviors, alone or in combination and interpreted in series or in parallel, to predict or detect a sick calf. Point estimates are the result of generalized estimating equations controlling for the random effect of pen. Presented are mean (95% CI).

		Diagnostic Test Characteristics[1]				
		Se	Sp	PPV	NPV	Ac
One Signal						
CON[2]		62.0 (53.3, 70.0)	35.1 (25.9, 45.6)	50.9 (37.6, 64.0)	43.9 (31.8, 56.8)	54.1 (48.5, 59.6)
DS[3]		54.3 (47.5,60.9)	43.9 37.2, 50.9)	52.5 (39.8, 64.9)	46.4 (33.7, 59.7)	49.6 (45.3, 53.9)
TDT[4]		66.6 (60.1, 72.5)	31.5 (23.6, 40.6)	52.9 (39.5, 65.8)	46.8 (34.7, 59.2)	49.6 (42.5, 56.7)
Two Signals						
DS and CON	Parallel[5]	78.7 (73.9, 82.7)	19.9 (17.3, 26.0)	52.1 (39.5, 64.4)	43.9 (31.4, 57.2)	53.2 (45.9, 60.4)
	Series[6]	39.7 (32.4, 47.6)	59.2 (50.6, 67.2)	51.2 (37.6, 64.6)	45.9 (33.7, 58.7)	49.5 (44.0, 55.1)
DS and TDT	Parallel	76.8 (70.4, 82.3)	19.2 (13.9, 26.0)	52.3 (39.7, 64.6)	44.3 (31.6, 57.9)	49.3 (41.5, 57.2)
	Series	44.3 (37.9, 50.8)	56.0 (47.5, 64.2)	53.5 (39.5, 67)	47.4 (35.1, 60.0)	48.8 (44.1, 53.5)
CON and TDT	Parallel	87.9 (84.4, 90.7)	10.1 (6.0, 16.6)	52.6 (39.6, 65.2)	43.7 (29.3, 52.9)	51.4 (40.4, 62.3)
	Series	41.1 (34.0, 48.6)	56.9 (47.9, 65.4)	50.9 (37.4, 64.4)	45.7 (33.6, 58.4)	49.7 (46.1, 53.4)
Three Signals						
DS, CON, TDT	Parallel	89.4 (86.2, 92.0)	7.7 (4.5, 12.9)	52.5 (39.8, 64.9)	41.3 (27.1, 56.7)	51.1 (40.2, 61.9)
	Series	31.1 (24.6, 38.5)	68.9 (60.3, 76.4)	51.8 (36.9, 66.3)	46.5 (34.4, 59.1)	48.9 (42.9, 55.0)

[1] Diagnostic Test Characteristics: Se = sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; Ac = accuracy
[2] DS = visit average drinking speed (ml/min)
[3]CON=visit average milk consumption (L/d)
[4]TDT = visit average time at the feeder while consuming milk (sec)
[5]Parallel = The SPC signal had to be positive for DS, CON or both for the test to be considered positive
[6]Series = The SPC signal had to be positive for DS and CON for the test to be considered positive

8 CHAPTER SIX

Preliminary evaluation of an automated indwelling rumen temperature bolus measurement system to detect pyrexia in preweaned dairy calves

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8.1 SUMMARY

Indwelling rumen temperature bolus (**RTB**) systems have the potential to offer a convenient and timely method of detecting pyrexia, indicative of active infectious disease. The aim of this pilot study was to evaluate the utility of using RTB systems in preweaned dairy calves. First, an in-vitro study was performed to evaluate the accuracy of the RTB in its immediate environment. Thirteen RTBs were immersed in a hot water bath (**WB**). Variably collected RTB temperatures were then matched to WB temperatures, which varied from 36°C to 41°C, with one hr spent at each temperature. Second, an in-vivo study was performed to evaluate the ability of the RTB to predict a rectal thermometer (**RT**) temperature. 10 healthy heifer calves less than one week of age were administered an RTB. RT and matched RTB temperatures were taken hrly, over a 6 hr period, one day per week during weeks 1, 3, 5, 7 and 9 of age. During each 6 hr observation period, calves were offered both water and milk feedings, and temperatures recorded every 15 minutes for 1 hr thereafter.

For both studies, the relationship between RTB and either WB (in-vitro) or RT temperature (in-vivo) was described by calculating a concordance correlation coefficient (**CCC**), and by use of a multivariable linear regression model with repeated measures. For the in-vivo study, the model also controlled for week and breed. Diagnostic test characteristics were calculated for the ability of individual RTB measures to detect pyrexia (RT $\geq$ 39.5°C). Results showed that for the in-vitro study, the association between the RTB and a known temperature was strong (CCC = 0.95), but the RTB measures underestimated the temperature of the water bath by 0.43 ± 0.08˚C. For the in-vivo study, the association between RT and the RTB temperature measurement in a calf was weaker (CCC = 0.29); the average RTB temperature was 0.33 ± 0.06˚C lower than the RT temperature. The sensitivity (29%) and PPV (17%) of using individual RTB measures to detect a fever was low. The results of this pilot study suggest that an individual RTB measurement may not be a good diagnostic test to detect pyrexia in calves.

8.2 TECHNICAL NOTE

Early detection and treatment of calfhood diseases (e.g. scours, pneumonia) should result in a better short- and long-term outcome for the animal (McGuirk, 2008). Therefore, systems or management tools that can aid in early detection (and treatment) of calfhood disease are of interest to producers and veterinarians for animal well-being and the sustainability of the dairy enterprise. Rectal temperature is one of the most commonly used and most useful indicators of the relationship between a dairy calf, her disease status (e.g. diarrhea, pneumonia), and her physical environment (Liang et al, 2014) although

daily screening of rectal temperatures in dairy calves is labor intensive and so is not routinely performed on dairies.

Rumen temperature bolus (**RTB**) systems, which utilize an indwelling temperature sensing device to automatically monitor individual animal rumen temperature over time, have the potential to facilitate a more timely detection of some disease states. RTB systems are available commercially for use in adult cattle, and are newly available but not yet validated for use in calves. The Bella Ag Cattle Temperature System® (Bella Ag LLC, Loveland, CO) consists of the bolus, a data collector (antenna), and a software monitoring system. The calf bolus is 7 x 3 cm, weighs 45 grams, and contains a magnet, a battery, a temperature sensing device, and a data transmitter in a silicone base. It is orally administered soon after birth, sits in the reticulo-rumen of the calf and has a reported precision of ± 0.05˚C between 32.2 - 43.3˚C and an accuracy of ± 0.25˚C. The bolus automatically takes reticulo-ruminal temperatures and variably transmits these temperatures to a software monitoring program. Current software algorithms alert the user when the rumen temperature fluctuates by 1.1˚C above or below the average bolus reading for the calf. Though validation has been completed for various automated body temperature sensing systems, including rumen boluses, in adult dairy cows (Bewley et al, 2008a; Tismet et al, 2008; Rose-Dye et al, 2011), little is known about the accuracy and potential utility of using a RTB system to aid producers in detecting morbidity in preweaned dairy calves.

To address this question, our first objective was to describe the ability of the Bella Ag calf RTB system to accurately measure the temperature of its immediate environment, when placed in a water bath (**WB**). We hypothesized that the RTB measures would be within ± 0.25˚C of the WB temperature. A difference of 0.25˚C was chosen for both the in-vitro and in-vivo studies because it is considered by the authors to be both biologically and clinically important, and is within the company reported accuracy of the bolus. This study began with an in-vitro phase where thirteen RTBs were placed in a metal rack and completely submerged into a water bath (Blue M Magni-Whirl, Blue M Electric Company, Blue Island, IL). The water bath contained 8L of tap water, was continually agitated, and was heated to 36˚C and maintained at this temperature for one hr. Every hr, the WB temperature was increased by 1˚C covering a biologically important range from 36-41˚C (Smith, 2009). The temperature of the water was measured and recorded every 5 minutes using a liquid in glass (**LIG**) thermometer (Fisherbrand 76mm Immersion, Fisher Scientific, Pittsburg, PA) with a resolution of 1˚C and an accuracy of ± 0.5˚C. We expected each RTB to variably transmit a temperature measure to the computer software at least every 15 minutes. RTB readings that occurred in the 15 minutes after increasing the water bath temperature by 1˚C were removed from analysis to account for potential bolus temperature lag as described by Vickers et al., 2010. Time stamped RTB temperature measures were downloaded from computer software, and each WB temperature reading was then matched to RTB readings that were within +/- 2.5 minutes.

Our second objective was to describe the relationship between rectal thermometer (**RT**) and RTB temperature measures in growing dairy calves from 1 week of age to 3 weeks

post-weaning. Secondary objectives were to describe whether the relationship between rectal temperature and RTB measurements changed significantly with other factors, including the ingestion of water or milk meals, and to determine the test characteristics of the RTB to detect pyrexia when pyrexia was defined as a rectal temperature ≥ 39.5˚C (referent test). We hypothesized that the RTB measurement would be within ± 0.25˚C of the RT measurement, regardless of calf age, that ingestion of water or milk would not significantly change this relationship, and that RTB measures would accurately detect naturally occurring pyrexia in preweaned calves. The in-vivo study was conducted at the University of Minnesota Saint Paul Teaching Dairy, St. Paul, MN, from October 24, 2014 to January 16, 2015. The use of animals in this study was approved by the University of Minnesota Institutional Animal Care and Use Committee #1409-31832A. Ten female Holstein or Crossbred (Holstein x Jersey x Swedish Red) calves less than one week in age were examined by a licensed veterinarian and were enrolled if they were healthy. Eligible calves were orally administered a lubricated (KY Jelly, Johnson & Johnson, Markham, ON, Canada) Bella Ag calf RTB by the study technician at the time of enrollment. The bolus ID and calf ID number were recorded in the Bella Ag Temperature Monitoring Software (Bella Ag Cattle Temperature System®, Bella Ag LLC, Loveland, CO). Data collection for study calves occurred on one day every two weeks (during weeks 1, 3, 5, 7, 9 and 11 of age). On each data collection day, calves were moved from their outside hutches at 1100 hrs, weighed with an electronic scale, and brought to an indoor study area with the Bella Ag antenna mounted in the room. Calves were observed for 6 hrs, from 1200 to 1800 hrs. During this time, calf rumen temperature was variably transmitted to Bella Ag software and rectal temperatures were collected in duplicate

using a digital thermometer (Medline Industries Inc., Mundelein, IL; Accuracy: ± 0.1˚C) once per hr. Calves were offered 2L of room temperature (average T = 24 ˚C) water at 1400 hrs, and the evening milk feeding (average T = 38.1 ˚C) was offered at 1600 hrs. Time of feeding, milk/water temperature (˚C), and amount consumed (L) was recorded. Rectal temperatures were taken immediately prior to the milk/water meal and at approximately 15 minute intervals over the next hr. Average ambient temperature (˚C) within the barn was recorded. After each data collection day, automatically captured time stamped RTB temperature measurements were downloaded from farm computer Bella Ag software and manually matched to RT measurements that were collected within ± 10 min of each other.

Data analyses for both studies were performed in SAS (v 9.3 SAS Institute, Cary, NC) except where noted. In-vitro analysis included descriptive statistics to describe (mean, SD, range), WB temperature (˚C) and RTB temperature (˚C) and to check for normality and outliers. A Pearson's correlation coefficient (**R^2**) and a concordance correlation coefficient (**CCC**) were calculated to describe the correlation between WB temperature and RTB temperature measurement, plus a CCC plot was generated (v 14 STATA, College Station, TX). The difference between WB temperature and RTB temperature was described using a mixed linear model that explained the difference in temperature by device (WB/RTB). The random effect of experimental day and repeated measures for each bolus were accounted for in the model. Final significance was determined at $P<0.05$.

For the in-vivo portion of the analysis descriptive statistics were generated to describe (mean, SD, range) all continuous predictors and outcomes of interest including: RTB temperature (˚C), RT temperature (˚C), temperature difference (RT – RTB (˚C)), calf weight (kg), ambient temperature (˚C) during the data collection period, milk temperature (˚C), and water temperature (˚C). Strong outliers (defined as > 3 Inter-quartile ranges from the first and third quartile) were removed from data set as described by Tukey (1977). A Pearson's correlation coefficient, a CCC, and associated plots were generated for the association between RT and RTB temperature (v 14 STATA, College Station, TX). The difference in temperature measurement (dependent variable) between the RT and RTB (explanatory variable) was explored using a mixed effects linear regression model, with repeated measures on calf. The final main effects model included device, breed and week. Interactions were explored with the main predictor of interest (device x breed and device x week). Both interactions were significant and a stratified analysis was performed. Final significance was determined at $P < 0.05$.

Finally, diagnostic test characteristics (Sensitivity (**Se**), Specificity (**Sp**), Positive predictive value (**PPV**), Negative predictive value (**NPV**), and Accuracy (**Ac**)) of using a single unadjusted RTB measurement to diagnose pyrexia in a calf (RT $\geq$ 39.5˚C) were calculated using generalized estimating equations, accounting for repeated temperature measures, with calculations performed as described by Dohoo et al. (2009). True prevalence and apparent prevalence of fever, plus a kappa statistic to measure agreement between RTB and RT were also calculated (Dohoo et al., 2009). The referent test was defined as the RT temperature measurement.

Because the diagnostic test characteristics for using an unadjusted single RTB measurement to diagnose a fever were poor, additional secondary analyses were conducted to recalculate the diagnostic test characteristics after adjusting the RTB temperature measures using both the in-vitro and in-vivo model adjustments and evaluating different RTB cut-points, ranging from 37.5 to 39.5˚C in 0.25˚C increments, to declare a fever present. For the latter approach the Se, Sp and Ac for each RTB cut-point were calculated and then a Receiver Operator Characteristic (**ROC**) analysis was performed to identify the RTB temperature cut-point that maximized both Se and Sp to detect a pyrexic calf (v 14 STATA, College Station, TX).

In- vitro data were analyzed for 945 bolus temperature readings over 6 hrs (range: 144 – 176 readings/hr) and two experimental days (Day 1, n = 452; Day 2, n = 493). The average WB temperature was 38.6 ± 1.7˚C (range: 36.0 – 42.0) and the average RTB temperature was 38.2 ± 1.7˚C (range: 35.0 – 41.5). 98.2% of the variation in the RTB temperature reading could be explained by the WB temperature ($R^2 = 0.982$). The CCC was 0.952 (95% CI: 0.947, 0.957, $p < 0.001$). The RTB temperature was estimated to be 0.43 ± 0.08˚C lower (95% CI: - 0.58, -0.28, $p < 0.0001$) than WB temperature.

Seven Holstein and three Crossbred heifer calves were enrolled in the in-vivo study, and represent 235 and 84 temperature measurement observations respectively. No calves were treated for clinical disease during the study period (1 to 11 weeks of age). 319 RT measurements had a corresponding RTB measurement (within +/- 10 minutes). Week 11

consisted of only 7 observations and exploratory data analysis revealed 9 strong outliers in all remaining weeks (Tukey, 1977). These 16 observations were removed, leaving 303 total matched observations for the final analysis. Removed observations were equally distributed across week and calf. Mean (SD) RT and RTB measurements over the study were 38.9 ± 0.32˚C and 38.6 ± 0.43˚C, respectively. The Pearson's correlation coefficient for the relationship between RT and RTB temperature measurements was 0.40. The CCC for this relationship was 0.29 (95% CI: 0.21, 0.36, $p < 0.0001$).

The multivariable model estimated that RTB temperature was 0.33 ± 0.06˚C (95% CI: -0.44, -0.21, $p < 0.0001$) lower than the RT (Table 1). After stratifying by breed, analysis showed that the difference between RT and RTB temperature measurements was significant in Holstein calves (-0.30 ± 0.07˚C, $p = 0.004$) but this was not true for Crossbred calves (-0.04 ± 0.11˚C, $p = 0.74$). After stratifying by week, analysis showed that, during all study weeks but week 5 (-0.09 ± 0.07˚C, $p = 0.23$), the RTB significantly under-estimated the RT temperature measurement. Ingestion of water or a milk meal was not associated with temperature readings.

