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PREDICTING TOMORROW: OPTIMIZING THE EARLY DETECTION OF DISEASE AND DISEASE RECOVERY IN DAIRY CALVES USING PRECISION TECHNOLOGIES

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PREDICTING TOMORROW: OPTIMIZING THE EARLY DETECTION OF DISEASE AND DISEASE RECOVERY IN DAIRY CALVES USING PRECISION TECHNOLOGIES

DISSERTATION

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

By

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2021

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ABSTRACT OF DISSERTATION

PREDICTING TOMORROW: OPTIMIZING THE EARLY DETECTION OF DISEASE AND DISEASE RECOVERY IN DAIRY CALVES USING PRECISION TECHNOLOGIES

The leading causes of morbidity and mortality in preweaned dairy calves are diarrhea and bovine respiratory disease (BRD). The delayed detection of these diseases in calves can also delay intervention and disease recovery. The overarching objective of this dissertation was to follow a cohort of calves daily for the first 90 days of life for naturally occurring clinical BRD bouts and diarrheal bouts. The objective answered if feeding behaviors and activity levels were different in at-risk calves during BRD development and BRD recovery from an antimicrobial intervention. Furthermore, the potential of colostrum replacer as a feeding intervention strategy to ameliorate disease bouts and improve performance in calves was explored. Thus, the objective of the first study was to investigate the relationship between feeding behavioral patterns and activity levels in preweaned calves for the five days before clinical BRD diagnosis using precision technologies to document these behaviors. Next, we investigated if dairy calves presented different behavioral responses after antimicrobial intervention for BRD depending on the outcome of the treatment (recovered or relapsed). Finally, we assessed if providing an early intervention of colostrum replacer to at-risk calves (e.g., at-risk by algorithm classification) could ameliorate disease bouts (e.g., diarrhea and BRD). A cohort of socially housed, Holstein calves born on one research facility (n=120) were health-scored daily from birth to 90 days of age (e.g., 14 days after weaning). Calves were fed by automated feeders which recorded daily milk and calf starter intakes, drinking speed, rewarded and unrewarded visits. All calves wore a commercial pedometer (IceQube, IceRobotics, Scotland) on their left rear leg. The pedometer recorded daily activity levels (lying time, lying bouts, step count, and an index based on rate of acceleration and step count). Prior to a BRD bout, destined-to-be-sick calves had negative relative changes in their calf starter intakes, and treatment day interacted with BRD status for relative changes in unrewarded visits to the feeder, step count, and the activity index. For BRD calves at-risk for relapse, there were lower starter intakes and lower unrewarded visits compared to recovered calves. Furthermore, within the 10-days after antimicrobial intervention, relapsed calves had lower activity levels compared to recovered calves. Colostrum intervention ameliorated the likelihood of a BRD bout, but not diarrhea, without affecting performance in dairy calves. These results suggest that in calves, negative relative changes in starter intake, and decreased step counts may be powerful indicators of BRD and recovery status. Colostrum intervention may also ameliorate BRD bouts in calves.

KEYWORDS: automated feeder, Bovine Respiratory Disease, calf health, colostrum, diarrhea, lying behavior

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07/28/2021

Date

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DEDICATION

To Aviva and my family

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PREFACE

I completed this thesis under the supervision of Dr. Joao H.C. Costa and co-supervision of Dr. Eric Vanzant. In addition to my supervisors, my supervisory committee included Dr. Roberta Dwyer, Dr. Morgan Hayes, and Dr. Richard Coffey, all from the University of Kentucky. Also, Dr Dave Renaud from the University of Guelph and Dr Heather W. Neave participated in the analysis and editing of the projects presented here. The studies described in this thesis were approved by the University of Kentucky's Animal Care Committee (2018: 2864).

Chapter 1 is a literature review and is my unique work. Dr. Costa was the supervisory editor, and content manager. All literature review research conduct was originally performed by me.

A version of chapter 2 was submitted to J. Dairy Sci. Cantor, M.C., and J.H.C. Costa. Daily feeding and activity behavioral patterns collected by precision technology are associated with Bovine Respiratory Disease status in preweaned dairy calves. This research concept, data collection, statistical analysis and writing were conducted by me under the supervision and editing of co-author, Dr. Costa.

A version of chapter 3 was published. Cantor, M.C., D.L. Renaud, and J.H.C. Costa. Nutraceutical intervention with colostrum replacer: can we reduce disease hazard, ameliorate disease severity, and improve performance in preweaned dairy calves? J. Dairy Sci. 104(6): 7168-7176. DOI: 10.3168/jds.2020-19654. This research concept, data collection, statistical analysis and writing were conducted by me under the supervision and editing of co-author, Dr. Costa. Dr. Renaud co-authored with statistical analysis and editing.

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CHAPTER 1. LITERATURE REVIEW: DIARRHEA, BOVINE RESPIRATORY DISEASE AND PRECISION DAIRY TECHNOLOGY DEVICES: OPPORTUNITIES FOR DAIRY CALF MANAGEMENT

Introduction

Diarrhea and bovine respiratory disease (**BRD**) are significant causes of morbidity in calves and are the leading causes of mortality in dairy calf operations (USDA, 2018). Mitigating disease in calves may increase farm efficiency and improve calf welfare. According to a national producer survey, diarrhea and BRD affected 33% of preweaned dairy calves, with a high majority of affected calves receiving antibiotics [diarrhea 76% and BRD 95% (USDA, 2018)]. The treatment and labor associated with managing BRD was estimated to cost $42 per calf (Dubrovsky et al., 2020), while treatment for diarrhea was estimated to cost a median of $56 per calf (Goodell et al., 2012). Thus, the cost of BRD and diarrheal treatment have economic implications on farm, especially since the preweaning period is a major investment for the dairy farm (Hawkins et al., 2019). Economic costs aside, diarrhea and BRD compromise calf welfare. These diseases are metabolically demanding (Pardon et al., 2013), and there are long-term effects of disease on calf survival and future productivity (Buczinski et al., 2021). Furthermore, BRD calves often relapse from the initial antimicrobial intervention which extends disease duration (Welling et al., 2020). It is fundamental to investigate methods to improve the detection of BRD and diarrheal bouts, classify recovery status, and investigate the potential of interventions to ameliorate disease bouts in preweaned calves. Exploring these avenues may improve a calf's affective state by using early interventions to decrease disease duration (Binversie et al., 2020) and mitigate the negative effects of disease on calf productivity (Buczinski et al., 2021).

Sickness behavior indicates a motivational state that is activated (i.e. by prostaglandin secretion) in conjunction with the febrile response associated with disease in mammals (Hart and Hart, 2019). For dairy calves, sickness behaviors have been passively captured by precision dairy technology devices **(PDT)**. The most researched PDT available for calf management is the automated feeder, which captures feeding behavior, and accelerometers, which capture locomotory activity as reviewed by Costa et al., 2020. However, there are a myriad of PDT options already available and many more in development, as reviewed by Costa et al., 2020. One major benefit of PDT is that mammals typically exhibit sickness behaviors a few days prior to clinical detection of disease (Dantzer and Kelley, 2007). This delay between sickness behavior and the presence of clinical symptoms associated with a disease provides a window of opportunity for earlier intervention. Moreover, it is fundamental to implement antimicrobial intervention earlier to increase the likelihood of achieving recovery status, especially for BRD bouts in calves (Binversie et al., 2020). This is important beyond improving the calf's welfare, there is also consumer pressure on the dairy industry to judiciously limit the use of antimicrobials, or eliminate their use altogether (Wemette et al., 2021). This consumer pressure is potentially linked to antimicrobial resistance, which has been observed on dairy calf operations (Langford et al., 2003). This process between consumer and government policy has led to the adoption of strict antimicrobial regulations for managing dairy calves in other countries (Johnsen et al., 2021). Furthermore, it is imperative to reduce antimicrobial resistance in calves to ensure antimicrobials remain effective for future generations. Thus, the development of strategies to reduce antimicrobial use are fundamental for the sustainability of the dairy industry in North America.

Indeed, the adoption of such strict antimicrobial practices in other countries often occurs at the same time as animal welfare recommendations are enforced by government policy (Johnsen et al., 2021). The promotion of dairy calf welfare is essential and it is a key component of making an animal's life worth living (Mellor, 2016). Since disease is a challenging welfare problem for the dairy industry, additional methods should be explored to detect behavioral indicators of sickness in calves. This would improve calf welfare by detecting disease earlier to potentially explore non-antimicrobial alternatives (i.e., intervention strategies). This would lower the dependence on antimicrobial therapies for managing calves, improving the sustainability of the dairy industry.

One potential early intervention strategy is the use of nutraceuticals to ameliorate disease bouts in calves. Nutraceuticals, such as bovine colostrum, are thought to have positive effects on physiological status and health outcomes (Cantor et al., 2021). Bovine colostrum and lactoferrin are the most widely researched nutraceuticals in human medicine due to the diverse, positive health outcomes observed (Francesco et al., 2016). Indeed, feeding bovine colostrum to animals turned off the expression of genes associated with necrosis during induced colitis in mice (Menchetti et al., 2020), lowered the duration of pneumonia in juvenile racehorses (Fenger et al., 2016), and lowered the incidence of diarrhea and use of antimicrobial treatments in calves (Berge et al., 2009 a) when compared to controls. This suggests that preventative feeding of bovine colostrum may serve as a preventative disease therapy in mammals. However, the potential of bovine colostrum as an early intervention strategy for calves already at-risk for disease (e.g., expressing sickness behavior) has yet to be explored.

This literature review has two objectives, first, introduce that diarrhea and BRD are challenging diseases for dairy calves while exploring the potential of an early intervention strategy to ameliorate disease. Second, comprehensively review the potential of PDT research to detect behaviors associated with the development of disease in dairy calves. The specific perspective of this literature review is to first, highlight the welfare challenges and industry sustainability issues associated with the high prevalence of diarrhea and BRD bouts in calves. Since antimicrobial resistance is a sustainability issue, what is currently known about recovery status in BRD cattle in relation to recent antimicrobial treatments is also addressed. Then, a comprehensive evaluation of the potential for colostrum as a nutraceutical to ameliorate disease is reviewed. Finally, a comprehensive literature review covers the potential for PDT to identify disease development in calves. Where possible, gaps in the literature for future research are mentioned.

Diarrhea, Bovine Respiratory Disease (BRD) and BRD recovery status in preweaned daily calves, what do we know?

Dairy calves are commonly removed from the dam and raised artificially around the world. Calves are housed either individually, in pairs, or in groups, but individual housing is most common. According to dairy producer reported surveys, 60% of farms raise pre-weaned calves in individual housing in western and central Europe (Marcé et al., 2010), 63% in the United States (USDA, 2018), 88% in Quebec, (Vasseur et al., 2010), and 70% in Southern Brazil (Hötzel et al., 2014). Individual housing for preweaned dairy calves was designed to limit or prevent direct contact between calves. This limited contact provides the calf's immune system time to develop immunocompetence (Cortese, 2009). Moreover, calves are especially susceptible to pathogenic diarrhea during the first 21 days of life (Cho and Yoon, 2014), and susceptibility to BRD can last as long as 90 days (Griffin et al., 2010). Thus, individual housing was argued as beneficial to promote the biological functioning of the calf since it minimizes the horizontal spread of disease (McGuirk and Peek, 2014). However, despite these claims, disease prevalence is high in dairy calves (USDA, 2018). Moreover, researchers observed that pair housing (Mahendran et al., 2021) and managing calves in social groups less than ten calves does not increase disease risk (Svensson and Liberg, 2006). This is due to the complexity of disease, and management factors on farm.

There are multiple management factors associated with disease likelihood on dairy farms and these are extensively covered in the literature. Briefly, feeding variables can affect the prevalence of disease in calf barns such as poor rates of passive transfer of immunity in the maternal colostrum as reviewed by Raboisson et al., 2016, inadequate milk feeding strategies and or abrupt weaning as reviewed by Palcynski et al., 2020 and the presence or absence of feed additives such as probiotics as reviewed by Camiloti et al., 2012. Passive transfer of immunity occurs when a calf is fed colostrum containing at least 150 g of immunoglobulins shortly after birth (Henderiana, 2009). Once born, a calf's small intestine is capable of absorbing immunoglobulins from colostrum passively; however, as soon as anything is ingested, and as time passes after birth, the small intestinal junctions commence closure (Henderiana, 2009). The achievement of appropriate passive immunity positively impacts a calf's likelihood of recurrent disease, survival, and future productivity on a farm (Raboisson et al., 2016). Moreover, passive transfer rates in calves were improved on farms when benchmarking tools were implemented to motivate producers (Atkinson et al., 2017). This evidence suggests that passive transfer of immunity in calves can be positively affected by management factors, consequently improving calf welfare. In fact, passive immunity has such profound effects on a calf's biological functioning that calves who failed were observed to have a greater likelihood of morbidity and mortality during the first lactation; hence, it is recommended by veterinary epidemiologists to achieve herd passive transfer rates of 80 to 90 % to promote herd immunity (Shivley et al., 2018a). According to the most recent assessment, United States dairy producers were achieving passive transfer rates within this range (Shivley et al., 2018a), though an Australian survey suggested passive immunity was lower at 70% (Cuttance et al., 2017). However, the American survey was only performed on heifer calves, and evidence on veal farms suggests that the prevalence of passive immunity is much lower for bull calves (Hulbert and Moisá, 2016). Thus, from a welfare perspective, dairy herds should strive to improve passive immunity rates on their dairy farms to improve the biological functioning of calves and to decrease the number of calves experiencing disease. For sustainability, dairy farms should also increase passive transfer rates on farm to lower their loss of replacement heifers and decrease their reliance on antimicrobials.

There are also environmental factors affecting disease likelihood in calves. These environmental factors include poor housing design such as infrequent maternity barn cleaning and low air exchanges per hour in the housing as reviewed by Ollivett, 2020. Furthermore, the most well-established environmental factors affecting calf health are temperature and humidity as neonatal calves are susceptible to temperature extremes due to a low body surface area and minimal body fat reserves (Smith et al., 2020). There is also evidence which suggests that stress can affect disease likelihood in calves. For example, the performance of painful procedures without analgesics such as dehorning or castration as reviewed by Costa et al., 2019, may be associated with increasing the prevalence of BRD in cattle (Pardon et al., 2015). It is also suggested that social isolation, which occurs in individually housed dairy calves, may be a factor indirectly affecting susceptibility to stress. Calves are naturally gregarious, and calves are highly motivated to access peers, it is possible that social isolation may create less resilience to stress as reviewed by Cantor et al., 2019. Thus, it is suggested that while individual housing was developed with disease control in mind, there are a plethora of management factors involved that can increase the prevalence of disease on dairy farms.

Individual housing was developed to minimize disease prevalence on farm, yet many calves are still experiencing disease. For example, the most recent USA-wide survey found that diarrhea has a producer reported prevalence of 21%, and was responsible for 56% of calf deaths pre-weaning (USDA, 2018). Similarly, BRD was reported to affect 12% of calves preweaning (USDA, 2018). Moreover, BRD is a major contributor to calf mortality, reported to be responsible for around a quarter of calf deaths (USDA, 2018). Another disease affecting dairy calves in North America is infection of the umbilical cord, which can lead to sepsis (Urie et al., 2018b). While the prevalence of umbilical infections was reported at 10%, there is a high likelihood of mortality (Urie et al., 2018b). Furthermore, this welfare concern is largely preventable with sterilization of the cord shortly after birth and providing clean bedding (Mee, 2008). Thus, for the purpose of this literature review, we will not discuss umbilical infections further.