The prevalence of pyrexia (RT ≥ 39.5˚C) measurements in this study population was 4.6% (14/303) (Table 2). The following diagnostic test characteristics for RTB are reported as proportions (95% CI). Without adjustment, the crude Se and PPV of the RTB to predict fever was 0.14 (0.03, 0.48), and 0.25 (0.13, 0.43) respectively. After the RTB temperature adjustment the Se, and PPV of the RTB was 0.29 (0.07, 0.67) and 0.17 (0.09,

0.27) respectively. Results of other adjustments and test characteristics are reported in Table 2.

Results of this study suggest that the Bella Ag RTB measure of temperature is a good predictor of a known temperature in an in-vitro (water bath) model. The two measures were highly correlated, with RTB temperature estimated to be 0.43 ± 0.08˚C lower than the WB temperature. It is difficult to compare the results of this study to the results of others, given that different temperature sensing devices are not necessarily directly comparable. One possible explanation for why the RTB underestimated the WB temperature by 0.43˚C in the current study could be a systematic bias (error) in the calibration of the bolus device. Another explanation could be compounded imprecision of the bolus and the thermometer (Accuracy of ± 0.5˚C) used to measure the water bath temperature. The LIG thermometer was validated prior to use, but the large accuracy range could result in the bolus appearing to perform worse than it actually did.

In the in-vivo study a weaker relationship existed between the RT and RTB in calves (CCC = 0.29), as compared to the work of others studying adult cattle (Bewley et al, 2008a; Rose-Dye et al 2011; Timsit et al 2011). However, these former studies tested different devices and were performed in older animals, so it is difficult to compare across studies. The RTB underestimated the rectal temperature of calves by approximately 0.33 ± 0.06˚C. In adult animals, the rumen can be anywhere from 0.45˚C to 2˚C (Dale et al, 1954; Bewley et al, 2008a; Timsit et al, 2011) higher than RT, due to heat generating fermentation in the rumen. Conversely, Rose-Dye et al (2011) found that beef steers aged

6-8 months have a slightly higher RT than rumen temperature (0.13˚C), in agreement with our results. Sparse data at week 11 prevents us from concluding about the relationship after 9 weeks of age in this study population. Additionally, for this study we chose to use a simple digital rectal thermometer, typical of what would be used under field conditions by producers or veterinarians. However, it is possible that this instrument may have had different results as compared with a more precise measurement instrument.

We found that the difference between RT and RTB temperature was not significantly different in crossbred calves as compared to Holstein calves. Liang et al (2014) found that crossbred lactating dairy cattle have a lower reticulo-ruminal temperature as compared to purebred Holsteins. The interaction observed between device temperature measurement and week is difficult to explain, but may be in part due to a decreased number of RTB measurements recorded on week 5. There were issues with the system reading the bolus signals during some weeks. This is important to note, as reliability is an important factor affecting the utility of a heath management technology, potentially affecting the ability of the system to find sick animals in a timely manner.

Ingestion of water or milk did not cause an important change in RTB temperature measurement or an important change in the relationship (difference) between RTB and RT measures. Water ingestion has been shown to affect rumen temperature in adult cattle (Bewley et al, 2008b), but calves drink very little water (Kertz et al, 1984), so bolus temperature fluctuations due to water intake may not be a problem in preweaned animals. Additionally, the milk (average T = 38.1˚C) meal was not associated with a difference in

temperature measures during the hr immediately after ingestion, which is plausible as milk is typically fed at calf body temperature.

The prevalence of pyrexia measures in this study population of calves was low (5% (14/303) of measurements; 5/10 calves had a least one pyrexic event). Despite adjustment, the RTB had a low sensitivity to detect pyrexia in calves with an increased rectal temperature. This will be problematic if individual RTB measurements (either crude, model-adjusted, or cut-point adjusted) are to be used as a disease screening test on farms, as it will result in many calves with increased rectal temperatures being missed (false negatives). The predictive power of individual RTB measure to detect a fever was also quite low, which is undesirable for a screening test. However, infectious disease often results in prolonged increased temperatures and it is therefore conceivable that a calf with a prolonged increase in rectal temperature could be identified by this system with repeated RTB measures reported over an extended period of time. This hypothesis requires further investigation.

The current pilot studies provide some very useful preliminary information regarding the ability of the Bella Ag Cattle Temperature System® to accurately measure temperature using an in-vitro model, and is the first study that we are aware of to describe the relationship between RT and RTB temperature measures in growing dairy calves. More research is needed to investigate if evaluation of automated RTB temperature measures collected repeatedly over time may have more utility to detect pyrexic calves under field conditions.

Table 13. Final multivariable linear regression model describing the relationship between rumen bolus and rectal temperature measures in calves between 1 to 9 weeks of age.

Outcome Variable	Adjusted Mean (SE)	*b* Coefficient (SE)	95% CI	Type III *P* Value
Intercept	.	39.04 (0.06)	.	<0.0001
Device				
Bolus	38.74 (0.03)	-0.33 (0.06)	(-0.44, -0.21)	<0.0001
Rectal	38.96 (0.03)	referent		
Breed				
Crossbred	38.92 (0.04)	-0.031 (0.05)	(-0.13, 0.07)	0.013
Holstein	38.77 (0.24)	referent		
Time				
Week 1	38.69 (0.04)	-0.12 (0.08)	(-0.27, 0.03)	0.0002
Week 3	38.75 (0.04)	-0.13 (0.08)	(-0.28, 0.02)	
Week 5	38.86 (0.04)	-0.019 (0.09)	(-0.19, 0.15)	
Week 7	38.95 (0.06)	-0.066 (0.07)	(-0.21, 0.07)	
Week 9	38.94 (0.05)	referent		
Device*Breed Interaction				
Bolus*Crossbred	.	0.36 (0.05)	(0.27, 0.45)	<0.0001
Bolus*Holstein	.	referent		
Rectal*Breed	.	referent		
Device*Week Interaction				
Bolus*Week				
Week 1	.	-0.28 (0.07)	(-0.42, -0.14)	<0.0001
Week 3	.	-0.15 (0.07)	(-0.28, -0.01)	
Week 5	.	0.13 (0.08)	(-0.04, 0.29)	
Week 7	.	-0.04 (0.07)	(-0.18, 0.09)	
Week 9	.	referent		
Rectal*Week	.	referent		

Table 14. Test characteristics of the rumen temperature bolus to detect a fever (rectal temperature ≥ 39.5˚C) (rectal temperature = gold standard). Reported are the proportion and 95% Confidence intervals.

Test Characteristics	Unadjusted[1]	Model Adjusted[2]	Optimal RTB Cut-point[3]
True Prevalence	0.05 (0.02, 0.07)	0.05 (0.02, 0.07)	0.05 (0.02, 0.07)
Apparent Prevalence	0.03 (0.01, 0.04)	0.08 (0.05, 0.11)	0.49 (0.44, 0.55)
Sensitivity	0.14 (0.03, 0.48)	0.29 (0.07, 0.67)	0.71 (0.42, 0.89)
Specificity	0.98 (0.94, 0.99)	0.93 (0.82, 0.98)	0.52 (0.34, 0.69)
Positive Predictive Value	0.25 (0.13, 0.43)	0.17 (0.09, 0.27)	0.07 (0.02, 0.17)
Negative Predictive Value	0.96 (0.90, 0.97)	0.96 (0.89, 0.99)	0.97 (0.93, 0.99)
Accuracy	0.94 (0.89, 0.97)	0.90 (0.81, 0.94)	0.53 (0.36, 0.69)
Kappa	0.15 (0.11, 0.19)	0.16 (0.12, 0.20)	0.04 (0.02, 0.06)

[1]Unadjusted = unadjusted RTB temperature measures

[2]Adjusted = adjusted RTB temperature measures (Holstein = bolus temp + 0.3; Crossbred = bolus temp + 0.04) using the correction factor estimated from the final breed stratified multivariable regression model.

[3]Optimal RTB Cut-point = RTB fever defined as a RTB temperature ≥ 38.75˚C using the temperature cut-point that maximizes sensitivity and specificity to detect a rectal temperature ≥ 39.5˚C from the receiver operator characteristic analysis.

9 CHAPTER SEVEN

Evaluation of the diagnostic utility of an indwelling rumen temperature bolus system to predict and detect disease in automatically fed group housed preweaned dairy calves.

W.A. Knauer and S.M. Godden

9.1 SUMMARY

The objective of this observational cohort study was to evaluate the diagnostic utility of using an indwelling rumen temperature bolus (**RTB**) system to predict and detect disease in group-housed preweaned dairy calves. From Feb – Aug 2014, a random subset of Holstein heifer calves on 2 farms in MN were administered an RTB bolus in the first days of life, and were enrolled into the study upon entrance to the group pen. Morbidity and mortality events were recorded by farm personnel, who were blinded to the RTB data being collected. Herds were visited weekly by a study technician to collect calf enrollment, treatment, and individual calf RTB data. RTB temperature data was averaged by hour and six hour (**QDAY**) increments and described over a 24 hour period by hour, QDAY, farm and season. Multivariable generalized mixed models were built to describe the difference in RTB temperature for matched pairs of sick and healthy calves in the 16 days (-8 to +8) surrounding a treatment event. Final models controlled for the effect of month, farm, calf age at treatment, disease diagnosis, and repeated

measurements by calf. Models were also stratified by disease diagnosed. Statistical process control (**SPC**) and deviation and threshold analysis was then performed to determine the diagnostic test characteristics and timing of an RTB temperature alert to detect a sick calf. 281 calves were enrolled in the study, representing 155,473 hourly and 33,185 QDAY temperature readings. One hundred sixty two (57.7%) enrolled calves had a first treatment event. Diarrheal disease, respiratory disease and ill thrift represented 41.3%, 11.7%, and 47% (76/162) of first treatments respectively. Overall, sick calves had an increased RTB temperature (0.2 ± 0.09ºC) at the time of clinical diagnosis, which was detectable up to 24hrs prior to detection of the sick calf by farm personnel, but this difference varied by clinical diagnosis. When SPC and other deviation and threshold limits were applied to RTB temperature data to evaluate diagnostic test characteristics, SPC analysis of the RTB data provided the most sensitive 54.3% (50.7, 57.8) and timely signal, finding a sick calf an average of 2.4 ± 6.1 (-32, 19) days prior to clinical diagnosis by farm personnel. Using a different approach to RTB data analysis, exceeding a simple threshold value of ≥ 38.75ºC provided excellent sensitivity (98.6% (93.2, 99.7)) to predict disease, finding a sick calf 5.7 days prior to an illness event. However, the specificity was too low (0.8% (0.3, 2.3)) for this to be considered a useful test. For all methods of analyzing RTB data, positive and negative predictive values were low (Positive predictive value range: 57% to 65%; Negative predictive value range: 22% to 58%). The results of this study suggest that an indwelling RTB temperature measuring device may have limited utility when used as a diagnostic test to predict or detect disease in group-housed preweaned dairy calves.

9.2 INTRODUCTION

Group housing and computerized feeding of dairy calves is growing in popularity in North America (USDA, 2014; Medrano – Galarza et al., 2017) due to labor benefits for the farm (Kung et al, 1997), and nutritional and social benefits for the calf (Jensen et al, 1997; Berberich and Grimm, 2013). However, disease incidence is higher in group housed dairy calves as compared to calves housed individually (Svensson and Liberg, 2006). Furthermore, disease detection can be more challenging when calves are housed in a group (Steenkamer, 1982). In theory, precision dairy technologies, such as automatically captured feeding behavior, could be used as a monitoring tool to detect disease in group housed calves. However, no automated method of monitoring behavior or other biological parameters described thus far has been proven a useful predictor or indicator of disease onset in the field (Svensson and Jensen, 2007; Borderas et al, 2009; Cramer et al, 2016, Knauer et al, in preparation).

Pyrexia (fever) is a common physiological reaction to acute bacterial or viral invasion, and plays an integral role in host defense (Hart, 1988; Walter et al, 2016). Respiratory and diarrheal diseases, affecting approximately 36% of all preweaned dairy calves (USDA, 2007), are often accompanied by fever (Godihno et al., 2005; Rose-Dye et al., 2011; McGuirk, 2008). Fever has been shown to precede clinical signs of bovine respiratory disease by up to 4 days (Schaeffer et al., 2004), indicating that body temperature may be a useful indicator of disease onset in dairy calves. However, pyrexia is not exclusively caused by an inflammatory reaction to infection; exercise and elevated ambient temperatures can also result in elevated body temperature. Alternately, decreases

in body temperature in calves can occur in response to cold stress (Olson et al, 1980) or as a response to decompensation prior to death (Hankenson et al., 2013). As such, monitoring to detect derangements in body temperature could be a useful means of detecting disease or other ailments in calves. However, daily screening of rectal temperature in dairy calves is labor intensive, and so is not routinely performed on dairy farms, regardless of housing system.

Indwelling rumen temperature bolus monitoring systems are a precision dairy technology that are newly available for calves, and can automate continual temperature capture in individual animals. The Bella Ag Cattle Temperature System® (Bella Ag, LLC, Loveland, CO) consists of the rumen temperature bolus (**RTB**), an antennae and a software monitoring system. The calf RTB (7 x 3cm, 45g) is administered to the calf in the first days of life, and then continually records and transmits a reticulo-ruminal temperature to computer software. At the time of the present study, the cost of the system was approximately $1500 for hardware and software, with each bolus costing $45. Current software monitoring algorithms alert the calf manager when the reticulo-rumen temperature of the calf fluctuates by 1.1˚C above or below the average for an individual calf. Recent work has reported the sensitivity and positive predictive value of this RTB to detect a rectal temperature ≥ 39.5ºC in preweaned dairy calves to be low (Knauer et al, 2016). However, this system has not yet been formally evaluated to predict and detect disease in preweaned calves in a field setting.

Evaluating thresholds or deviations from average are methods commonly applied to sensor derived livestock data to detect disease or to monitor processes over time (Gonzalez et al, 2008; Stangaferro et al, 2016). Statistical Process Control (**SPC**) offers

an advantage to these methods in that SPC utilizes control charts to differentiate common case from special cause variation in a process over time (Hawkins and Olwell, 1998). Traditionally used in manufacturing, SPC has been newly applied to livestock data, and has been shown to be predictive of disease onset when applied to feeding (Quimby et al., 2001) and drinking (Madsen and Kristensen, 2005) behaviors, and to RTB temperature readings in feedlot bulls (Timsit et al, 2011). Self-starting cumulative sum (**CUSUM**) control charts are useful when there is no historical data available (Hawkins and Olwell, 1998). In this procedure, the first several observations are used to establish the mean and variation, and the mean is then updated with each new observation. CUSUM charts also have an advantage over other charting methods in that they are sensitive to small shifts (< 1.5SD), particularly of interest in biological processes (Mertens et al, 2011). Chart sensitivity is determined by upper and lower control limits, defined as ± SD by which the mean is allowed to vary. When the mean falls outside the upper or lower limits, as pre-determined by the user, the process is out of control and corrective actions are taken.

The first objective of this study was to complete a matched pair analysis to describe the association between calf morbidity and RTB temperature measurement in group housed preweaned dairy calves. We hypothesized that RTB temperatures will be increased before and during an illness event as compared to healthy calves. The second objective of this study was to apply SPC and other data analysis techniques to automatically captured RTB temperature data to describe the diagnostic test characteristics and timing of signaling, relative to disease diagnosis by a trained herdsman, when using these techniques to detect a sick preweaned group-housed dairy

calf. We hypothesized that SPC and other data analysis techniques of RTB data would provide a sensitive and timely diagnostic test to detect ill calves.

9.3 METHODS

The use of animals in this study was approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol #1308-30844A).

9.3.1 Herd Selection

This prospective observational cohort study was conducted on a convenience sample of two commercial MN dairy farms. Herds were selected based on their willingness to install the Bella Ag Cattle Temperature System® (Bella Ag LLC, Loveland, CO) in the calf barn, and their agreement to allow study technicians to administer RTB boluses to calves. Herds were further selected based on their use of an automated milk feeding system (Förster-Technik, Engen Germany) which must have been in place for greater than one year. Herds must also have provided a daily milk allowance of ≥ 7L per day at a total solids level of >125g/L (g powder added to 1L water).