Diagnosis of BRD in calves is complex. Thus, there is disagreement on the reported data around prevalence of BRD affecting dairy calves. For example, an epidemiological study found that BRD affected approximately a third of pre-weaned calves on Californian dairy farms (Dubrovsky et al., 2019b), suggesting BRD may be higher than producer-reported prevalence (USDA, 2018). Alternatively, a recent cross-sectional study in Germany suggested that BRD may be less prevalent, affecting 9% of calves assessed, though navel infections were reported as high as 33% on this study (Dachrodt et al.2021). There are likely disparities between studies due to the complexity of disease involvement and different diagnostic criteria used to define a disease. For BRD, it is theorized that bacterial pneumonia occurs after viral invasion in the calf; stressors immunocompromise the calf, leading to an opportunistic infection of the respiratory tract (Pardon and Buczinski, 2020). Regardless, this evidence suggests that calfhood disease is a problem for dairy producers, and the development of these diseases is discussed further.

Diarrhea in neonatal dairy calves

Diarrhea is considered a symptom associated with lower gastrointestinal tract (GIT) upset. Diarrhea in calves can be caused by alterations in GIT permeability, colonization of pathogens, and or pathogen toxin excretion (Cho and Yoon, 2014). All of these alterations can lower GIT functionality, leading to frequent excretion of fecal material, and altered water absorption in calves (Renaud et al., 2020). Briefly, as reviewed by Cho and Yoon (2014), calfhood diarrhea can be caused by short-term viruses (i.e., the host combats the pathogen and resolves infection) such as Rotavirus and

Coronavirus. However, the host might also become a carrier and or spreader of the virus for its lifetime such as BVD. Gram positive bacteria such as *Clostridium perfringens spp.*, and gram-negative bacteria such as *E. coli spp*., and *Salmonella spp*. can also cause diarrhea in calves. Finally, protozoan species such as *Cryptosporidium parvum, Coccidia* and *Giardia duodenalis* can also cause virulent infections that often lead to persistent shedding of the cysts in calves; managing protozoan species are considered a major welfare challenge when raising calves since they compromise growth (Shivley et al., 2018b). Hence, ionophores are often fed to manage *Giardia duodenalis* and coccidia in calves (USDA, 2018). Despite this, *Cryptosporidium parvum* is the most prevalent pathogen associated with diarrhea in dairy calves (Shivley et al., 2018b). This evidence suggests that there are multiple pathogens which affect prevalence of diarrhea in dairy calves. Furthermore, management factors may affect the prevalence of diarrhea associated pathogens.

The prevalence of different diarrhea associated pathogens in calves is partially associated with management factors. For example, a USA-wide survey observed that the prevalence of *E. coli* in young calves was low, and mostly associated with poor passive transfer of immunity rates (Stenkamp-Strahm et al., 2018). Furthermore, a USA-wide survey observed that nearly half of the fecal samples taken on preweaned calves were positive for *Cryptosporidium parvum*, with larger herd sizes and greater temperature humidity indexes increasing the likelihood of positive samples (Urie et al., 2018a). A third of the fecal samples taken on calves were positive for *Giardia duodenalis*, with smaller herd sizes and lower temperature humidity indexes increasing the likelihood of a positive sample (Urie et al., 2018a). In contrast, *Salmonella* spp. associated with diarrhea in calves was considered an endemic problem, and virulence was more associated with genetic mutations of the pathogen's genome (Mohler et al., 2009). Thus, it is likely that the pathogen prevalence on farm is impacted by the management factors employed. While antimicrobials are the most common treatment method to ameliorate symptoms of diarrhea in calves, there are not any commercial antimicrobials effective against protozoan species (i.e., only ionophores are used); thus, it is hypothesized that diarrhea promotes overuse of antimicrobials (Tzipori, 1983). This evidence suggests that pathogen associated diarrhea is partially management factor related, and alternative methods to ameliorate diarrhea bouts in calves should be explored.

Diarrhea bouts are commonly treated with antimicrobials in calves (Urie et al., 2018b). However, this increases sustainability concerns about antibiotic resistance in dairy farms and beyond. Diarrheal bouts are easily detected in calves since only one symptom, fecal consistency, is assessed. For example, one system validated for scoring calves for diarrheal bouts only requires the presence or absence of feces with a watery consistency (Renaud et al., 2020). The feasible detection of diarrhea may lead to overuse of antimicrobials by producers, which is heavily scrutinized by the dairy consumer (Knowlton and von Keyserlingk, 2018). However, beyond consumer concern, the use of antimicrobials to treat diarrheal bouts in calves was associated with shedding *E. coli* strains genetically resistant to commercial antimicrobials (Khachatryan et al., 2004). Thus, the overuse of antimicrobials to treat diarrheal bouts in calves extends beyond consumer concern, and is a relevant issue needing attention in the dairy industry.

While there are clear benefits for using antimicrobials to treat certain diarrhea-associated pathogens in calves, interventions are recommended by veterinarians only when a pronounced febrile response is observed (Berge et al., 2009, b). For example, Berge et al., (2009, b) observed that targeted antimicrobial therapy using extreme depression and a pronounced fever as symptoms accompanying diarrheal bouts in calves reduced the duration of diarrhea compared to the preventative feeding of antimicrobials in milk to calves. Similarly, a veterinary review suggested that the preventative feeding of antimicrobials in milk to calves increased antimicrobial resistance, and was minimally effective across studies (Smith, 2015). Smith (2015) emphasized the discontinuation of preventively feeding antimicrobials to calves and to implement targeted therapy based on evidence of systemic infection (i.e., a pronounced fever and extreme lethargy). The preventative feeding of antimicrobials in milk is thought to reduce the likelihood of disease in calves. However, recently, Buss et al., (2021) observed that there were no justifiable benefits for calves fed antimicrobials in milk. Furthermore, the practice of preventative feeding of antimicrobials in milk was associated with the increased shedding of antimicrobial resistant *E. coli* in calves (Khachatryan et al., 2004). This is a calf welfare and industry sustainability issue as antimicrobial resistant *E. coli* causes systemic infection in calves with diarrhea (Khachatryan et al., 2004). Furthermore, Langford et al., (2003) observed that when calves are preventatively fed antimicrobials in milk, a dose specific prevalence of antimicrobial resistance was shed in the feces by the calves (Langford et al., 2003). This is a sustainability concern as antimicrobials used for preventative feeding strategies to prevent diarrhea in calves has promoted antimicrobial resistance.

This also has welfare consequences for the calf. More recently, administering therapeutic antimicrobials to calves was associated with turning on genes in the lower gut associated with antimicrobial resistance, a delayed development of lower gut taxa diversity, and a less robust microbial population in the small intestine was observed (Ma et al., 2020). There is some evidence which suggests that treatment of calf manure may neutralize persistence of these species in the calf's environment, but this was observed in research settings (Oliver et al., 2020). Thus, it is recommended for producers to judiciously move away from preventative antimicrobial therapy and focus on targeted therapies for calves with diarrhea. Collectively, this evidence suggests that limiting the use of antimicrobials to treat diarrhea in calves is fundamental for the GIT health of the developing calf and to decrease the prevalence of antimicrobial resistance.

There are also multiple welfare challenges associated with diarrheal bouts for preweaned calves. For example, a systematic review observed that diarrhea in preweaned calves was associated with ataxia, dehydration through loss of fluids, and a poor suckle, indicating weakness (Meganck et al., 2014). More recently, diarrhea in calves was also associated with disrupted and altered microflora colonies in the lower gut (Ma et al., 2020), suggesting diarrhea may have additional physiological disturbances beyond dehydration in calves. Minor to moderate dehydration can be rectified in calves through provision of oral solutions (Taylor et al., 2017). Rehydration of calves is imperative, as dehydration is compounded by an increased risk of blood acidosis through loss of potassium (Golbeck et al., 2018), bicarbonate, and D-lactate (Trefz et al., 2012). Correcting blood acidosis in calves often requires invasive intervention. For example, Trez et al., (2012) observed that an intravenous solution of sodium bicarbonate can correct blood acidosis in calves. However, blood acidosis is likely painful for calves, calves positive for blood acidosis were depressed, had a lethargic corneal reflex, and were less capable of standing securely (Trefz et al., 2012). In summary, symptoms associated with diarrheal bouts in calves such as dehydration and blood acidosis are painful, and lead to poor affective states in calves. Thus, mitigating diarrheal severity in calves, and lowering the duration a calf experiences diarrhea will improve calf health and welfare.

Diarrhea preweaning may affect calf performance. For example, diarrhea preweaning was associated with poor performance in calves, such as a reduced average daily gains in dairy calves (Windeyer et al., 2014), and in veal calves, there was a greater risk of mortality, and a lower hot carcass weight (Pardon et al., 2013). There is also the possibility that diarrhea bouts in preweaned calves have long-term effects; one study observed a lower likelihood of pregnancy and reduced milk production during the first lactation in calves identified with a diarrheal bout preweaning (Aghakeshmiri et al., 2017). Others have observed that calves with a diarrhea bout preweaning were older at first calving and smaller (Heinrichs et al., 2005). Since the average case of diarrhea in calves in one prospective cohort study was around 6 days (Renaud et al., 2019), research needs to identify additional behavioral changes associated with diarrhea in calves to minimize the compounding effects of diarrhea, and provide a timely intervention.

In summary, diarrhea is problematic for the calf and for the dairy industry. There is a need to detect diarrhea earlier for timely intervention to decrease the duration a calf experiences disease. However, preventative feeding of antimicrobials may have long-term consequences on the health of the calf's GIT and may promote antimicrobial resistance. Thus, there is a need to investigate alternative methods for early intervention to ameliorate diarrhea in calves. However, diarrhea is not the only major player in calfhood disease. Bovine respiratory disease, a disease of the upper or lower respiratory tract in cattle, is another disease that has welfare and sustainability implications for calves and the producer.

Bovine respiratory disease in neonatal dairy calves

Bovine respiratory disease is likely a painful disease for cattle causing physiological changes such as inflammation of the respiratory tract (Bednarek et al., 1999), which leads to coughing (McGuirk and Peek, 2014), activation of the febrile response, and activation of the HPA-immune axis, which leads to sickness behavior (Hart and Hart, 2019). Bovine respiratory disease leads to an immunocompromised status in cattle, it is considered a failure of lung epithelia integrity where native microbiota in the nasopharynx colonize the respiratory tract leading to bacterial pneumonia in calves (Raabis et al., 2021). Indeed, poor transfer of passive immunity in calves is one reason BRD prevalence rates are different between dairies (Dubrovsky et al., 2019a). Specifically, Dubrovsky et al., (2019a) observed in a cross-sectional study that BRD prevalence rates on dairies were largely explained by maternal factors such as being a twin, or inadequate immunoglobulin content of the dam's colostrum and colostrum management factors such as bacterial contamination and delayed delivery after a calf was born. There were also environmental management factors associated with a higher BRD prevalence on farm including poor air exchanges per hour, the prevalence of dust, and the use of wooden hutches (Dubrovsky et al., 2019a). Thus, it is likely that the prevalence of BRD in calves is partially explained by variation in maternal factors and management factors on farm.

The etiology (Bednarek et al., 2012), and pathophysiology (Autio et al., 2007) of pathogens causing BRD in cattle has been extensively reviewed. Briefly, the most common pathogens isolated from nasopharyngeal swabs of dairy calves living on farms with a high BRD prevalence were viral such as bovine coronavirus and bovine respiratory syncytial virus, gram negative bacteria such as *Pasteurella multocida*, *Mannhaemia haemolytica*, and *Histophilus somni*, and gram positive bacteria such as *Mycoplasma bovis* (Francoz et al., 2015). Due to the complexity of BRD, multiple clinical symptoms of BRD are used to diagnose the disease.

A systematic scoring system, such as the Wisconsin scoring system, is commonly used to detect BRD bouts in calves (McGuirk and Peek, 2014). Use of this system involves assigning cumulative scoring from two abnormal categories (e.g., 0 normal to 3 severely abnormal) such as cloudy nasal discharge, colored eye discharge, presence of ear flick or head tilt, elevated rectal temperature (e.g. higher temperatures receive higher scores), and the presence of spontaneous, or repeated coughing to diagnose BRD (McGuirk and Peek, 2014). However, Aly et al., (2014) suggested that the Wisconsin scoring system was labor intensive (e.g., 20 hours per hundred health checks). Thus, a dichotomous clinical scoring system (e.g. UC Davis Chart) was validated to reduce labor and detect BRD bouts in calves using the clinical symptoms of the Wisconsin scoring system, but this system included labored breathing as a symptom (Love et al., 2016). One limitation to the UC Davis Chart was that the sensitivity rate was similar (e.g., 70%) to the Wisconsin scoring system (Love et al., 2016). However, Aly et al., (2020) observed that using the UC Davis Chart to score calves for BRD bouts daily was also labor intensive (Aly et al., 2020). This suggests that both clinical scoring systems validated to assess for BRD bouts in calves are labor intensive, and they potentially miss sick calves. For example, the average sensitivity for detecting BRD bouts in calves using the Wisconsin scoring system was reported at 62%, while the average specificity was 74% (Buczinski et al., 2015). In comparison, Buczinski et al., (2015) observed that the validated thoracic lung ultrasonography scoring system increased sensitivity and specificity for detecting BRD bouts in calves (e.g., average sensitivity was 79% and average specificity was 94%). Furthermore, very recently, the lung ultrasonography scoring system was validated as no different in average sensitivity (e.g., 84%) or average specificity (e.g., 74%) when compared to chest thoracic radiography to diagnose pneumonia in hospitalized calves (Berman et al., 2021). This suggests that the lung ultrasonography scoring system is a gold standard for diagnosing BRD bouts in calves. There is also evidence that when both the Wisconsin scoring system and lung ultrasonography scoring system were used together for BRD diagnosis, a higher sensitivity was achieved in diagnosing BRD bouts in preweaned calves (Buczinski et al., 2014). Thus, it is suggested that herds can assess the prevalence of BRD in calves by hiring a veterinarian to use lung ultrasonography scoring system and the Wisconsin scoring system collectively. However, though lung ultrasonography scoring system is sensitive to detect BRD bouts in calves, we suggest that the cost of an ultrasound, the training required, and the labor involved with scanning calves are barriers for incorporation into daily use on farm. Thus, additional BRD identification devices which limits the use of manual labor are needed.

There are welfare consequences to the calf who incurs a BRD bout. For example, in the short term BRD was associated with a lower average daily gain preweaning, suggesting calves who experienced BRD may use nutrients poorly, a biological functioning concern (Cramer and Ollivett, 2019). In the long-term, BRD at weaning was associated with lower milk production during the first lactation (Dunn et al., 2018 and Teixeira et al., 2017), and a lower likelihood of pregnancy (Teixeira et al., 2017). More recently, a scoping review and meta-analysis by Buczinski et al., (2021) observed that BRD incurred preweaning in dairy calves was associated with a greater likelihood of reduced average daily gain, dying, not completing the first lactation, and a greater likelihood of lower milk production in the first lactation compared to healthy counterparts.(Buczinski et al., 2021). Therefore, detecting calves at risk for a BRD bout in a timely manner may also potentially mitigate the effect of BRD on calf productivity.

In summary BRD is a complex disease with many pathogens involved. Due to the complexity of BRD, multiple symptoms are required to detect the disease. However, the lung ultrasonography scoring system can provide an accurate diagnosis of a BRD bout in a calf on farm. The disease causes short-term consequences, affecting calf productivity, and long-term consequences, including lowered chance of survival, and potentially less milk compared to healthy conspecifics. It is also likely that there is a real impact on a calf's affective state when a calf experiences a BRD bout and the time a calf experiences the disease should be minimized (Aly et al., 2020). However, another major challenge with BRD is the threat this disease poses to sustainability when calves do not respond to the initial antimicrobial intervention **(relapse)**. Evidence suggests that up to a quarter of calves can relapse with BRD (Heins et al., 2014), and this may be in part due to delayed disease detection, and antimicrobial resistance of the pathogens involved (Lysnyansky and Ayling, 2016).