9.3.2 Calf Management and Data Collection

Data collection occurred from Feb to Aug 2014. A questionnaire was administered at the beginning of the study to describe calf facilities and calf management. For an enrolled calf entering the group pen, farm personnel recorded calf id, birth date, and pen entry date. Trained farm personnel identified sick calves based on daily subjective evaluations and recorded the time, diagnosis, and treatment for each morbidity event. Mortality events were also recorded in this fashion. Case definitions were

standardized across farms through the use of a visual scoring system that evaluated ocular and nasal discharge, coughing, head tilt, fecal score and attitude (https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring _chart.pdf).

At each weekly farm visit, a random subset of healthy Holstein heifer calves over 35kg were orally administered a Bella Ag Calf Rumen Temperature Bolus (Bella Ag LLC, Loveland, CO) that were least 1d prior to entrance to the group pen. Briefly, the bolus (7 x 3cm, 45g) was lubricated (Priority Care ® General Lube, First Priority, Inc, Elgin, IL), the calf was gently encouraged to stand, and once standing, the RTB was manually administered. The bolus ID and calf ID were recorded and then entered into the Bella Ag Temperature monitoring software. Calves were enrolled into the study when they entered the group pen, and exited the study when they were weaned from milk. While at the farm, the study technician collected calf enrollment data, treatment records and mortality data. RTB temperature measurements were downloaded from Bella Ag Software each week. Farm personnel were blinded to sensor derived RTB data throughout the study period. Daily temperature information during the same period as the study was downloaded from MN Department of Natural Resources weather stations located within 10 miles of study farms for reporting of average ambient temperatures during the study period.

Case Definitions. Disease diagnosis was based on visual assessment by farm personnel. A case of diarrhea (**DIA**) was defined as visible diarrhea (very loose or watery feces; fecal score of 2 or 3 on a 0-3 scale) as well as treatment with antibiotics,

electrolytes, or IV fluids, or a combination of the three. A case of respiratory (**RESP**) disease was defined as a calf with an increased respiratory rate or effort, cough, plus treatment with antibiotics. A case of ill thrift (**ILL**) was defined as either: 1) a calf that had a rectal temperature > 39.5˚C; 2) a calf that was depressed but for which the caregiver did not have a clear diagnosis or; 3), a calf with other miscellaneous illnesses such as umbilical infection, joint infection, or injury, and treatment with antibiotics and/or non-steroidal anti-inflammatories. Treatment events known to be prophylactic (i.e. antibiotic administered to all calves at pen entry) occurred on one farm and were excluded from analysis. Duration of treatment was defined as the period between the first and last treatment. A new treatment event was defined as; an event that occurred for the first time or; an event that occurred at least 5 days after conclusion of treatment for a previous event or; an event that occurred within 5 days of the conclusion of a previous event but represented a different disease diagnosis.

9.3.3 Statistical Analysis

Sample Size. 281 calves were enrolled on 2 MN dairy farms. Of these, 57.7% of calves had a first treatment event, resulting in 162 treated calves and 119 untreated calves, respectively. This sample size (>115 calves per group) provided in excess of 90% power and 95% confidence to detect a 0.5ºC difference reticulo-ruminal temperature between sick and healthy calves (Assumed a SD = 0.25ºC, one tailed test) for objective 1.

Descriptive Statistics. All statistical analysis was performed in SAS (v 9.4; SAS Institute, Cary, NC) except where noted. Temperature measurements that occurred before the date

of pen entry or after weaning were excluded from descriptive analysis. Descriptive statistics (mean, median, SD, range) were generated to describe overall and by farm: (1) general calf management (age at entry to pen, group size, days in pen, age at weaning); and (2) calf health according to farm personnel (diagnosis, day of week at treatment, time of treatment, days of treatment, day of age at treatment, proportion treated both overall and by disease treated). Because some boluses were lost (eructated), specific descriptors were generated to describe the proportion of boluses lost (%) and age at bolus loss (mean, median, SD, range). R (v. 3.3.0, R Core Team, Vienna, Austria) was used to average individual calf RTB temperature measurements by hour, and by 6 hour increments (**QDAY**; 4 data points/day; (12AM – 6AM, 6AM – 12PM, 12PM – 6PM, 6PM – 12AM). Individual calf RTB measurements were then summarized at the hour and QDAY level overall, by farm, and by season (Winter = start of study (Feb 18) – Mar 20; Spring = Mar 21 – Jun 20; Summer = Jun 21 – end of study (Aug 22)). Average daily max and min ambient temperatures reported by local weather stations for both farms were averaged by month and by season. Plots were generated and visually assessed to check for normality of continuous variables.

Matched Pair Analysis. Because no data exists to describe RTB measurements in sick and healthy preweaned calves, we used a matched pair analysis to determine the relationship between RTB temperature measurement and morbidity around the time of a first treatment event. Cases were defined as a calf that had a first treatment event during the time it was in the group pen. Control calves were pen and age (± 7 days) matched to a case, and were defined as a calf that did not experience a treatment even from birth to

weaning. The QDAY of illness diagnosis (based on farm personnel) was designated as QDAY "0", and then 32 QDAYs before and after diagnosis were matched to a healthy control (-8d to +8d). This time period was chosen based on work by Timsit et al (2011) and Schaeffer et al (2004) reporting that increased body temperature can precede clinical signs of disease by up to 7 days. Case calves were matched to healthy controls in a 1:1 ratio. Treated calves that did not have a pen and age matched untreated calf available to serve as a control were excluded from this analysis.

Univariable analysis was first performed to describe the relationship between the occurrence of a treatment event (treated; Y/N) and potential predictors (explanatory variables) of interest, including RTB temperature (ºC), age at disease diagnosis, QDAYs from treatment (-32 - +32), disease diagnosed, month, farm, feeding machine, and pen. Predictors that were significant at the $P < 0.20$ level in the univaraible analysis were carried forward to offer to the multivariable analysis. Multivariable generalized mixed models were used to describe the difference in temperature for matched pairs of healthy and sick calves on the days leading up to and during a treatment event (QDAY -32 to +32). All predictors significant at the $P < 0.20$ level in the univariable models were offered to the full main effects model. Backwards stepwise variable section was then used until all remaining predictors were significant at $P < 0.05$. Repeated measurements by calf were accounted for in the model and an autoregressive correlation structure was chosen based on the model with the lowest Akaike's Information Criteria. An interaction term (QDAY from treatment*treatment Y/N) was added to the model to describe the association between illness and RTB temperature measurement in the 8 days surrounding

a treatment event. Models were then stratified by disease diagnosis (DIA, RESP or ILL). Because there were very few RESP calves (n=4 pairs), and because preliminary analysis showed that the RTB results from RESP and ILL calves behaved very similarly, the two categories of disease were considered together in the stratified model. Adjusted means were plotted and final significance was determined at the $P < 0.05$.

Diagnostic Test Characteristics When Using SPC Analysis of RTB Data to Detect Illness in Calves. Self-starting CUSUM charts were generated using QDAY average RTB temperature data for all calves during the milk fed period. In a self-starting CUSUM chart, the time to a positive signal (average run length (**ARL**)) is defined by three parameters: Δ, k and h. Δ is defined at the smallest shift in the process mean to be detected. "k" is the magnitude of the deviation of an observation from the target, and is often referred to as the reference value. In self-starting CUSUMs, "k" is typically set to 0.5 (Hawkins and Olwell, 1998). "h" is the magnitude required to conclude that the process is out of control and defines the upper control limit (UCL) and lower control limit (LCL). "h" is typically referred to as the decision interval, and "h" values of 4.5, 4.0. 3.5, 3.0, 2.5, 2.0 and 1.5 were tested with these QDAY RTB measures. Self-starting CUSUM charts were created for each calf at each decision interval for QDAY RTB temperature averages. A data base was then created that contained the date, QDAY and type of SPC signal (**PM** = positive mean; **NM** = negative mean; **PV** = positive variance; **NV** = negative variance) at each of the decision intervals. General descriptive statistics were generated to describe the direction and type of signal generated by the control chart, as well as to describe the SPC signal date as compared to the date that a first treatment

event was recorded for treated calves. Based on an increase in temperature around the time of illness, a PM signal was chosen as the index test. The reference standard was defined as a producer reported first treatment event. Treatments were defined as DIA, and RESP/ILL.

Diagnostic test characteristics for a PM SPC signal to detect a sick calf as compared to a human observer were calculated at each of the decision intervals from 2 x 2 tables. Diagnostic test characteristics included sensitivity (**Se**), specificity (**Sp**), accuracy (**Ac**), positive predictive value (**PPV**) and negative predictive value (**NPV**). Diagnostic Se was defined as the proportion of truly sick calves (as identified by the producer) that had a positive SPC test. Diagnostic Sp was defined as the proportion of healthy calves (never diagnosed by the producer as sick) that were negative on SPC (no PM signal). Accuracy was defined as the total proportion of tests that correctly identified a sick or healthy calf. The PPV was defined as the proportion of positive PM tests that correctly identified a sick calf, and NPV was defined as the proportion of negative PM tests that correctly identified a healthy calf (Dohoo et al., 2009). The test characteristics for each decision interval were determined. For those calves that achieved a positive SPC test and were truly sick (true positives), the timeliness of the signal was assessed based on subtracting the date of the SPC signal from the date that the producer identified the calf as sick. After completing the SPC analysis for all treatment events, this analysis was repeated for each individual disease diagnosed (DIA or RESP/ILL. The two decision intervals with the optimal sensitivity and timing (3.0, 2.5) were then carried forward to the multivariable model.

As a next step, univariable models were generated to describe the relationship between a PM Sig (Y/N) and predictors of interest including calf treatment (Y/N; main predictor of interest), calf age at signal, farm, machine, pen, signal month, QDAY, and signal timing (night/day). Variables that were significant $P < 0.20$ in the univariable model were then offered to the full main effects model and backwards stepwise elimination was used until all variables remaining were significant at $P < 0.05$. The main predictor and outcome were then switched in the model to determine predictive values. Farm was controlled for as a random effect in all models. Test characteristics and 95% confidence intervals were calculated from model output as described by Dohoo (2009). All statistical modeling was performed in SAS (V 9.4, SAS Institute Inc, Cary, NC).

Diagnostic Test Characteristics When Using Deviation Thresholds of RTB Data to Detect Illness in Calves. Because SPC analysis of RTB data might not be the most accurate or timely means of evaluating RTB data to detect sick calves, we also evaluated test characteristics when using simple deviations and threshold levels to declare a positive test, assuming that positive deviations reflected pyrexia associated with an infectious disease event. Two different deviation thresholds were tested for their ability to detect disease from a rolling two-day average of QDAY RTB temperature readings, > 1ºC and > 0.5ºC. Three absolute thresholds in RTB measures were also tested, and a signal was determined if a single QDAY RTB temperature reading was greater than or equal to 39.5ºC, 39.17ºC, or 38.75ºC. A reading ≥ 39.5ºC was chosen based on its typical clinical indication of calf fever in the field (McGuirk, 2008). Readings of ≥ 39.17ºC and ≥

38.75ºC were chosen as thresholds based on RTB bolus correction factors determined in a validation of the RTB system and described elsewhere (Knauer et al., 2016).

Diagnostic test characteristics (Se, Sp, Ac, PPV, NPV) for each of the five deviation or threshold (**DT**) values to detect a sick calf, as compared to a trained human observer (reference test), were calculated from 2 x 2 tables. The index test was the first temperature to cross the previously defined threshold. The reference test was a producer reported first treatment event. Timeliness of the signal was calculated by subtracting the date of the DT signal from the date that the producer identified the calf as sick. These analyses were completed both overall and by disease treated (DIA or RESP/ILL). Univariable models were then generated to describe the relationship between a DT signal (Y/N) and predictors of interest including calf treatment (Y/N; main predictor of interest), calf age at pen entry, month, farm, machine, and pen. Variables that were significant $P < 0.20$ in the univariable model were then offered to the full main effects model and backwards stepwise elimination was used until all variables remaining were significant at $P < 0.05$. The main predictor and outcome were then switched in the model to determine predictive values. Farm was controlled for as a random effect in all models. Test characteristics and 95% confidence intervals were calculated from model output as described by Dohoo (2009). All statistical modeling was performed in SAS (V 9.4, SAS Institute Inc, Cary, NC).

9.4 RESULTS

9.4.1 General Farm Management.

Two MN herds were enrolled in the present study. Farm A was a contract heifer grower receiving calves from four sites. Calves were housed in a greenhouse style hoop barn in four straw bedded pens that were approximately 117m^2 in size. Farm A had two automatic calf feeders (DeLaval CF 1000; DeLaval, Tumba, Sweden) and fed milk replacer (Cow's Match ColdFront®/WarmFront®, Land O'Lakes Animal Milk Products, Shoreview, MN) at a maximum feeding level of 8L/d fed at a concentration of 150g/L (grams milk powder added to 1L water). Calves on farm A were housed individually for approximately 12d at which point they entered the group pen. Group size on farm A averaged 21 ± 4, and calves were weaned from milk at approximately 51d of age. Farm B milked approximately 780 cows. Calves were housed in four pens in a four sided converted pig barn with tube ventilation. Pens were bedded with a combination of sawdust and straw and were approximately 76m^2. Farm B fed pasteurized whole milk through two automatic calf feeders (Lely Calm Combi; Lely North America, Pella, IA) at a maximum feeding level of 10L/d. Calves on farm B were housed individually for approximately 7d at which point they entered the group pen and were housed in groups of 21 ± 4 calves per group. Calves were weaned from milk at approximately 50d of age. Pens on both farms were managed as all in all out.

9.4.2 Calf Level Description and Calf Health

RTB boluses were administered to 302 calves from Feb to Aug 2014. Calves that were administered a bolus but never reached the group pen (n=17) and calves that had less than 15 temperature reads (n=4) were excluded from the study leaving 281 calves (Farm A: n=146; Farm B; n=135) with RTB temperature data available for analysis.

RTB bolus loss due to eructation occurred in 23% of calves (65/281) at an average of 35 ± 8d of age. Data from calves that lost their bolus was used for all analysis until the time that their bolus was lost. A RTB was defined as "lost" if 1) readings on the calf stopped suddenly and the calf had not died; 2) temperature readings dropped below 34.4ºC for > 5 consecutive readings and the calf had not died; or 3) temperature readings were above 41.7ºC for >5 consecutive readings and the calf had not died. Missing data was also present in 9% (25/281) of calves enrolled, mostly due to farm electrical issues that caused the RTB software to malfunction from approximately 4/26/2014 to 5/23/2014. Calves that entered the group pen during this time (n=17) were excluded from SPC and DT analysis, but their captured RTB temperatures contributed to all descriptive temperature data presented.

A total of 57.7% (162/281) of enrolled calves had a first treatment event, with 15.3% (43/281) and 2.5% (7/281) of calves treated for a second and third time, respectively. Of the first treatments, 41.3% (67/162) were treated for DIA, 11.7% (19/162) were treated for RESP, and 47% (76/162) were treated for ILL. The group pen mortality rate over the study period was 2.5% (7/281). There were 58 pairs of calves available for the matched pair analysis. Of these, 50% (29/58) were calves treated for DIA, 43% (25/58) were calves treated for ILL, and 7% (4/58) were calves treated for RESP.

9.4.3 Descriptive Data for RTB Temperature Measures

There were 572,154 total RTB temperature readings collected over the observation period. Upon data compression to hourly and QDAY databases, RTB

readings less than 34.4ºC and greater than 41.7ºC were excluded from analysis due to biological implausibility, leaving 155,473 and 33,185 hour and QDAY averages, respectively, for analysis. Over a 24hr period, average RTB temperatures showed a monophasic diurnal pattern, with the temperature nadir (reduced by 0.23ºC) between 8AM and 11AM, then steadily increasing over the rest of the day. However, this result varied by farm. There was a much larger daily change in RTB measures in Farm A as compared to Farm B (Figure 1). This diurnal variation also varied by season. Calf RTB measurements on both Farm A and Farm B had the highest peaks during the summer, while winter and spring were more similar. However, Farm A had the highest peak RTB temp in the spring, whereas Farm B had the higher peak temperatures in the winter (Figure 2). The average weather station captured minimum and maximum ambient temperatures for Farm A by season were -17.9 ± 8.2ºC (range: -31.7, 3.8) and -4.9 ± 7.5ºC (range: -17.2, 12.2) for Winter, 4.4 ± 7.9ºC (range: -12.8, 18.8) and 17.0 ± 8.8ºC (range: -3.8, 31.1) for Spring, and 14.6 ± 3.3ºC (range: 8.3, 22.8) and 26.8 ± 2.6ºC (range: 20.5, 31.7) for Summer. The average weather station captured minimum and maximum ambient temperatures for Farm B by season were -17.3 ± 8.2ºC (range: -29.4, 2.2) and -6.9 ± 7.8ºC (range: -20, 8.8) for Winter, 3.5 ± 8.6ºC (range: -16.1, 17.8) and 14.1 ± 9.2ºC (range: -7.2, 30) for Spring, and 14.7 ± 2.6ºC (range: 9.4, 20) and 25.1 ± 2.5ºC (range: 17.2, 31.1) for Summer.

9.4.4 Matched Pair Analysis of RTB Measures Between Sick and Healthy Calves

The final model describing the difference in RTB temperature measurements between sick and healthy calves (n = 58 pairs) in the 16 days (64 QDAYs) surrounding a treatment event controlled for the effect of month, farm, calf age at treatment, disease diagnosis, and repeated measurements by calf. The overall difference in RTB temperature measurement in matched pairs of sick and healthy calves was 0.02 ± 0.09ºC (P = 0.21) with sick calves having a lower temperature overall. On the QDAY of diagnosis for any disease, sick calves had a RTB measurement that was 0.2 ± 0.09ºC higher than healthy calves (P = 0.017). Sick calves had a higher RTB temperature measurement on QDAYs -4, -3, -1 to 1, 5, and 7 as compared to their healthy matched controls (Figure 3). In the DIA stratified model, which controlled for the effect of farm, age at treatment, month, and repeated measurements by calf, the overall difference in RTB temperature measurement between matched pairs of calves was 0.02 ± 0.13ºC (P = 0.007), with healthy calves having a lower RTB temperature. On the QDAY of treatment, DIA calves had an average RTB temperature measurement that was 0.10 ± 0.13 ºC higher than healthy controls, but this result was not significant (P = 0.42). DIA calves did not have higher RTB temperature measurements on any of the 64 QDAYs surrounding treatment as compared to control calves. (Figure 4). In the RESP/ILL stratified model, which controlled for the effect of farm, age at treatment, month, and repeated measurements by calf, healthy calves had a RTB temperature that was 0.05 ± 0.13ºC higher, but this result was not significant (P = 0.35). On the QDAY of illness detection, RESP/ILL calves had a RTB temperature that was 0.32 ± 0.12 ºC (P = 0.007) higher than matched healthy calves. RESP/ILL calves followed roughly the same pattern as all treated calves, having a higher RTB temperature on QDAYs -4, -3, -1 to 1, 7, 13, and 18 (Figure 5).

9.4.5 Signal Timing when Using SPC Analysis or Deviation Analysis of RTB Measures to Identify Sick Calves

There were 264 calves available for determination of signal timing and diagnostic test characteristics. The following reports the total number of signals, then average day of signal relative to either day in pen or day of treatment ± SD. When use of SPC was considered, the signal occurred earliest in the feeding period when using a control limit of 2.5 (n=126; 4.3 ± 2.8d), then 3.0 (n=128; 5.1 ± 3.8d). A control limit of 2.5 also resulted in the most timely signal as compared to day of treatment in treated calves (n=84; -2.9 ± 5.7d), then 3.0 (n=84; -2.4 ± 6.1d).

When considering the use of a deviation from a two day average, a deviation of >0.5ºC was the earliest signal, both overall (n= 249; 10.3 ± 7.2d) and when considering days from treatment (n=146; 2.3 ± 9.1d) as compared to a deviation of 1ºC.

When considering a threshold temperature, a temperature threshold of ≥ 38.75ºC resulted in the earliest (n=262; 2.3 ± 4.4d) and most timely (n=151; -5.7 ± 7.9d) signal (Table 1). The proportion of calves that signaled relative to when they were treated for all SPC Threshold, and Deviation signals is reported in Table 2.

9.4.6 Diagnostic Test Characteristics when Using SPC analysis or Deviation analysis of RTB Measures to Identify Sick Calves

Reported are the point estimates and 95% confidence intervals of the diagnostic test characteristics estimated from generalized estimating equations (Table 3). When considering the ability of an SPC signal on a quarter day average RTB temperature measurement to detect a sick calf, a signal at the 3.0 control limit had the highest Se at

54.3% (50.7, 57.8), a Sp of 59.8% (45.2, 72.9), a NPV of 47.7% (26.3, 70.0), and a PPV of 64.1% (52.2, 74.4).

When considering a RTB temperature deviation from a 2 day average, a positive signal on a 0.5ºC increase in RTB temperature had the highest Se to detect a sick calf with a Se of 94.7% (94.3, 95.0) and a PPV of 58.7% (39.1, 75.9). However, the Sp and NPV were only 9.4% (8.8, 10.1) and 57.2% (54.1, 60.4), respectively.

When considering an increase over a RTB threshold temperature, a threshold of $\geq$ 38.75ºC resulted in a Se of 98.6% (93.2, 99.7), a Sp of 0.8% (0.3, 2.3), a PPV of 57.7% (38.4, 74.9), and a NPV of 22.7% (11.2, 40.5). The remaining diagnostic test characteristic results are reported in Table 3.

9.5 DISCUSSION

Housing dairy calves in groups is gaining in popularity in the North America (USDA, 2014; Medrano – Galarza et al., 2017), but disease detection remains a challenge in these systems as compared to individual housing (Steenkamer, 1982). Body temperature can be a useful indicator of infectious disease. Compared to temperature monitoring via rectal thermometer, automated indwelling temperature systems do not require additional labor than the initial bolus administration, making them a potentially useful disease detection tool. This is the first study to evaluate the diagnostic utility of using an indwelling rumen temperature bolus system for disease detection in group housed preweaned dairy calves.

General Calf Management and Calf Health

Housing and feeding management on the two study farms is similar to that described for group-housed computer feeder herds in the Upper Midwest (Jorgensen et al., 2017). Though the two farms had minor differences in feeding levels, type of milk offered and space allowed per calf, perhaps the most important difference between the two farms with the potential to influence calf temperature measures was barn design. Farm A was a greenhouse style hoop barn, which has the potential to be influenced more by radiant heat from the sun than a closed barn with 3m ceilings. However, we controlled for season and farm in our methodology, so our results should not be affected by these factors, but rather only how an individual calf changes her temperature over time. Additionally, treatment rates on these two farms are within the disease incidence that has previously been reported for group housed calves (Svensson and Jensen, 2007; Roth et al., 2009).

Descriptive Data for RTB Temperature Measures

Boluses were lost, presumably due to eructation, in 23% of the study population at an average of 35d of age. Such a high occurrence of bolus loss will reduce the usefulness of this monitoring system on commercial dairy farms. Timsit et al (2011) did not report any bolus loss when an RTB was administered to 25 feeder bulls. However, that study was conducted in older animals and using a different bolus system (Thermobolus, Medria SAS). In the present study, calves on both farms were offered grain immediately upon entrance to the group pen, and calves can begin to eructate as early as 2 weeks of age (Swanson and Harris, 1958). We hypothesize that several factors could have contributed to the high incidence of bolus loss in the current study. First, the

reticulo-rumen of the young calf is roughly 1/3 the size of the abomasum (Heinrichs and Jones, 2003). It is possible that this small reticulo-rumen, combined with the beginning of eructation, may make it easier for the bolus to be lost. Second, the bolus weighs 45g and is lighter than the bolus designed for an adult dairy cow. It could be that the weight of the bolus is not sufficient to keep it firmly in the reticulum. In addition to boluses lost due to presumed eructation, an additional 7% of calves had approximately 1 month of RTB information lost due to farm electrical issues and software malfunction. While this would potentially be noticed on a farm that was actively using the software to monitor calves, software malfunction is important to report as a potential disadvantage to using precision technologies to monitor health. A handful of boluses were recovered and replaced on Farm A, but with the financial investment of these types of systems, the large rate of bolus loss observed in this study represents a challenge for successful commercial adoption of this technology.

In the present study, we observed a monophasic diurnal pattern to calf RTB temperature measurements over a 24 hour period. Overall, calves experienced their peak RTB temperatures from 9PM – 6AM, with the nadir occurring from 8AM - 11AM. Macaulay et al (1995) reported a maximum tympanic temperature measurement from 12PM – 6PM, with the minimum occurring from 6AM – 9AM in calves housed individually in either a polymer hutch, polyethylene dome, or a wooden hutch. Hill et al (2016) reported findings similar to the present study, where the calf tail temperature nadir occurred around 8AM with the peak temperature from 5PM – 10PM. Conversely, Wrenn et al (1961) reported a diphasic pattern to calf vaginal temperatures, with temperature elevations in the morning (5 – 7AM) and again in the afternoon (1 – 6PM). These same

authors reported that body temperatures were lowest in the evening and early hours of the morning, but these animals were housed in heated barns, and were not subjected to hot summer or severe winter weather. There was also a substantial difference in diurnal variation by farm. Farm A and B had much different calf building types, and we speculate that the barn ambient temperatures fluctuations were much different in the two environments, though we did not measure this in the present study. Local weather stations for the two herds reported daily temperatures were similar in all seasons when comparing Farm A and Farm B, but we did not measure daily or hourly barn temperatures and so should be cautious inferring that outside ambient temperature accurately reflected conditions within the barns. Peña et al (2016) reported higher rectal temperatures in the afternoon in calves that were housed in Calf-Tel hutches compared to calves housed in wire hutches. Spain and Spires (1995) also reported a positive correlation between hutch micro-environmental temperature and calf rectal temperatures, further suggesting that the calves' immediate environment can influence body temperature.

RTB temperature measures also varied by season. Calves on Farm A were the most influenced by changes in ambient temperature corresponding to season. Beakley and Findlay (1955) reported an increase in calf rectal temperature corresponding to increasing ambient temperature and humidity. Lactating dairy cattle have been reported to have the highest average daily reticulorumen temperature in the summer, with the lowest temperature in the winter (Liang et al., 2013). Interestingly, calves on Farm B had the lowest RTB temperature measurements during the spring. We hypothesize that this may be due to more temperature regulation capabilities in the winter on Farm B as

compared to Farm A due to facility design. Indwelling temperature devices have the potential to help us better understand nuances of calf environmental comfort, though this remains to be investigated.

Matched Pair Analysis of RTB Measures between Sick and Healthy Calves

In the present study, the RTB temperature measures of sick calves were higher than matched healthy calves 24 hours prior to clinical diagnosis of illness by trained farm personnel. However, this overall difference was driven by calves diagnosed with ill thrift (ILL) and respiratory disease (RESP), as calves diagnosed with diarrhea (DIA) did not have different temperatures as compared to matched healthy calves. Clinical signs of respiratory disease are often preceded or accompanied by a fever (Schaefer et al., 2004; Timsit et al., 2011). Additionally, many of the cases of ill thrift in the present study (24/29) were calves that were treated for a rectal temperature ≥ 39.5ºC. RTB temperature measures were not predictive of diarrheal disease, however Figure 4 suggests that calves treated for DIA did have a higher RTB temperature, this result was just not statistically significant. Diarrheal disease in preweaned dairy calves can have a complex etiology (McGuirk, 2008), and not all causative agents will induce a fever reliably (Van Metre et al, 2008). It is therefore conceivable that the use of RTB temperature measurements to detect DIA in calves might depend on the causative agent, and will not be reliable as a predictor of disease onset. The former hypothesis requires further investigation.

Diagnostic Test Characteristics Using SPC analysis or Deviation analysis of RTB Measures to Identify Sick Calves

We described the diagnostic test characteristics and timing of a signal when using a variety of data analysis techniques to describe the ability of QDAY average RTB temperature measures to predict and detect a first treatment event in an individual group housed preweaned dairy calf. No one RTB data analysis method was found to be optimal in the present study. Though the threshold methods were able to achieve sensitivities ranging from 78 to 98%, specificity estimates were extremely low, and the positive and negative predictive values of all methods were poor. Negative predictive values are the most important test characteristic for the determination of utility and use of a screening test in the field, because it represents out confidence in a negative result. In this present study, NPVs ranged from 22.7 to 57.2%, meaning that for a calf not signaling, we can only reliably predict her as being healthy approximately half of the time. This is unacceptable if producers are going to solely rely on RTB alerts for disease detection. One potential advantage to SPC analysis of RTB data (vs the other approaches investigated) was in the timing of the signal compared to the day the calf was detected and treated by herd personnel. The majority of SPC signals (69.1%) occurred in the week leading up to and the day of clinical diagnosis, suggesting that these may potentially be more useful alerts for detection of illness.

No comparable works exists that describes the use of indwelling temperature devices in pre-ruminant calves. Timsit et al (2011) used similar methodology to report that a reticulorumen hyperthermia episode as detected by an indwelling temperature bolus could predict the onset of bovine respiratory disease (**BRD**) clinical signs in young feeder bulls. These authors reported that this hyperthermia event preceded nasal discharge by 19hrs, depression by 51hrs, and cough by 64hrs, and reported a PPV of

73%. In another study using infrared orbital temperature to predict BRD in feeder calves as compared to diagnosis based on clinical and inflammatory markers (gold standard), Schaefer et al (2011) found a PPV and NPV of 80 and 65% respectively in the 4-6 days before clinical illness was observed. However, the Se and Sp to detect disease at the same time was 69 and 77%, respectively. These reported predictive values are higher than those that we found in the present study, possibly because these authors were working with a single, more predictably behaved disease complex in an older class of animal.

Strengths and Limitations

This is the first study to investigate the diagnostic utility of an automated indwelling temperature bolus system to predict or detect disease in preweaned dairy calves. The field study was conducted on 2 commercial dairy farms using calf rearing facilities typical of many North American farms, over multiple seasons, and provides a good basis for future work in this field. However, due to budget constraints, we could only install the RTB system on two farms in this study. Future studies should reevaluate the system in herds with very different housing types, including both group or individual housing systems, and in different regions. Though we did not measure it in our study directly, others have reported the effect of ambient temperature on body temperature, which could be important when using these types of temperature monitoring devices in other climates or housing conditions.

Another potential study limitation is the use of producer reported clinical disease diagnosis as the reference test. Other studies have reported that calf caregivers have a poor sensitivity of pre-mortem disease diagnosis (Sivula et al, 1996). A cross-sectional

validation on a subset of study farms supports this hypothesis, with a reported Se and Sp of producer detection to be 26% and 97%, respectively, as compared to an experienced veterinarian (Knauer et al., 2017). However, these results should be interpreted with caution, as this only represents a one day snap shot of producer performance.

In spite of having trained the herd staff involved with this study, it is possible that producer errors in disease detection could affect our results: First, if calves that were truly sick were missed by farm personnel, then in reality there would be fewer false positives than it seems, resulting in a higher Sp and PPV for the SPC, Threshold, or Deviation alert methods of RTB data analysis. Conversely, if calves were over-treated by farm personnel and the true incidence of first treatment events was lower, then the relative Se and NPV of the diagnostic tests evaluated might be underestimated in the current study. If this methodology were used as a screening tool, in this situation, the user would be more confident in a negative result (improved NPV) and less concerned about missing a sick calf.