Who fails to recover from Bovine respiratory disease?

Bovine respiratory disease is a welfare and sustainability problem for both the dairy and beef industries. In veal calves, nearly a fifth of BRD calves required additional antimicrobial interventions and some of these calves died (Welling et al., 2020). In dairy calves, a third of dairy calves relapsed to their initial antimicrobial treatments for BRD, and re-treatment was associated with mortality (Heins et al., 2014). In beef cattle, nearly half of the cattle initially treated for BRD with antimicrobials relapsed with BRD, which required a re-treatment (Rooney et al., 2005). However, others suggested that beef cattle relapse rates for BRD might be closer to a third of the cattle treated (Burciaga-Robles et al., 2010, Holland et al., 2010). One of the largest challenges with relapsed BRD cases is that these cattle are at a much higher risk for chronic pulmonary disease and mortality compared to BRD calves who recovered from the initial antimicrobial intervention (Welling et al., 2020). Unfortunately, each additional antimicrobial intervention increases the cumulative probability of chronic pulmonary disease in cattle, and these animals seldom remain profitable (Holland et al., 2010). This evidence suggests that BRD calves who relapse and fail to respond to antimicrobial treatment for BRD are a prevalent problem.

Antimicrobial intervention effectiveness is less than optimal for BRD bouts in calves. The pathogens involved and the class of antimicrobials used can affect the cure rate, suggesting a need to promptly quantify if these BRD calves are responding to antimicrobial intervention (e.g., hereafter referred to as recovery status). For example, a review by Lysnyansky and Ayling (2016) observed that multiple *in vitro* studies found that some of the most common pathogens associated with BRD had a high minimum inhibitory concentration for most of the commercially available antimicrobials used to treat BRD in beef cattle. This may partially explain why relapsed calves are common and are at a much greater risk for chronic pulmonary disease and mortality. However, there is also a lag between antimicrobial intervention and resolution of clinical symptoms in recovered BRD calves, complicating detection as reviewed by Cramer and Ollivett, 2020. One potential solution is to use behavioral changes (i.e., sickness behaviors) to identify recovery status in these calves and provide prompt interventions.

There are benefits to using early antimicrobial interventions to treat BRD bouts in calves. For example, using the lung ultrasonography scoring system with the Wisconsin scoring system to detect and treat BRD bouts in early development improved calf productivity, increased the likelihood of cure, and reduced the likelihood of a calf requiring an additional antimicrobial intervention (Binversie et al., 2020). Early intervention on a BRD bout in dairy calves is imperative so that calves can recover and resume normal behavioral activity. While the detection of a relapsed BRD bout is important for the dairy and for the calf, it is impractical from an economic perspective. Assessing every preweaned calf every day for clinical symptoms associated with BRD is too labor intensive for larger farms (Aly et al., 2020). Similarly, every case of BRD was estimated to cost $42 per case per calf, suggesting the disease is costly (Dubrovsky et al., 2020). It is likely that delayed antimicrobial intervention reduces dairy calf productivity and may comprise survival as has been seen in veal calves (Welling et al., 2020) and beef cattle (Rooney et al., 2005). However, one study on relapse rates in BRD dairy calves suggested average daily gain was not affected (Heins et al., 2014). Methods which decrease reliance on human labor should be explored to find BRD calves at risk for relapse status to promote early intervention.

Bovine respiratory disease causes physiological changes such as inflammation of the respiratory tract which leads to coughing, pain, and a febrile response, resulting in the display of sickness behavior by the inflicted animals (Hart and Hart, 2019). Some examples of sickness behavior related to BRD bouts in dairy calves can be passively recorded by precision technology devices such as depressed feeding behavior measured by automated feeders (Morrison et al., 2021), and reduced activity levels measured by accelerometers (Costa et al., 2020). Sickness behavior in calves can also be monitored by directly looking for self-isolation from the herd (Cramer et al., 2016), depression (Cramer et al., 2019) and signs of physical pain such as hunched shoulders, labored breathing (Love et al., 2016), and lateral lying (Hixson et al., 2018). However, using direct signs of disease to watch for sickness behaviors in cattle requires labor, and is sometimes impractical for dairy farms (Aly et al., 2020). Thus, the use of PDT to monitor daily behavioral patterns of dairy calves has great potential.

In summary, BRD, and relapsed calves who do not respond to the initial antimicrobial intervention contribute to welfare and sustainability issues for the dairy industry. Calf welfare is compromised due to the pain experienced during a BRD bout, and the longer-term reduced likelihood to thrive as an adult. Similarly, the prevalence of relapsed BRD cattle suggests that the prevalence of antimicrobial resistance is high, the disease is viral, or there is a high inhibitory concentration of the pathogens involved; this suggests a high dose of antimicrobials required to eliminate the infection. There is the potential to use sickness behavior to detect recovery status in BRD calves, but research is needed. Similarly, there is a need to investigate the potential of antimicrobial alternatives to manage BRD, particularly with early intervention strategies regarding calfhood disease.

Colostrum: a potential nutraceutical to ameliorate disease in calves

The dairy industry is under pressure to lower reliance on antibiotics, particularly for sub-therapeutic use (Knowlton and von Keyserlingk, 2018); thus alternative therapies should be considered to mitigate disease risk in calves. One potential opportunity is feeding bovine colostrum as a nutritional strategy to older calves since it has a high content of immunoglobulin and lactoferrin (Francesco et al., 2016), and has anti-inflammatory properties (Lee et al., 2019). Moreover, using first milking bovine colostrum as a dietary supplement may ameliorate disease bouts as was observed in human medicine (Bagwe et al., 2015).

In healthy humans, supplementing dietary colostrum as a disease-preventative strategy was associated with a lower likelihood of developing the flu compared to those that were vaccinated and served as controls (Cesarone et al., 2007). Similar immune improvement was observed in immunocompromised mice (Menchetti et al., 2020), and in recently weaned piglets, though leukocyte populations were higher in the piglet’s small intestine rather than circulating in the blood (Boudry et al., 2007). Moreover, healthy dogs fed a 0.1% colostrum supplement had improved fecal IgA, and higher circulating IgG 40 weeks after immunization for distemper compared to controls (Satyaraj et al., 2013). The benefit of colostrum to improve immune response was also observed in other mammal neonates. A low dose (3g/d) of colostrum supplementation for 12 weeks to 550 chronically ill (diarrhea or pneumonia) human children ameliorated disease bouts, and lowered antibiotic intervention (Patel and Rana, 2006); however, this pioneer work used the children receiving the treatment as their own control. Hence, there are limitations that can be inferred from the results of this study. However, feeding 100 young Thoroughbred horses a colostrum supplement at (50 g/d) for 4 months ameliorated disease duration (2 weeks) versus soy flour controls (Fenger et al. 2016). This research collectively suggests that dietary colostrum supplementation may improve immune function in healthy and at-risk humans, mice, piglets, and horses. To date, there is no work that has investigated the efficacy of colostrum in older dairy calves and research is needed.