9.6 CONCLUSIONS

The results of the present study show that individual calf RTB temperature measurements have diurnal and seasonal variation, and this difference varies by farm. RTB measurements can change up to 24hrs before clinical diagnosis of disease, but this varies by type of disease treated. When applying different approaches to analyze RTB data, including SPC and deviation/threshold methods, as a test to predict or detect disease in calves, no one method is superior, with the predictive values being poor overall. As

such, this study suggests that RTB data may have limited utility when used as a stand-alone method of disease prediction or detection in group-housed preweaned dairy calves.

Table 15. The timing of a RTB temperature alert for different types of signals, for treated preweaned automatically fed group housed female Holstein calves (n=264 calves).

Signal type	N	Days on Feeder	N	Days from Treatment
SPC Control Limit				
3.0	128	5.1 ± 3.8 (1, 30)	84	-2.4 ± 6.1 (-32, 19)
2.5	126	4.3 ± 2.8 (1, 18)	84	-2.9 ± 5.7 (-32, 8)
Deviation[1]				
>1.0ºC	101	19.2 ± 10 (4, 40)	61	12.4 ± 11.6 (-13, 37)
>0.5ºC	249	10.3 ± 7.2 (2, 40)	146	2.3 ± 9.1 (-27, 29)
Threshold[2]				
≥ 39.50ºC	196	10.9 ± 8.8 (1, 37)	120	2.5 ± 11.5 (-42, 29)
≥ 39.17ºC	234	8.4 ± 7.5 (1, 39)	142	0.2 ± 10.2 (-39, 29)
≥ 38.75ºC	262	2.3 ± 4.4 (1, 26)	151	-5.7 ± 7.9 (-42, 13)

[1]Deviation = The first time a calf had an RTB temperature reading that was >1.0 or >0.5ºC as compared to her rolling two day average RTB Temperature. Calves had to accumulate at least 4 readings that contributed to the rolling average before a signal could occur.
[2]Threshold = A positive signal was considered the first RTB reading that was greater than or equal to the defined threshold temperature (39.5, 39.17, 38.75ºC) after the first observation.

Table 16. Timing of the first SPC, Deviation, or Threshold signal, relative to treatment date. Reported are number of signals (proportion of treated calves that signaled) at different intervals before, on the day of, or after the day of treatment.

	N[1]	N[2]	Signal 8 Days or more before Treatment	Signal 4 to 7 days before Treatment	Signal 1 to 3 days before Treatment	Signal on the Day of Treatment	Signal after the Calf was Treated
SPC Control Limit							
3.0	128	84	16 (19.0)	14 (16.7)	34 (40.5)	10 (11.9)	10 (11.9)
2.5	126	84	13 (15.5)	15 (17.9)	35 (41.7)	11 (13.1)	10 (11.9)
Deviation[3]							
> 1ºC	101	61	48 (78.7)	2 (3.2)	6 (9.8)	5 (8.2)	1 (1.6)
> 0.5ºC	249	146	66 (45.2)	15 (10.3)	39 (26.7)	13 (8.9)	14 (9.5)
Threshold[4]							
≥ 39.50ºC	196	120	47 (39.2)	13 (10.8)	27 (22.5)	18 (15)	15 (12.5)
≥ 39.17ºC	234	142	43 (30.3)	22 (15.5)	29 (20.4)	21 (14.8)	27 (19.0)
≥ 38.75ºC	262	151	19 (12.6)	40 (26.5)	31 (20.5)	8 (5.3)	53 (35.1)

[1] N= total number of alerts out of total calves (n=264)

[2] N = total number of alert calves out of total calves treated (n=162)

[3]Deviation = The first time a calf had an RTB temperature reading that was >1.0 or >0.5ºC as compared to her rolling two day average RTB Temperature. Calves had to accumulate at least 4 readings that contributed to the rolling average until a signal could occur.

[4]Threshold = A positive signal was considered the first RTB reading that was greater than or equal to the defined threshold temperature (39.5, 39.17, 38.75) after the first observation.

Table 17. Diagnostic test characteristics of the ability of an RTB temperature bolus signal based on either SPC, a Deviation, or a Threshold Temperature method to detect a sick calf using individual animal quarter day RTB temperature averages. Point estimates are the result of generalized estimating equations controlling for the random effect of farm. Presented are mean (95% CI).

	Diagnostic Test Characteristics[1]				
	Se	Sp	PPV	NPV	Ac
SPC Control Limit					
3.0	54.3 (50.7, 57.8)	59.8 (45.2, 72.9)	64.1 (52.2, 74.4)	47.7 (26.3, 70.0)	57.4 (51.4, 63.3)
2.5	51.8 (49.2, 54.4)	64.9 (50.0, 77.4)	65.4 (56.4, 73.5)	49.3 (70.2, 28.7)	58.1 (52.2, 64.1)
Deviation[2]					
> 1ºC	40.0 (39.9, 40.2)	64.3 (48.1, 77.8)	62.0 (32.3, 84.8)	44.1 (57.8, 31.2)	50.0 (44.0, 56.0)
> 0.5ºC	94.7 (94.3, 95.0)	9.4 (8.8, 10.1)	58.7 (39.1, 75.9)	57.2 (54.1, 60.4)	58.6 (52.7, 64.5)
Threshold[3]					
≥ 39.50ºC	78.4 (53.4, 92.0)	26.0 (35.1, 18.6)	59.5 (37.1, 78.6)	46.5 (51.3, 41.8)	59.0 (41.2, 74.7)
≥ 39.17ºC	93.9 (75.4, 98.7)	10.0 (6.9, 14.2)	59.2 (38.4, 77.2)	53.3 (45.7, 60.8)	59.8 (39.6, 77.2)
≥ 38.75ºC	98.6 (93.2, 99.7)	0.8 (0.3, 2.3)	57.7 (38.4, 74.9)	22.7 (11.2, 40.5)	57.4 (37.9, 74.7)

[1] Diagnostic Test Characteristics: Se = sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; Ac = accuracy

[2]Deviation = The first time a calf had an RTB temperature reading that was >1.0 or >0.5ºC as compared to her rolling two day average RTB Temperature. Calves had to accumulate at least 4 readings that contributed to the rolling average until a signal could occur.

[3]Threshold = A positive signal was considered the first RTB reading that was greater than or equal to the defined threshold temperature (39.5, 39.17, 38.75) after the first observation.

Figure 10. Hourly RTB readings (ºC) for all group housed preweaned dairy calves (n=281 calves; n=155,473 readings) and by farm (Farm A: n=146 calves, n = 73,545 readings; Farm B: n = 135, n=81,928 reading) over a period of 8 months (Feb - Aug 2014).

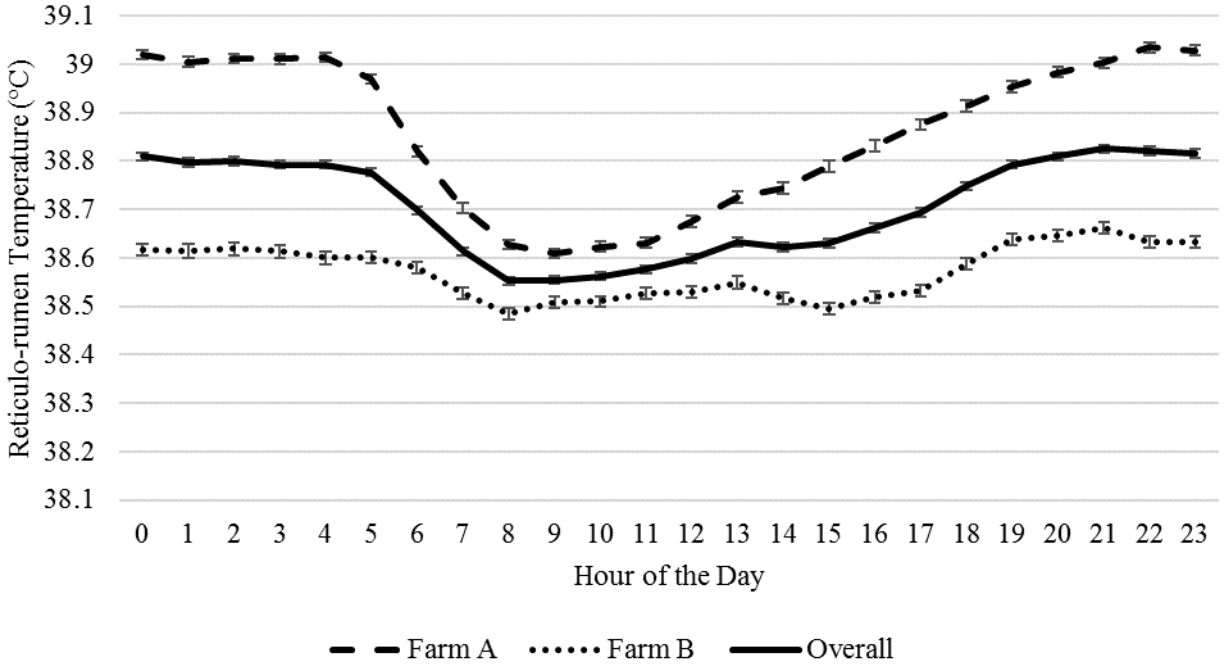


Figure 11. Hourly average reticulo-rumen temperature readings (ºC) (± SE) for group housed, preweaned dairy calves by season (Winter = Feb 18 – Mar 20; Spring = Mar 21 – Jun 20; Summer = Jun 21 – Aug 22) and farm.

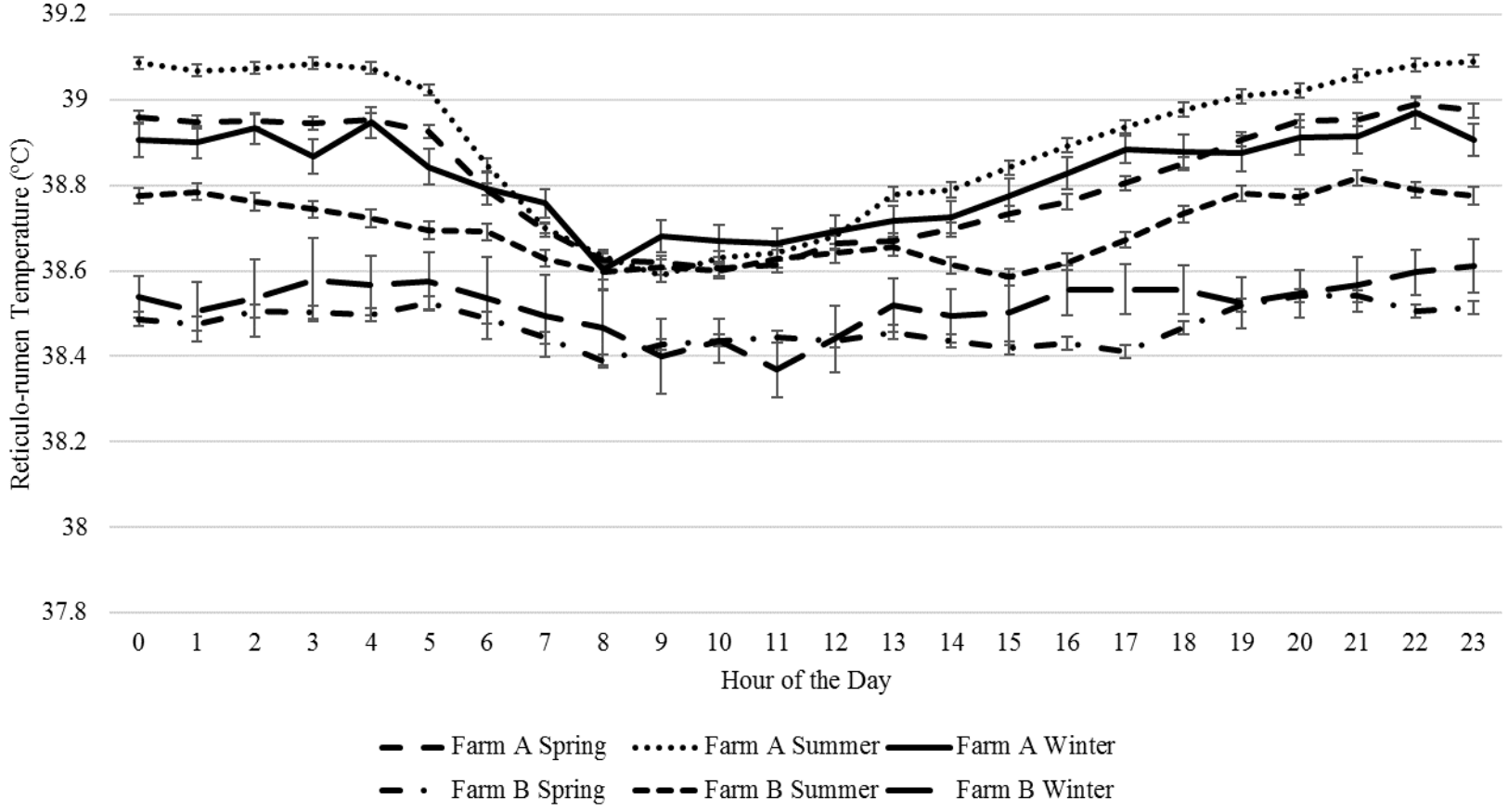


Figure 12. Results of a mixed model investigating the association between reticulo-rumen temperature (ºC) and treatment on the 8 days surrounding a treatment event in matched pairs of treated and healthy group housed preweaned dairy calves (n=58 pairs; n=6,240 RTB temperature measurements). The final model controlled for the effect of month, farm, calf age, disease diagnosis, and repeated measurements by calf. Presented are adjusted means and SEM. (* = $P < 0.05$)

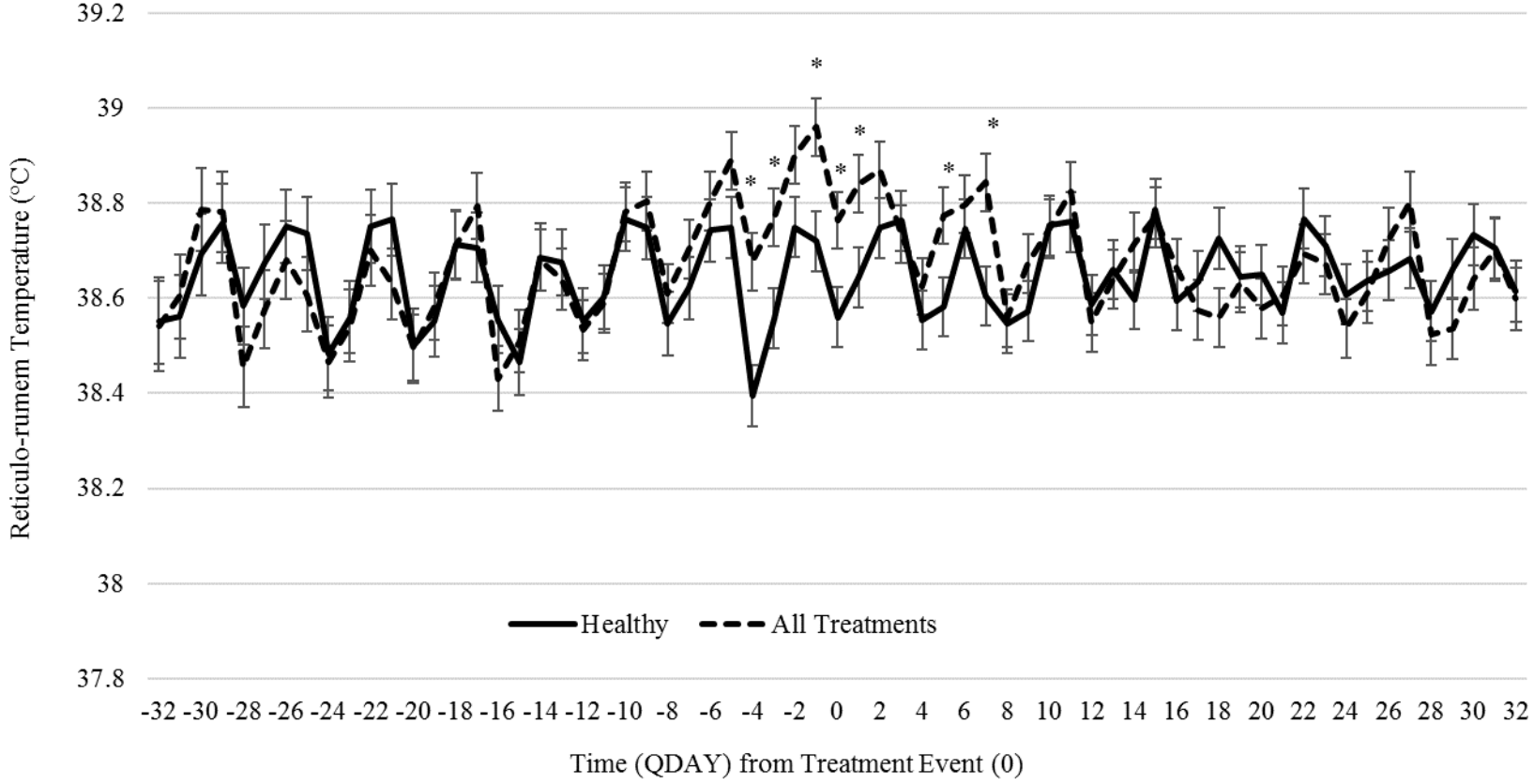


Figure 13. Results of a mixed model investigating the association between reticulo-rumen temperature (ºC) and treatment on the 8 days surrounding a diarrheal event in matched pairs of treated and healthy (n=29 pairs) group housed preweaned dairy calves. The final model controlled for the effect of month, calf age, farm, and repeated measurements by calf. Presented are adjusted means and SEM. (* = $P < 0.05$)

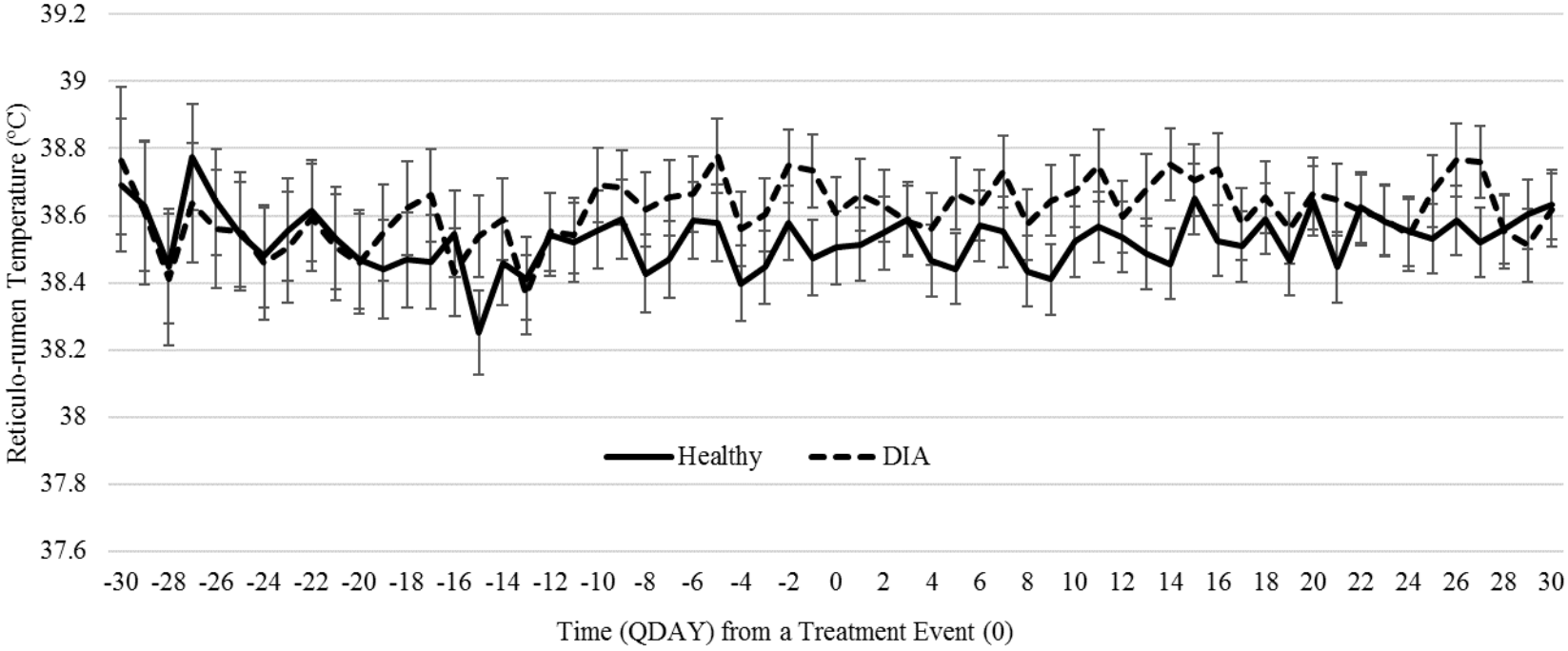
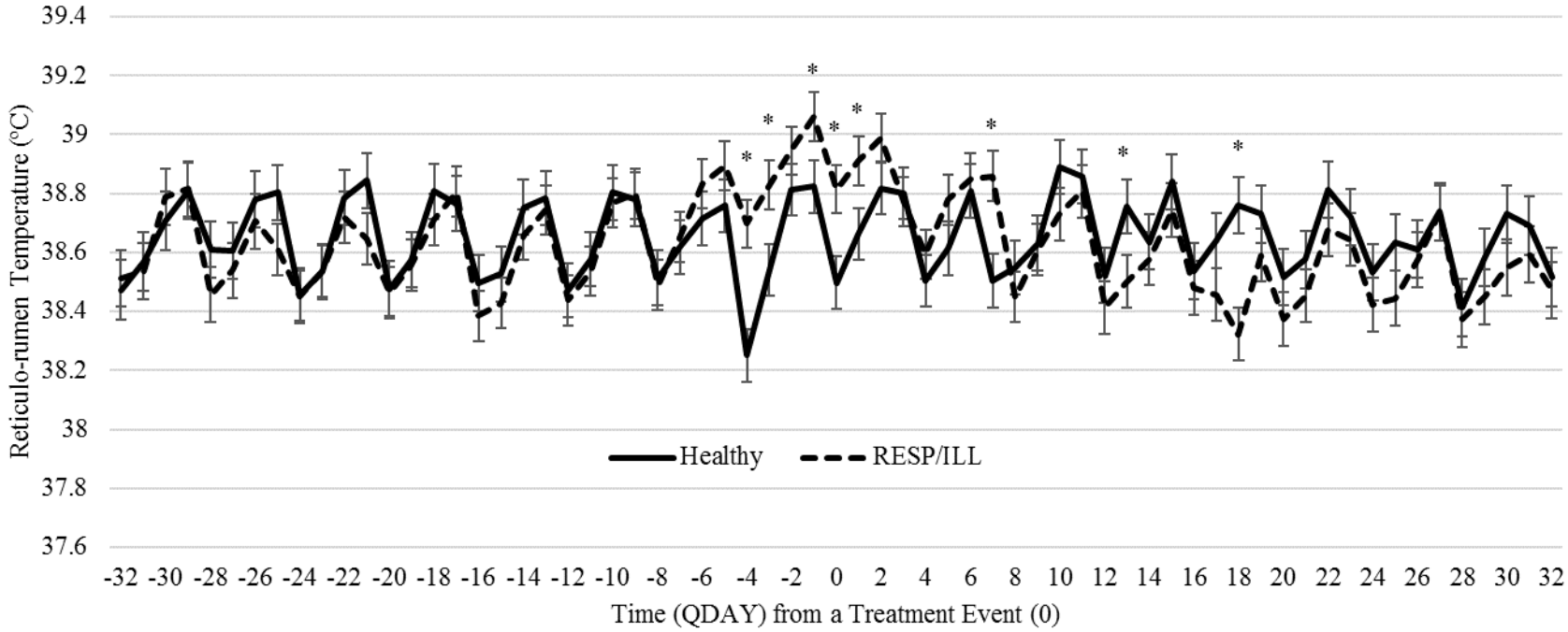


Figure 14. Results of a mixed model investigating the association between reticulo-rumen temperature (ºC) and treatment on the 8 days surrounding a RESP or ILL event in matched pairs of treated and healthy group housed preweaned dairy calves (n=29 pairs). The final model controlled for the effect of month, calf age, farm, and repeated measurements by calf. Presented are adjusted means and SEM. (* = $P < 0.05$)



10 CHAPTER EIGHT

Summary of Results, Implications, and Future Directions

10.1 INTRODUCTION AND OBJECTIVES

Housing preweaned calves in groups and feeding them automatically is increasing in popularity worldwide. Advantages to this management system include the reallocation of calf labor, earlier socialization of the calf as well as the ability to feed more milk more easily. Unfortunately, housing calves in groups can lead to an increased incidence of morbidity and mortality and delays in disease detection. The use of precision dairy technologies, namely automatically captured feeding behavior and body temperature data, may aid producers in earlier disease detection and intervention. However, research to date suggests that changes in feeding behavior, as currently calculated and reported by autofeeders, are neither timely nor sensitive when used screening tool to detect morbidity in group-housed calves. Furthermore, studies are lacking to evaluate the utility of using automatically captured (sensor derived) body temperature data to detect illness in calves. The overall aim of this thesis was to improve our understanding of how sensor derived observations, such as feeding behavior or rumen temperature measures, vary in sick (vs healthy) calves, and to describe the diagnostic utility of individual animal data collected from precision dairy technologies as a tool to predict and/or detect disease in group housed automatically fed pre-weaned dairy calves. We proposed to apply a different statistical methodology to individual calf feeding behaviors when summarized at both the day and visit level, and to indwelling rumen temperature bolus measures, to determine if illness events could be detected in a sensitive and timely manner as compared to clinical

diagnosis by trained farm personnel. Several objectives were set to accomplish this aim, the conclusions of which will be discussed in general terms in this chapter. Opportunities for future work in this area will be discussed at the conclusion of this chapter.

10.2 SUMMARY OF RESEARCH ACTIVITIES AND RESULTS

10.2.1 Objective One: The use of day level feeding behaviors to predict and detect disease in automatically fed group housed preweaned dairy calves.

The first objective of this study was to describe the use and utility of individual calf day level feeding behaviors to predict and detect disease. We conducted a prospective observational cohort study on 10 farms in Minnesota (n=4) and Virginia (n=6). Calves were enrolled upon entry to the group pen and exited the study at weaning. Study technicians visited the farms to collect enrollment, calf health data as recorded by farm personnel, and feeding behavior data from automatic feeder software. A matched pair analysis was performed to describe the difference in day level feeding behaviors and morbidity in the time before and during a treatment event. The results of this study show that calves drink less milk, drink more slowly, and visit the feeder without a milk meal (unrewarded visit) less frequently in the days surrounding a treatment event then age and pen matched healthy calves. There were no differences between sick and healthy calves when rewarded visits to the feeder were considered. These changes varied by clinical disease diagnosis by farm personnel, with the earliest and most consistent changes in calves diagnosed with diarrheal disease, followed by ill thrift calves, and finally calves diagnosed and treated for respiratory disease.

We then investigated the diagnostic test characteristic and timing of statistical process control (**SPC**) techniques applied to individual animal daily average drinking

speed, milk consumption, and unrewarded visit behavior to predict and detect clinical disease as compared to a farm personnel diagnosis. Self-starting CUSUM charts were parameterized for optimal sensitivity and timing in a test set of calves, then applied to all calves. The diagnostic test characteristic when evaluating single, two way and three way combinations of feeding behaviors were investigated. These results showed that the combination of drinking speed and milk consumption interpreted in parallel combination were the most sensitive (70.9%) and timely test to detect an illness event, signaling a sick calf an average of 3 days prior to a treatment event. However, none of the predictive values of any of the single, two way, or three way combinations of feeding behavior parameters had sufficient predictive ability to be used alone without daily observations by skilled calf caregivers.

The results of objective one contribute to the knowledge of daily average feeding behavior in group housed dairy calves, and is the first attempt at investigating the utility of using signals generated by statistical process control to predict and detect disease. The use of drinking speed and milk consumption in combination provide the most sensitive test, but none of the predictive values were sufficient to use this method of detection alone. Calf caregivers with good observational skills are still necessary to detect sick calves in group housing systems.

10.2.2 Objective Two: The use of visit level feeding behaviors to predict and detect disease in automatically fed group housed preweaned dairy calves.

The second objective of this thesis was to describe the use and utility of visit (or meal) level feeding behaviors to predict and detect disease in automatically fed group

housed preweaned dairy calves. Data collected from a subset of calves from objective one was used for this study, representing 8 farms in Minnesota (n=3) and Virginia (n=5). These eight farms had the institute function installed in automatic feeder hand held devices, which was used to record individual calf visit behavior. Visit level average behaviors were averaged into six hour increments (quarter day). A matched pair analysis was used to describe the difference in quarter day visit average feeding behaviors in sick and healthy calves around the time of an illness event. These results showed that sick calves had an increase in total drinking time at the feeder and a decrease in visit average drinking speed up to 24hrs prior to clinical disease diagnosis by farm personnel. Visit average milk consumption and total time at the feeder was only different between sick and healthy calves in the 6 hour time period prior to clinical diagnosis. (Knauer et al., 2017)

Statistical process control techniques were then applied to these same visit average feeding behaviors to understand the diagnostic test characteristics and timing of using this method to detect a sick calf. Self-starting CUSUM chart parameters were first optimized for sensitivity and timing in a testing subset of calves, then optimal parameters were applied to all calves in the data set. Diagnostic test characteristics and timing for visit average feeding behaviors were analyzed alone and in combination. A positive alert on a combination of drinking speed, total drinking visit time, and/or milk consumption provided a sensitively to 89% and was able to detect as sick calf an average of 6.5d prior to detection by farm personnel. However, the specificity was very poor (7.7%) and predictive values for all single and combination visit average feeding behaviors were also

poor, with negative predictive values ranging from 41 – 48% and positive predictive ability ranging from 50 – 54%.

The results of objective two contribute to the knowledge of visit (meal) average feeding behavior in group housed dairy calves. Overall, the use of visit average feeding behaviors had improved sensitivity and timing when compared to the aforementioned evaluation of daily average feeding behaviors. However, predictive ability of the test was not improved, suggesting that neither day-level of visit (meal) level feeding behavior data are sufficient to predict or detect disease when used as the sole method of detection. As such, daily visual observation by trained personnel will still be necessary to detect illness in calves.

10.2.3 Objective Three: The use of automatically captured indwelling rumen temperature bolus measures to predict and detect disease in automatically fed group housed preweaned dairy calves.

The third objective of this thesis was to investigate the diagnostic utility of an indwelling calf rumen temperature bolus system. As a first step, a validation study was performed to describe the performance of the bolus as compared to two reference standards. First, the bolus temperature was compared to a known water bath temperature. The bolus was well correlated to the water bath temperature over a range of biologically plausible temperatures. Second, a prospective cross sectional study was performed that compared the bolus temperature measurement to the rectal temperature in growing heifer calves and described the diagnostic test characteristics to detect a rectal temperature ≥ 39.5ºC. The bolus underestimated the rectal temperature of growing heifer calves by an

average of 0.33ºC and had a poor sensitivity (29%) and positive predictive value (17%) to detect a rectal temperature ≥ 39.5ºC (Knauer et al, 2016).

As a second step in this investigation, a field study was conducted to describe the use and utility of an indwelling rumen temperature bolus system to predict and detect disease in automatically fed group housed preweaned dairy calves. A prospective cohort study was performed on two farms in MN utilizing group housing and automatic feeding. Enrolled calves were administered boluses at birth and their temperatures were automatically captured during the time they were in the group pen. Temperatures were averaged by both hour and six hour time periods. We reported a monophasic diurnal pattern of individual calf bolus temperature measurements over a 24 hour period, which varied by farm and season. Results of a matched pair analysis showed that bolus temperature was elevated 24hrs prior to clinical diagnosis by farm personnel as compared to healthy control calves, though this varied by type of disease present. When specific diseases were investigated, calves diagnosed with pneumonia and ill thrift had a bolus temperature that was elevated 24hr prior to clinical diagnosis, but calves diagnosed with diarrhea did not have different bolus temperature measures than their healthy matched control calves.