There is preliminary evidence that the use of colostrum as a disease-preventative supplement fed to neonatal dairy calves during the first 14 d of life lowered the likelihood of abnormal feces, lowered the likelihood of abnormal respiration, and decreased the likelihood of antimicrobial intervention (Chamorro et al., 2017). Different results were observed at a lower dosage, where 30 calves receiving colostrum at 70 g/d had a lower likelihood of diarrhea, and a decreased likelihood of antimicrobial intervention, but the likelihood of developing BRD was not different compared to negative controls (Berge et al., 2009a). Moreover, a recent study observed that partially replacing the milk diet with different levels of colostrum at up to 700 g per day reduced the chance of diarrhea, improved performance, and tended to reduce the risk of BRD (Kargar et al., 2020). Similarly, there is evidence that extended colostrum feeding to calves during the first 21 days of life decreased BRD morbidity for the first year of life and decreased diarrhea risk, while milk production was higher for extended colostrum-fed calves during the first lactation (Armengol and Fraile, 2020). However, there is no work which has investigated the effect of intervening on calves already at risk for disease. Hence, there is the potential for colostrum to ameliorate disease in dairy calves when fed as a preventative therapy. However, it is unknown if colostrum fed in the short-term can be used as an intervention feeding strategy to mitigate disease risk in calves.

In summary, there is evidence that using colostrum as a disease-preventative supplement may improve immune status in other species. To date, there is no work that has investigated the efficacy of colostrum feeding as an intervention strategy in the short-term for dairy calves at-risk for disease. However, there is literature which suggests feeding colostrum to initially healthy dairy calves as a longer-term preventative feeding strategy may improve calf health outcomes when they do become sick. One limitation to feeding colostrum to calves across an extended period is cost. Therefore, it is of interest to determine if an intervention provided to a calf demonstrating sickness behavior can lower disease likelihood and ameliorate disease bouts. Since sickness behavior can be monitored with PDT, the possibility of early intervention of colostrum to calves during their first expression of sickness behavior should be explored.

Collectively, this evidence suggests that diarrhea and BRD are highly prevalent diseases on dairy farms, and antimicrobial interventions are not always effective. Thus, there is the real opportunity to explore alternative therapies for early intervention to ameliorate and prevent disease bouts in calves. One avenue of possibility is the use of PDT to monitor for sickness associated behaviors in calves. The cytokine-induced sickness response is an induced motivational state which precedes expression of clinical symptoms (Johnson, 2002). Thus, there is the potential to use PDT to monitor and detect the development of sickness in dairy calves. I will explore this topic in the second part of this literature review.

Accelerometers and automated feeders: precision technology devices with the potential to detect sickness behavior associated with disease in preweaned dairy calves

Precision livestock research encompasses two areas: (1) use of technology to increase farm efficiency and (2) use of technology to manage production parameters of livestock (Eckelkamp and Bewley, 2021). There is the potential for incorporation of PDT to manage production parameters of dairy calves in relation to disease diagnosis. Precision livestock researchers suggested that accelerometers and automated feeders, which can collect activity levels, and feeding behaviors in calves, respectively, may be useful for disease detection. Furthermore, accelerometers are already in common use to manage reproduction in older animals on dairies (Dolecheck et al., 2018) and automated feeders are already in use to improve farm efficiency when feeding calves (Medrano-Galarza et al., 2017). Thus, there is the potential for adding to the utility of PDT to manage disease in calves since producers already have these technologies on farm. Where possible, the potential for future research using PDT to manage dairy calf health is explored.

Three-dimensional accelerometers

Three-dimensional accelerometers measure tilt relative to the object wearing the device; this permits observation of the speed, direction, and acceleration rate of the object equipped with the device (Costa et al., 2020). Accelerometers are wearable PDT, the automated recording of behavioral positions of calves such as lying bouts, and rates of movement such as step counts are one reason this is the most researched PDT for calf management (Robert et al., 2009). Furthermore, an activity index based on acceleration rate and step counts was recently validated as an indicator of a play bout in calves (Gladden et al., 2020). Accelerometers come in many forms such as pedometers, ear-tags, collars, and sleep monitors and were developed to measure many behaviors in calves. A complete picture of the options available are presented in **Table 1.1.**

Accelerometers and calfhood diarrheal bouts

There is literature which suggests that accelerometers, which measure behavioral activity levels, including lying time, lying bouts, step counts, and rate of acceleration in calves, may be capable of monitoring activity levels associated with diarrhea bouts prior to disease in cattle. For example, in disease challenge models (e.g., *Salmonella* Spp.), calves decreased lying bouts and increased lying bout duration 3 days before diarrhea where lying times were not affected (Lowe et al., 2019b). In research with naturally occurring diarrhea bouts, lying bouts either tended to be lower with diarrhea (Sutherland et al., 2018, Belaid et al., 2020), were lower for 2 days prior to diagnosis (Belaid et al., 2020), or no relationship was observed (Studds et al., 2018). In contrast, Swartz et al., (2020) observed that calves increased their lying bouts, and decreased their lying bout durations from days 7 to 3 prior to diarrhea. A disagreement in the literature between studies is likely due to different statistical methodology, and the pathogens involved with diarrhea. For example, the *Salmonella* challenge resulted in virulent diarrhea bouts which infected the control calves since they were socially housed; a lack of independent control and the pathogen involved likely affected the lying behaviors of these calves (Lowe et al., 2019a). Thus, we suggest that further research is needed using carefully selected controls to investigate if lying bouts are associated with diarrheal bouts in calves.

In contrast to lying bout behavior, the literature on daily lying time patterns in calves prior to a diarrheal bout agrees. For example, tendencies for lower lying time (Studds et al., 2018), or a significantly lower lying time prior to diarrhea bouts in calves were observed (Sutherland et al., 2018, Swartz et al., 2020). Therefore, disease affects lying behavior in calves and this behavioral change can be detected with accelerometers. However, there is no literature available on the effect of acceleration rate (or an activity index) on diarrhea bouts in calves, and this warrants future investigation.

Accelerometers and calfhood Bovine Respiratory Disease

Precision livestock technology research has also used accelerometers to detect sickness behaviors for the few days before BRD bout diagnosis in dairy calves. For example, young steers experimentally induced with BRD had increased lying times (Toaff-Rosenstein et al., 2016) compared to control calves. Calves infected with *Mannhaemia haemolytica* demonstrated laterality of lying towards the left side compared with control calves, though lying time did not differ (Hixson et al., 2018). This evidence suggests that experimentally infected animals experience changes in their lying behavior, but which activity behaviors change is unclear.

There is moderate agreement in the literature on the effect of naturally occurring BRD on calf activity levels. For example, two studies agree that BRD bouts are associated with decreased lying bouts in the few days before diagnosis in calves (Swartz et al., 2020, Duthie et al., 2021). For step counts, only one study evaluated step counts, and step counts declined the day before BRD diagnosis in calves (Duthie et al., 2021). However, a study which did not stratify by disease type also observed a decline in step counts in the days before diagnosis (Belaid et al., 2020). Thus, this research suggests that reduced activity levels may indicate sickness behavior associated with BRD status in dairy calves.

The relationship with lying time and BRD bouts is less clear than activity levels. Lying time was either not affected (Swartz et al., 2017), only changed on day 10 prior to diagnosis (Belaid et al., 2020), or lying times were greater (Duthie et al., 2021) in calves diagnosed with a BRD bout. It is possible that differences between studies are explained by varying management factors known to affect lying times in calves. For example, lying behavior in calves can decrease as bedding dry matter content decreases (Camiloti et al., 2012), and as space allowances decline per calf (Jensen and Kyhn, 2000). For example, one study suggested that providing a minimal space allowance (e.g., 1.5 m^2) to group housed calves reduced locomotory play bouts compared to calves offered greater space allowances (Jensen and Kyhn, 2000). Thus, it is possible that the relationship between lying times in calves and BRD may be related to different management factors, but more research is needed. A study which replaces bedding biweekly and provides stocking density to calves at greater than 1 m^2 is needed to explore this relationship with lying time and BRD bouts. In summary, activity levels such as lying bouts and step counts decline prior to BRD diagnosis in calves.