Statistical process control techniques as well as threshold and deviation limits were then applied to individual calf temperature data to learn if these methods of data analysis could be applied to these bolus temperature measures to predict and detect disease in an accurate and timely manner. Results showed that no detection technique had a sufficient combination of acceptable diagnostic test characteristics and timing to be applied directly in the field. Positive and negative predictive ability of all detection

techniques were poor, indicating that caution should be used in considering these methods as the false positive rate may be unacceptable for producers using these systems. In addition to the poor diagnostic test characteristics, a high rate of bolus loss (23% of calves) would also limit the utility of this system if adopted on commercial dairy farms.

The results of objectives three represent the first study investigating the use and utility of an indwelling rumen temperature bolus for prediction and detection of morbidity in group housed pre weaned dairy calves. No detection method provided test characteristics that were sufficient to predict or detect disease. An unexpected result from this field study was the difference in diurnal variation in RTB measures by farm. More studies on more farms are needed to understand how ambient temperature and barn temperature are associated with calf body temperature, performance, and health.

10.3 IMPLICATIONS AND OPPORTUNITIES FOR FUTURE RESEARCH

The goal of sensor research is to provide farmers with tools to improve cow and calf health management. The central research question, therefore, should be what value the sensor system adds to farmer decision making. (Rutten et al 2013) This should not just be economic value, but also include considerations for the value of risk mitigation, animal health and wellbeing, and labor efficiency. The results of our study suggest that neither sensor-derived feeding behavior data nor automatically captured temperature measures provided sufficient test performance to be used as the sole means of disease detection in group housed preweaned dairy calves. As such, until better automated

disease detection tools are developed, it will still be important that trained herd personnel continue to conduct visual assessments of calves for the purpose of detecting illness.

The first next step will be to investigate other analytical techniques applied to this data to see if improved sensitivity and specificity can be achieved over what was found in the current study. One such method may be through combining feeding behavior data as well as bolus temperature signals to the algorithm. Lukas et al. (2009), reported an improvement in test sensitivity in the detection of some periparturient diseases in adult dairy cows when milk yield and electrical conductivity were monitored together, rather than just milk yield alone. To detect metabolic and digestive disorders in fresh cows, Stangaferro et al (2016) reported an improvement in overall test accuracy when using rumination time and activity in a combined health index score rather than using each alone. These studies suggest that multiple health measures could be useful in the development of a health index for dairy calves which includes feeding behaviors and temperature data. Another interesting addition to this index would be calf activity monitoring, which has not been explored in its association with morbidity in group housed dairy calves.

Another potential alternative detection method for these sensor-derived data is the potential application of machine learning (ML). In ML, a machine (an algorithm/model) improves its performance (predictive accuracy) in achieving a task (e.g. disease diagnosis) from experience (data). The goal is for the predicting model to generalize well, and make accurate predictions on previously unseen data. (Valletta, 2017). In this way, the algorithm improves over time as more data becomes available. An advantage of ML techniques over other classical statistical procedures is that ML has no underlying

assumptions and merely makes predictions based on the data provided. Very few examples of these types of applications to livestock sensor data exist. Borchers et al (2017) used ML techniques (random forest, linear discrimination analysis, and neural network) to predict calving time from activity and rumination data. They found that ML applied to the combination of parameters had good test characteristics when predicting the day of calving and the 8h time period prior to calving.

After developing the most accurate and timely algorithm, this information could be used to develop a calf health decision support model. This would provide advice on how to interpret the sensor alert information, and advice on how to proceed with the decision on the calf that signaled. One example of this is the breeding management tool developed by Giordano et al, (2011) that provides guidance on what breeding management strategy will result in the maximum net present value per cow. This type of model could be integrated into an easy to use app or computer program that provided the producer with a diagnosis (or predicted diagnosis) and could provide economic, treatment, and potential outcomes. This would all result in the producer (or calf caregiver) making a decision about the calf with as much information as possible.

The aim of this thesis work was to investigate the utility of the application of SPC to individual animal feeding behavior and temperature measures to predict and detect disease. However, one other potential application of SPC or other monitoring techniques to precision technologies is at the group or pen level. Monitoring pen level averages has been reported to have utility to detect disease outbreaks (Madsen and Kristensen, 2005), and could also be used to detect technology failure and/or human error.

Another interesting observation from the third objective of this thesis was the variation in calf rumen bolus temperature by farm. One interesting question that results from these findings is whether and/or how calf housing environment influences calf temperature. An interesting area of future research could be to investigate associations between body temperature fluctuations, calf health, and calf performance, which could ultimately result in recommendations for improvements in calf housing and heat abatement strategies. Understanding temperature fluctuations as it relates to calf housing could also help inform producers as to the best time of day to take calf rectal temperatures to reduce false positive results Another challenge is the cost of these systems, so an economic analysis, taking into consideration all of the benefits of continual monitoring of calf body temperature, is warranted.

11 REFERENCES

Appleby, M. 2001. Performance and feeding behavior of calves on ad libitum milk from artificial teats. App. Anim. Behav. Sci. 74: 191-201.

AlZahal, O., H. AlZahal, M.A. Steele, M. Van Schaik, I. Kyriazakis, T.F. Duffield, and B.W. McBride. 2011. The use of a radiotelemetric ruminal bolus to detect body temperature changes in lactating dairy cattle. J. Dairy Sci. 94: 3568-3574.

Beakley, W.R. and J.D. Findlay. 1955. The effect of environmental temperature and humidity on the rectal temperature of calves. J. Agric. Sci. 45: 353.

Berberich, N., and H. Grimm. 2013. Effects of two feeding systems on the development of dairy calves. Landtechnik. 68 (5): 333 – 338.

Bewley, J.M., M.E. Einstein, M.W. Grott, and M.M. Schutz. 2008a. Comparison of reticular and rectal core body temperatures in lactating dairy cows. J. Dairy Sci. 91: 4661-4672.

Bewley J.M., M.W. Grott, M.E. Einstein, and M.M. Schutz. 2008b. Impact of intake water temperatures on reticular temperatures of lactating dairy cows. J. Dairy Sci. 91: 3880 – 3887.

Bewley, J. 2010. Precision Dairy Farming: Advanced Analysis Solutions for Future Profitability. Proc: The First North American Conference on Precision Dairy Management.

Borchers, M.R., Y.M. Chang, K.L. Proudfoot, B.A. Wadsworth, A.E. Stone, and J.M. Bewley. 2017. Machine-learning-based calving prediction from activity, lying, and ruminating behaviors in dairy cattle. J. Dairy Sci. 100: 5664 – 5674.

Borderas, T.F., A.M. de Passille, and J. Rushen. 2008. Behavior of dairy calves after a low dose of bacterial endotoxin. J. Anim. Sci. 86: 2920 – 2927.

Borderas, T.F., J. Rushen, M.A.G. von Keyserllingk, and A.M.B. de Passille. 2009. Automated measurement of changes in feeding behavior of milk-fed calves associated with illness. J Dairy Sci. 92:4549-4554.

Bossuyt, P.M., J.B., Reitsma, D.E., Bruns, C.A., Gatsonis, P.P., Glasziou, L., Irwig, J.G., Lijmer, D., Moher, D., Rennie, H.C.W., de Vet, H.Y., Kressel, N., Rifai, R.M., Golub, D.G., Altman, L., Hooft, D.A., Korevaar, and J.F. Cohen, For the STARD Group. 2015. STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies. B.M.J. 351: h5527.

Broom, D.M. and J.D. Leaver. 1978. Effects of group-rearing on partial social isolation on later social behavior of calves. Anim. Behavior. 26: 1255 – 1263.

Chua, B., E. Coenen, J. van Delen, and D.M. Weary. 2002. Effects of pair versus individual housing on the behavior and performance of dairy calves. J. Dairy Sci. 85: 360 – 364.

Costa, J.H.C., R.K. Meagher, M.A.G.von Keyserlingk, and D.M. Weary. 2015. Early pair housing increases solid feed intake and weight gains in dairy calves. J. Dairy Sci. 98: 6381 – 6386.

Cornou, C.J., J. Vinther, and A.R. Kristensen. 2008. Automatic detection of oestrus and health disorders using data from electronic sow feeders. Livest. Sci. 118: 262 – 271.

Correa, M.T., C.R. Curtis, H.N. Erb, and M.E. White. 1988. Effect of calfhood morbidity on age at first calving in New York Holstein herds. Prev. Vet. Med. 6: 253 – 262.

Cramer, M.C., and A.L. Stanton. 2015. Associations between health status and the probability of approaching a novel object or stationary human in preweaed group-housed dairy calves. J. Dairy Sci. 98: 7298 – 7308.

Cramer, M.C., T.L. Ollivet, and A.L. Stanton. 2016. Associations of behavior – based measurements and clinical disease in preweaned, group-housed dairy calves. J. Dairy Sci. 99: 7434 – 7443.

Curtis, C.R., Erb, H.N., and M.E. White. 1988. Descriptive epidemiology of calfhood morbidity and mortality in New York Holstein herds. Prev. Vet. Med. 5: 293 – 307.

Dairy Calf and Heifer Association. Production and performance standards established for Holstein calves, from birth to 6 months of age, across the United States. http://calfology.com/sites/default/files/file-attachments/final_gold_standards_i.pdf Accessed 11/23/15.

Dale, H.E., R.E. Stewart, and S. Brody. 1954. Rumen temperature. 1. Temperature gradients during feeding and fasting. Cornell Vet. 44: 368 – 374.

Daros, R.R., J.H.C. Costa, M.A.G. von Keyserlingk, M.J. Hötzel, and D.M. Weary. 2014. Separation from the Dam Causes Negative Judgement Bias in Dairy Calves. PLoS ONE 9(5): e98429. https://doi.org/10.1371/journal.pone.0098429.

De Passille A.M., M. Rabeyrin and J. Rushen. 2016. Associations between milk intake and activity in the first days of a calf's life and later growth and health. App. Anim. Behav. Sci. 175: 2-7.

De Vries, A., and J. K. Reneau. 2010. Application of statistical process control charts to monitor changes in animal production systems. J. Anim. Sci. 88 (E. Suppl.): E11 – E29.

DeVries, T.J., M.A.G von Keyserlingk, and D.M. Weary. 2004. Effect of feeding space on the inter-cow distance, aggression, and feeding behavior of free-stall housed lactating dairy cows. J. Dairy Sci. 87: 1432 – 1438.

Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. VER, Inc., Charlottetown, Prince Edward Island, Canada.

Estevez, I., I.L. Andersen, and E. Naevdal. 2007. Group size, density and social dynamics in farm animals. App. Anim. Behav. Sci. 103: 185 – 204.

Faerevik, G., K. Tjentland, S. Lovik, I.L. Andersen, K.E. Boe. 2008. Resting pattern and social behavior of dairy calves housed in pens with different sized lying areas. App. Anim. Behav. Sci. 114: 54 – 64.

Fraser, David. 2008. Understanding animal welfare: The science in its cultural context. Wiley-Blackwell. Ames, IA.

Gaillard, C., R.K. Meagher, M.A.G. von Keyserlingk and D.M. Weary. 2014. Social housing improves dairy calves' performance in two cognitive tests. PLoS ONE 9(2): e90205. doi:10.1371/journal.pone.0090205.

Giordano, J.O., P.M. Fricke, M.C. Wiltbank, and V.E. Cabrera. 2011. An economic decision-making support system for selection of reproductive management programs on dairy farms. J. Dairy Sci. 94: 6216 – 6232.

Godinho, K.S., A. Rae, G.D. Windsor, N. Tilt, T.G.Rowan, and S.J. Sunderland. 2005. Efficacy of tulathromycin in the treatment of bovine respiratory disease associated with induced Mycoplasma bovis infections in young dairy calves. Vet. Therapeutics. 6: 96-112.

Gonzalez, L.A., B.J. Tolkamp, M.P. Coffey, A. Ferret, and I. Kyriazakis. 2008. Changes in feeding behavior as possible indicators for automatic monitoring of health disorders in dairy cows. J. Dairy Sci. 91: 1017 – 1028.

Graves, R.E., J.T. Tyson, D.F. McFarland, and T.H. Wilson. 2008. Penn State housing plans for calves and heifers, NRAES-201. 278.

Hankenson, F.C., N. Ruskoski, M. van Saun, G. Ying, J. Oh, and N.W. Fraser. 2013. Weight loss and reduced body temperature determine humane endpoints in a mouse model of ocular herpesvirus infection. J. of the Am. Ass. for Lab. Anim. Sci. 52: 277 – 285.

Hart, B.L. 1988. Biological Basis of the Behavior of Sick Animals. Neuroscience and Behavioral Reviews. 12: 123-137.

Hawkins, D.M., and G.H. Olwell. 1998. Cumulative Sum Charts and Charting for Quality Improvement. Springer-Verlag, New York, NY.

Heinrichs, A.J., and C.M. Jones. 2003. Feeding the newborn dairy calf. http://extension.psu.edu/publications/feeding-newborn-dairy-calf

Heinrichs, A.J., and B.S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. J. Dairy Sci. 94: 336 – 341.

Herskin, M.S., F. Skjoth, and M.B. Jensen. 2010. Effects of hunger level and tube diameter on the feeding behavior of teat-fed dairy calves. J. Dairy Sci. 93: 2053 – 2059.

Hill, T.M., H.G. Bateman II, J.M. Aldrich, and R.L. Schlotterbeck. 2011. Comparisons of housing, bedding, and cooling options for dairy calves. J. Dairy Sci. 94: 2138 – 2146.

Huuskonen, A., and H. Khalili. 2008. Computer-controlled milk replacer feeding strategies for group-reared dairy calves. Livestock Sci. 113:203-306.

Jasper J., and D.M. Weary. 2002. Effects of ad libitum milk intake on dairy calves. J. Dairy Sci. 85: 3054 – 3058.

Jensen, M.B., Vestergaard, K.S., Krohn, C.C., Munksgaard, L. 1997. Effect of single versus group housing space allowance on responses of calves during open-field tests. Appl. Anim. Behav. Sci. 54: 109 – 121.

Jensen, M.B., K.S. Vestergaard, and C.C. Krohn. 1998. Play behavior in dairy calves kept in pens: the effect of social contact and space allowance. App. Anim. Behav. Sci. 56: 97 – 108.

Jensen, M.B., L. Munksgaard, L. Mogensen and C.C. Krohn. 1999. Effects of housing in different social environments on open field and social responses of female dairy calves. Acta. Agric. Scand. 49:113-120.

Jensen, M.B. and R. Kyhn. 2000. Play behavior in group-housed dairy calves, the effect of space allowance. App. Anim. Behav. Sci. 67: 35-46.

Jensen M.B., and L. Holm. 2003. The effect of milk flow rate and milk allowance on feeding related behavior in dairy calves fed by computer controlled milk feeders. App. Anim. Behav. Sci. 82:87 – 100.

Jensen, M.B. 2004. Computer-controlled milk feeding of dairy calves: The effects of number of calves per feeder and number of milk portions on use of feeder and social behavior. J. Dairy Sci. 87: 3428 – 3438.

Jensen, M.B. 2007. Age at introduction to the group affects dairy calves use of a computer controlled milk feeder. App. Anim. Behav. Sci. 107: 22-31.

Jensen, M.B. 2009. Short communication: Milk meal pattern of dairy calves is affected by computer-controlled milk feeder set-up. J. Dairy Sci. 92: 2906 – 2910.

Jensen, M.B. and L.E. Larsen. 2014. Effects of level of social contact on dairy calf behavior and health. J. Dairy Sci. 97: 1-10.

Jensen, M.B., L.R. Duve, and D.M. Weary. 2015. Pair housing and enhanced milk allowance increase play behavior and improve performance in dairy calves. J. Dairy Sci. 98: 2568 – 2575.