Automated feeders

While accelerometers are the most researched PDT in calves, automated feeders are the most popular PDT used on commercial farms (Medrano-Galarza et al., 2017). It is likely that the popularity of automated feeders is linked to a study that supported increasing milk allowances without increasing labor costs when compared to individual housing (Kung et al., 1997). Automated feeders can also administer feed such as milk and calf starter, supplements such as probiotics, and some can even record the feeding behaviors of the calves. Feeding behaviors recorded by these systems include milk and solid feed intake, the intake rate, drinking speed, and how often a calf visits and receives milk (rewarded visits) or does not (unrewarded visits). Thus, with the recording of feeding behavior, there is the potential of this system to detect disease. This literature review covers the research regarding feeding behavioral depression as sickness behaviors associated with diarrheal bouts, and BRD bouts recorded by automated feeding systems.

Automated feeding systems and calfhood diarrheal bouts

Automated feeding systems automatically record the daily feeding behaviors of individual calves, suggesting the potential of this PDT to use behavioral deviations to detect sickness behaviors. However, the milk feeding strategy used on the automated feeder affected which sickness behaviors were observed in calves in the literature. For simplicity, studies which offered calves 7 L/d or less will be referred in this review as limit-fed calf studies. For example, calves allotted 12 L/d had lower milk intakes several days preceding illness detection, whereas calves offered limit-fed milk reduced milk intake only a day prior to diagnosis (Sutherland et al., 2018). In agreement, Borderas et al., (2009) observed that limit-fed calves changed milk intakes only on the day of diarrhea, whereas calves offered more milk decreased their intakes several days before diarrhea. It is possible that only calves offered higher milk allowances experience depressed milk intakes before diarrhea. This possibility may be due to the amount of milk offered, 7 L/d is less than half of what a calf drinks when milk is offered *ad libitum* (Welboren et al., 2018). Thus, it may be suggested that limiting milk intake precludes the ability to detect a diarrheal bout in calves compared to studies which offered calves higher milk allotments.

Drinking speed, or how quickly calves drink their meals, and unrewarded visits, or how often a calf visits the feeder without receiving milk might change earlier than milk intake in limit-fed calves approaching a diarrheal bout. For example, drinking speed decreased in limit-fed calves 3 days prior to a diarrheal bout (Knauer et al., 2017). However, Knauer et al., (2017) relied on producer reporting for diarrheal bouts in calves, thus more research is needed to ensure all diarrheal bouts are detected. Unrewarded visits decreased in limit-fed calves 2 to 3 days prior to diarrhea diagnosis (Lowe et al., 219b; Sutherland et al., 2018), or decreased for 4 days prior to diarrhea diagnosis (Knauer et al., 2017). This suggests that unrewarded visits may decline for several days for calves approaching a diarrheal bout. Unrewarded visits have no nutritive purpose, which may be why calves decrease this behavior prior to diarrhea. However, future research is needed to explore individual behavioral changes rather than looking at differences between average daily behaviors between healthy and sick calves.

Automated feeding behaviors and calfhood Bovine Respiratory Disease

One potential opportunity to improve BRD detection in calves is to use PDT to monitor for changes in behavioral patterns. For example, automated feeders can record feeding behaviors such as drinking speed, milk intake, and visits, and these behaviors may change before a BRD bout in calves (Morrison et al., 2021). However, it appears that milk feeding allowance affects which feeding behaviors are sickness signals for BRD bouts.

There is agreement in the literature that limit-fed, diseased calves changed milk intake on the day of BRD detection (Borderas et al., 2009, Knauer et al., 2017, Swartz et al., 2017). However, there is conflicting evidence on the relationship of milk intake and BRD for calves on non-restrictive diets. For example, no relationship was observed (Cramer et al., 2020), or milk intake declined a few days before BRD diagnosis in calves (Duthie et al., 2021). Evidence suggests, that like diarrhea, milk intake is affected, and changes are measurable for several days in calves offered a non-limiting milk allowance. However, for BRD bouts, more research is needed as there is a lack of agreement in the literature. Similarly, there is conflicting information about drinking speed as sickness behavior for BRD. Drinking speed either decreased on the day of BRD diagnosis in limit-fed calves (Knauer et al., 2017), or no relationship was found (Swartz et al., 2017). Moreover, for calves offered more milk, there were either lower drinking speeds observed for 3 days prior to BRD (Cramer et al., 2020) or no relationship was found (Duthie et al., 2021). Thus, evidence is conflicting on the utility of milk intake and drinking speed as sickness behaviors related to BRD in calves.

Like drinking speed, unrewarded visits may or may not be a sickness behavior indicative of a BRD bout. For example, unrewarded visits decreased several days prior to diagnosis in limit-fed calves (Svensson and Jensen, 2007), were lower on the day of disease diagnosis in calves offered more milk (Borderas et al., 2009), or decreased several days prior to BRD diagnosis in calves offered more milk (Duthie et al., 2021). It is likely that different disease definitions and milk feeding strategy affected the relationships observed. Future research needs to quantify disease definitions clearly, and relative changes in calf behaviors should be explored to investigate associations with feeding behavior and these relationships.

It is possible that the lack of agreement on milk intake, drinking speed and unrewarded visits is due to different disease definitions used. For example, Cramer et al., (2020) used lung ultrasonography scoring system as part of the BRD diagnosis criteria, which has higher operative sensitivity and is more accurate than the Wisconsin scoring system (Buczinski et al., 2015). It is recommended moving forward to use similar disease diagnosis criteria to eliminate these discrepancies in the literature (e.g., STROBE vet guidelines). Furthermore, very few studies use inter-observer agreement to ensure health exams were performed similarly between researchers. This can introduce considerable bias for under-or-over reporting disease and should be eliminated. Incorporation of a clinical scoring system, with a gold standard to diagnose disease such as lung ultrasonography scoring system and inter-observer validations across time should be incorporated into calf health studies.

In summary, accelerometers and automated feeders are often used on dairy farms, and the utility of using these PDT to evaluate sickness behaviors in calves brings a great opportunity to improve the welfare of the animals and to reduce the use of antimicrobials. Overall, calves may experience restlessness and decline their feeding behavior a few days before diarrhea diagnosis, but findings are dependent on milk feeding strategy. However, there is limited evidence which explored the relationship between diarrhea bouts and sickness behaviors in diarrheic calves. Similarly, there are limitations to this collective research as diagnostic criteria for diarrhea relied on producer reporting, potentially under reporting diarrhea (Knauer et al., 2017). Other studies had limitations, some lacked independent controls (Lowe et al., 2019b), or used calf cleanliness to diagnose disease (Sutherland et al., 2018) and this was reported as a poor diagnostic criterion for diarrhea in calves (Graham et al., 2018). Moving forward, researchers need to incorporate standardized reporting, and the recent validation of diarrhea scoring criteria (Renaud et al., 2020) should be used to standardize reporting for future research. Future research should evaluate the potential of accelerometers and automated feeders to detect diarrhea bouts in calves when they are followed every day using standardized reporting and diagnostic criteria.

Overall, for BRD, precision livestock researchers observed that calves may experience reduced activity levels and feeding behavior depression a few days before diagnosis, but findings are dependent on milk feeding strategy. There is a lot more evidence to suggest that feeding behavior depression and reduced activity levels occur for the few days before BRD diagnosis compared to literature exploring PDT and diarrhea bouts in calves. However, there is disagreement in the literature on which activity levels and feeding behavior variables change before a BRD bout.

It is suggested that a limitation to this collective evidence is that disease definitions differ, and frequency of clinical scoring differs, despite often using the same clinical scoring system (McGuirk and Peek, 2014). For example, one study relied on producer reporting, potentially under reporting BRD (Knauer et al., 2017), another used collective consecutive days of BRD, potentially delaying reporting (Duthie et al., 2021), while another used lung ultrasonography scoring system a more sensitive diagnostic tool that may have caught BRD bouts sooner (Cramer et al., 2020). This makes comparisons difficult, though the evidence collectively suggested accelerometers and automated feeders can record behavioral changes associated with BRD in calves. This is not an encompassing list of differences between studies. Future research should follow calves daily to diagnose BRD development and define its onset. There is also the possibility to incorporate diagnostic tools such as lung ultrasonography scoring system as a gold standard to improve the reliability of the diagnosis. Furthermore, there is the potential to use PDT to detect recovery from the initial antimicrobial intervention for BRD in calves, and this should be explored. Precision livestock researchers may improve calf welfare using these PDT by detecting disease sooner for earlier interventions, and by promoting calf recovery status. Similarly, use of PDT to detect calves at risk for disease provides a sustainable option to explore colostrum as an intervention strategy to ameliorate disease severity. However, a study is needed which follows calves daily to capture all health events. Furthermore, a study is needed to explore the potential of an individual calf's relative changes in behavior to indicate a disease event.