Johnson, RW. 2002. The concept of sickness behavior: a brief chronological account of four key discoveries. Vet. Imm. and Immunopath. 87: 443-450.

Jorgensen, M.W., A. Adams-Progar, A.M. de Passille, J. Rushen, S.M. Godden, H. Chester-Jones, and M.I. Endres. 2017. Factors associated with dairy calf health in automated feeding systems in the Upper Midwest United States. J. Dairy Sci. 100: 5675 – 5686.

Kertz, A.F., L.F. Reutzel, and J.H. Mahoney. 1984. Ad libitum water intake by neonatal calves and its relationship to calf starter intake, weight gain, fecal scores, and season. J. Dairy Sci. 67: 2964 – 2969.

Keys, J.E., R.E. Pearson, and P.D. Thompson. 1978. Effect of feedbunk stocking density on weight gains and feeding behavior of yearling Holstein heifers. J. Dairy Sci. 61: 448 – 454.

Kluger, M.J., and L.K. Vaughn. 1978. Fever and survival in rabbits infected with Pasteurella multocida. J. Physiol. 282: 243 – 51.

Knauer, W.A., S.M. Godden, and N. McDonald. 2016. Technical note: Preliminary evaluation of an automated indwelling rumen temperature bolus measurement system to detect pyrexia in preweaned dairy calves. J. Dairy Sci. 99: 9925 – 9930.

Knauer, W.A., S.M. Godden, A. Dietrich, and R.E. James. 2017. The association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. J. Dairy Sci. 100: 5642 – 5652.

Knauer, W.A., S.M. Godden, D.M. Hawkins, A. Dietrich, and R.E. James. The application of statistical process control techniques to daily average feeding behaviors to detect disease in automatically fed group-housed pre-weaned dairy calves. *In preparation for J. Dairy Sci.*

Knauer, W.A., S.M. Godden, A. Dietrich, and R.E. James. The association between visit level feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. *In preparation for J. Dairy Sci.*

Kung, L., S. Demarco, L.N. Siebenson, E. Joyner, G.F.W. Haenlein, and R.M. Morris. 1997. An evaluation of two management systems for rearing calves fed milk replacer. J. Dairy Sci. 80: 2529 – 2533.

Liang, D., C.L., Wood, K.J. McQuerry, D.L. Ray, F.D. Clark, and J. M. Bewley. 2013. Influence of breed, milk production, season, and ambient temperature on dairy cow reticulorumen temperature. J. Dairy Sci. 96: 5072 – 5081.

Losinger, W. C. and A.J. Heinrichs. 1997. Management practices associated with high mortality among preweaned dairy heifers. J Dairy Res. 96: 1-11.

Lukas, J.M., J.K. Reneau and J.G. Linn. 2008a. Water Intake and Dry Matter Intake Changes as a Feeding Management Tool and Indicator of Health and Estrus Status in Dairy Cows. J. Dairy Sci. 91: 3385-3394.

Lukas, J.M., J.K. Reneau, C. Munoz-Zanzi, and M.L. Kinsel. 2008b. Predicting somatic cell count standard violations based on herd's bulk tank somatic cell count. Part II: Consistency Index. J. Dairy Sci. 91: 433 – 441.

Lukas, J.M., J.K. Reneau, R. Wallance, D. Hawkins and C. Munoz-Zanzi. 2009. A novel method of analyzing daily milk production and electrical conductivity to predict disease onset. J. Dairy Sci. 92: 5964 – 5976.

Maatje, K., J. Verhoeff, W.D.J. Kremer, A.L.M. Cruijsen, and T.S.G.A.M. van den Ingh. 1993. Automated feeding of milk replacer and health control of group-housed veal calves. Vet Rec. 133: 266- 270.

MaCaulay, A.S., G.L. Hahn, D.H. Clark, and D.V. Sisson. 1995. Comparison of calf housing types and tympanic temperature rhythms in Holstein calves. J. Dairy Sci. 78: 856 – 862.

Madsen, T.N., and A.R. Kristensen. 2005. A model for monitoring the condition of young pigs by their drinking behavior. Computers and Electronics in Agriculture. 48: 138 – 154.

Martin, S.W. and A.D. Wiggins. 1973. A model of the economic costs of dairy calf mortality. Am. J. Vet. Res. 34: 1027 – 1031.

McGuirk, S. 2008. Disease management of dairy calves and heifer.Vet. Clin. North Am. Food Anim. Pract. 24 : 139-153.

Medrano – Galarza, C., S.J. LeBlanc, T.J. Devries, A. Jones – Bitton, J. Rushen, A.M. de Passille, and D.B. Haley. 2017. A survey of dairy calf management practices among farms using manual and automated milk feeding systems in Canada. J. Dairy Sci. 100: 6872 – 6884.

Mertens, K., E. Decuypere, J. De Baerdemaeker, and B. De Ketelaere. 2011. Statistical control charts as a support tool for the management of livestock production. J. Ag. Sci. 149 (3): 369 – 384.

Miekley, B., E. Stamer, I. Traulsen, and J. Krieter. 2013. Implementation of multivariate cumulative sum control charts in mastitis and lameness monitoring. J. Dairy Sci. 96: 5723 – 5733.

Millman, S.T. 2007. Sickness behavior and its relevance to animal welfare assessment at the group level. Animal Welfare. 16: 123-125.

Moallem, U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, and A. Shamay. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93:2639–2650.

Montgomery, D.C. 2013. Introduction to Statistical Quality Control. 7th Ed. John Wiley & Sons, Inc. Hoboken, NJ.

Murray, M.J. and A.B. Murray. 1979. Anorexia of infection as a mechanism of host defense. Am. J. of Clin. Nurtr. 32: 593 – 596.

Neave, H.W., R.R. Daros, J.H.C. Costa, M.A.G. von Keyserlingk, and D.M. Weary. 2013. Pain and Pessimism: Dairy Calves Exhibit Negative Judgement Bias following Hot-Iron Disbudding. PLoS ONE 8(12): e80556. https://doi.org/10.1371/journal.pone.0080556.

Odde, K.G., G.H Kiracofe, and R.R. Schalles. 1985. Suckling behavior in range beef calves. J. Anim. Sci. 61: 307 – 309.

O'Driscoll, K., M.A.G. von Keyserlingk, and D.M. Weary. 2006. Effects of mixing on drinking and competitive behavior of dairy calves. J. Dairy Sci. 89: 229 – 233.

Olson, D.P., C.J. Papasian, and R.C. Ritter. 1980. The effects of cold stress on neonatal calves I. Clinical condition and pathological lesions. Can. J. Comp. Med. 44: 11 – 18.

Pena, G., C. Risco, E. Kunihiro, M-J.Thatcher, and P.J. Pinedo. 2016. Effect of housing type on health and performance of preweaned dairy calves during the summer in Florida. 99: 1655 – 1662.

Quimby, W.F., B.F. Sowell, J.G.P. Bowman, M.E. Branine, M.E. Hubbert, and H.W. Sherwood. 2001. Application of feeding behaviour to predict morbidity of newly received calves in a commercial feedlot. Can. J. Anim. Sci. 81 (3): 315 – 320

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reneau, J.K. and J. Lukas. 2006. Using Statistical Process Control Methods to Improve Herd Performance. Vet. Clin. North Am. Food Anim. Pract. 22: 171-193.

Rose-Dye, T.K., L.O. Burciaga-Robles, C.R. Krehbiel, D.L. Step, R.W. Fulton, A.W. Confer, and C.J. Richards. 2011. Rumen temperature change monitored with remote rumen temperature boluses after challenges with bovine viral diarrhea virus and *Mannheimia haemolytica*. J. Anim. Sci. 89: 1193-1200.

Roth, B.A., E. Hillman, M. Stauffacher and N.M. Keil. 2008. Improved weaning reduces cross-sucking and may improve weight gain in dairy calves. App. An. Behav. Sci. 111: 251-261.

Roth, B.A., N.M. Keil, L. Gygax and E. Hillman. 2009. Influence of weaning method on health status and rumen development in dairy calves. J. Dairy Sci. 92: 645 – 656.

Rutten, C.J., A.G.J. Velthuis, W. Steeneveld, and H. Hogeveen. 2013. Invited Review: Sensors to support health management on dairies. J. Dairy Sci. 96: 1928 – 1952.

Rushen, J., A.M. de Passille, and L. Munksgaard. 1999. Fear of people by cows and effects on milk yield, behavior, and heart rate at milking. J. Dairy Sci. 82: 720 – 727.

Rushen, J., A.M. de Passille, M.A.G. von Keyserlingk, and D.M. Weary. 2008. The welfare of Cattle. Springer. Dordrecht, The Netherlands.

Schaeffer, A.L., N. Cook, S.V. Tessaro, D. Deregt, G. Desroches, P.L. Dubeski, A.K.W. Tong, and D.L. Godson. 2004. Early detection and prediction of infection using infrared thermography. Can. J. Anim. Sci. 84: 73-80.

Schaeffer, A.L., N.J. Cook, J.S. Church, J. Basarab, B. Perry, C. Miller, and A.K.W Tong. 2011. The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves. Res. In Vet. Sci. 83: 376 – 384.

Sivula, N.J., T.R. Ames, W.E. Marsh, and R.E. Werdin. 1996. Descriptive epidemiology of morbidity and mortality in Minnesota dairy heifer calves. Prev. Vet. Med. 27: 155-171.

Smith, B. 2009. Manifestations and management of disease in neonatal ruminants. Page 276 in Large Animal Internal Medicine. 4th ed. Mosby Elsevier, St. Louis, MO.

Soberon, F., and M.E. Van Amburgh. 2013. The effect of nutrient intake from milk or milk replacer of preweaned dairy calves on lactation milk yield as adults: A meta-analysis of current data. J Anim. Sci. 91:706-712.

Sowell, B.F., M.E. Branine, J.G.P. Bowman, M.E. Hubbert, H.E. Sherwood, and W. Quimby. 1999. Feeding and Watering Behavior of Healthy and Morbid Steers in a Commercial Feedlot. J. Anim. Sci. 77: 1105 – 1112.

Spain, J.N., and D.E. Spiers. 1996. Effects of supplemental shade on thermoregulatory response of calves to heat challenge in a hutch environment. J. Dairy Sci. 79: 639 – 646.

Stangaferro, M.L., R. Wijma, L.S. Caixeta, M. A. Al-Abri, and J.O. Giordano. 2016. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part 1. Metabolic and digestive disorders. J. Dairy Sci. 99: 7395 – 7410.

Steeneveld, W., L.C. van der Gaag, W. Ouweitjes, H. Mollenhorst, and H. Hogeveen. 2010. Discriminating between true-positive and false-positive clinical mastitis alerts from automatic milking systems. J. Dairy Sci. 93: 2559 – 2568.

Steenkamer, N. 1982. Alternative housing systems for veal calves, their effect on welfare and performance and their economic feasibility. In: Signoret, J.P. (Ed.), Welfare and Husbandry of Calves: Current Topics in Veterinary Medicine and Animal Science. 19: 226 – 234.

Steiger, M., M. Senn, G. Altreuther, D. Werling, F. Sutter, M. Kruezer and W. Langhans. 1999. Effect of a prolonged low-dose lipopolysaccharide infusion on feed intake and metabolism in heifers. J. Anim. Sci. 77: 2523-2532.

Svensson, C., K. Lundborg, U. Emanuelson, and S.O. Olsson. 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Prev. Vet. Med. 58:179-197.

Svensson, C., and P. Liberg. 2006. Effect of group size on health and growth rate of Swedish dairy calves housed in pens with automatic milk-feeders. Prev. Vet. Med. 73:43-53.

Svensson, C., and M.B. Jensen. 2007. Short Communication: Identification of Diseased Calves by Use of Data from Automatic Feeders. J. Dairy Sci. 90: 994 – 997.

Sweeney, B.C., J. Rushen, D.M. Weary, and A.M. de Passille. 2010. Duration of weaning, starter intake, and weight gain of dairy calves fed large amounts of milk. J. Dairy Sci. 93: 148 – 152.

Swanson, E.W. and J.D. Harris. 1958. Development of rumination in the young calf. J. Dairy Sci. 41: 1768 – 1776.

Timsit, E., S. Assie, R. Quiniou, H. Seegers, and N. Bareille. 2011. Early detection of bovine respiratory disease in young bulls using reticulo-rumen temperature boluses. Vet. Journal. 190: 136-142.

Tukey, J.W. 1977. Exploratory Data Analysis. Addison – Wesley, Reading MA.

Urton, G., M.A.G. von Keyserlingk, and D.M. Weary. 2005. Feeding behavior identifies dairy cows at risk for metritis. J. Dairy Sci. 88: 2843 – 2849.

US Department of Agriculture. National Animal Health Monitoring Services. Dairy 2007. Heifer calf health and management practices on U.S. dairy operations, 2007. http://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_ir_CalfHeath.pdf. Accessed 8/1/2017.

USDA-NAHMS. 2011. Dairy Heifer Raiser, 2011. An overview of operations that specialize in raising dairy heifers. Accessed 8/3/2017. https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairyheifer11/HeiferRaiser.pdf

US Department of Agriculture. National Animal Health Monitoring Services. Dairy 2014. Dairy Cattle Management Practices in the United States, 2014. https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy14/Dairy14_dr_PartI.pdf. Accessed 8/1/2017.

USDA National Animal Statistical Service. Accessed 7/31/2017. http://usda.mannlib.cornell.edu/usda/current/AgriPric/AgriPric-07-31-2017.pdf

Valletta, J.J., C. Torney, M. Kings, A. Thornton, and J. Madden. 2017. Review: Applications of machine learning in animal behavior studies. Animal Behavior. 124: 203 – 220.

Van Metre, D.C., B.C. Tennant, and R.H. Whitlock. 2008. Infectious diseases of the Gastrointestinal Tract. In: Rebhun's Diseases of Dairy Cattle 2nd Edition: 200 – 229.

Vickers, L.A., O. Burfeind, M.A.G. von Keyserlingk, D.M. Veira, D.M. Weary, and W. Heuwieser. 2010. Technical note: Comparison of rectal and vaginal temperatures in lactating dairy cows. J. Dairy Sci. 93: 5246 – 5251.

Vieira, A.D.P., A.M. de Passille, and D.M. Weary. 2012. Effects of early social environment on behavioral responses of dairy calves to novel events. J. Dairy Sci. 95: 5149 – 5155.

Virtala, A.M., G.D. Mechor, Y.T. Grohn, and H.N. Erb. 1996. The effect of calfhood diseases on growth of female dairy calves during the first 3 months of life in New York state. J. Dairy Sci. 79: 1040 – 1049.

Von Keyserlingk, M.A., J. Rushen, A.M. de Passille, and D.M. Weary. 2009. Invited review: The welfare of dairy cattle – Key concepts and the role of science. J. Dairy Sci. 92: 4101 – 4111.

Walter, E.J., S. Hanna-Jumma, M. Carraretto, and L. Forni. 2016. The pathophysical basis and consequences of fever. Critical Care. 20: 200.

Waltner-Toews, D., S.W. Martin, and A.H. Meek. 1986. Dairy calf management, morbidity and mortality in Ontario Holstein herds. III. Association of management with morbidity. Prev. Vet. Med. 4: 137 – 158.

Warnick, V.D., C.W. Arave, and C.H. Mickelsen. 1977. Effects of group, individual, and isolation rearing of calves on weight gain and behavior. J. Dairy Sci. 60: 947 – 953.

Weary, D.M., J.M. Huzzey, and M.A.G von Keyserlingk. 2009. Board-Invited Review: Using behavior to predict and identify ill health in animals. J. Anim. Sci. 87: 770 – 777.

Wrenn, T.R., J. Bitman, and J.F. Skyes. 1961. Diurnal patterns of bovine body temperature. J. Dairy Sci. 44: 2077 – 2080.

Zwald, A., T.L., Kohlman, S.L. Gunderson, P.C. Hoffman and T. Kriegl. 2007. Economic costs and labor efficiencies associated with raising dairy herd replacements on Wisconsin dairy farms and custom heifer rearing operations. UW Extension Publications.