Conclusions

In conclusion, diarrhea and BRD are common diseases during calfhood with real implications for affecting a calf's well-being. Similarly, while antimicrobial interventions to ameliorate these diseases are most common, many pathogens associated with diarrheal bouts and BRD in calves are antimicrobial resistant. Thus, methods which detect sickness behaviors in calves prior to clinical disease onset, and to detect calves in recovery are needed to increase sustainable practices in the dairy industry. One potential for early intervention is to determine if colostrum can be fed to calves as a feeding intervention strategy (e.g., when feeding behavior declines). Early evidence in parallel from other species, and in calves suggest that feeding colostrum as a preventative strategy improves immune status and decreases disease risk. However, no research has explored the utility of colostrum an intervention strategy to ameliorate disease in calves at risk for disease, and research is warranted.

One opportunity to detect calves at risk for disease is to passively monitor sickness behaviors in calves with PDT, such as accelerometers and automated feeding systems. Accelerometers are validated for step activity, lying bouts, lying time, feeding time, rumination, and sleep in calves. Automated feeding systems can record a variety of feeding behaviors including milk and solid feed intake, feeding rate, feeding time, and visits. Indeed, preliminary evidence suggests that calves have feeding behavior depression prior to clinical diagnosis of diarrhea and BRD bouts in calves. Similarly, calves exhibit reduced activity levels in the days before diagnosis with a BRD bout, and restless activity levels prior to diarrhea diagnosis. However, inconsistencies between studies in reporting, sampling frequency, and even diagnostic criteria for disease leads to a need for future research. Future research is needed to follow a cohort of calves daily for true disease onset using validated clinical definitions for disease, including consistent antimicrobial interventions, inter-observer reliability agreement, and gold standards for diagnostic reporting.

The dissertation objective

The overarching objective of this dissertation is to follow a cohort of calves daily from birth until 14 days past weaning (first 90 days of life) for disease bouts to determine if feeding behavior and activity levels decline prior to BRD in preweaned dairy calves. Since calves often relapse to the initial antimicrobial treatment for a BRD bout, the potential of PDT to indicate recovery status in calves will also be explored. Specifically, it is of interest to quantify if daily feeding behaviors such as milk intake, calf starter intake, drinking speed, and/or rewarded and unrewarded visits recorded by an automated feeder indicate feeding behavior alterations prior to BRD diagnosis as well as if this indicates recovery status in calves (relapsed status). Furthermore, it is of interest to quantify if activity levels such as daily lying time, lying bouts, step counts, and average acceleration rates indicate lethargy in calves prior to BRD diagnosis as well as if this indicates recovery status in calves (relapsed status). It is expected that recovered calves who respond to the initial antimicrobial intervention for BRD would no longer express sickness behavior, thus making relapsed calf behavior different. Specifically, it is expected that recovered calves will have greater activity levels and greater starter intake as they approach convalescence. However, this has never been explored in calves and offers a potential additional benefit for the technology.

Finally, it is of interest to determine if feeding colostrum to calves who decline in their feeding behavior can ameliorate a disease bout and improve performance. Providing nutritive support to calves during a time of metabolic challenge and immune activation is a proof-of-concept approach. This is important since the dairy industry should explore antimicrobial alternatives moving forward, and antimicrobial resistance in calves should be mitigated. Since the collective evidence of this literature review suggests that both milk feeding strategies, and management factors influence disease likelihood, calves on this study received the highest level of welfare available to artificially raise dairy calves. For example, calves on this study were provided a high caloric diet (intermediate milk levels, and free choice starter, and hay), to ensure essential needs were met and calf welfare was not self-limiting regarding biological functioning prior to becoming sick. Furthermore, calves were provided social housing, and enrichment items such as a ball, and calf brush in their enclosure to enhance expression of natural behavior to prevent boredom. This optimal welfare environment was provided to these calves so that changes in calf behavior associated with disease were not bound by a limit-fed milk diet, boredom, or external management factors (such as dirty bedding, dehorning etc.). However, we still used an artificial environment as this research needed to remain relevant for the commercial dairy industry. Thus, this dissertation assessed the ability of an accelerometer, and an automated feeder to indicate sickness behavior prior to disease onset, and to indicate recovery status in dairy calves with BRD. Furthermore, the potential of colostrum to ameliorate disease bouts in calves who had depressed feeding behavior prior to disease onset was explored.

Table 1.1 Validation of accelerometers to monitor calf activity levels and feeding behavior (adapted from Costa et al., 2020[1])

Studies (n=24) which evaluated the validity of accelerometers for measuring activity-related behavior in calves including lying, standing, walking and locomotory play(n=11), sleep (n=1) or feeding behaviors (n=9). Feeding behaviors rumination time, drinking, or feeding time in young calves. Studies were selected from manuscripts published in the last decade after full text screening. Electronic databases searched included ScienceDirect, PubMed, Ebscocost, Google Scholar and Web of Science.

Behavior Citation	Device Manufacturer Attachment	Calf sample size and observation period	Device sampling strategy	Validation Method
Lying time (Trénel et al., 2009)	IceQube Ice Robotics, Edinburgh, Scotland pedometer	N=9, 12 hours	1 reading/second	Mean Sensitivity (99.6%) Specificity (98.0%) with video
Lying time (Bonk et al., 2013)	HOBO Pendant G data logger Onset Computer Corp., Bourne, MA pedometer	Study I N=8, 37 2-hour periods Study II N=19, 19 24-hour periods	30-seconds or 60-seconds intervals; processed as unfiltered. 1-minute or 2-minute filters	Best correlation with direct observation (= 0.99) for 60-second intervals Bland-Altman (mean difference = 0.0 ± 3.1 min) Predictability (99.3%) Sensitivity (99.2%) Specificity (97.7%)
Lying time (Swartz et al., 2016)	AfiTag II Afimilk Ltd., Kibbutz Afikim, Israel pedometer	N=5 ,7 hours	Nearest 1 min, reported in 3 min intervals	Correlation with video (= 0.99) and HOBO (= 0.99)
Lying time (Finney et al., 2018)	IceQube Ice Robotics, Edinburgh, Scotland pedometer	N=12, 96 consecutive hours	1reading/second summed 15 min intervals unfiltered and filtered	Bland-Altman against video difference=3.6 seconds; SD not reported Filtering method did not change outcome.

Table 1.1 Continued

Lying time (Roland et al., 2018b)	SMARTBOW ear tag Smartbow GmbH, Weibern, Austria Ear tag	N=15, 4 hours	10 readings / second	Cohen's kappa with video (=0.88) Sensitivity (94.4%) Specificity (94.3%) Accuracy (94.3%) Precision (95.8%)
Lying bouts (Bonk et al., 2013)	HOBO Pendant G data logger Onset Computer Corp., Bourne, MA pedometer	Study I N= 8, 37 2-hour periods Study 2 N= 19, 19 24-hour periods	30-second or 60-second intervals processed as unfiltered. 1-minute or 2-minute filters	Best correlation with direct observation (= 0.85 for 60-s interval Bland-Altman (mean difference = 0.68 ± 0.75 bouts)
Lying bouts (Swartz et al., 2016)	AfiTag II (Afimilk Ltd., Kibbutz Afikim, Israel) pedometer	N=5, 7 hours	1 bout = minimum of 3 minutes lying down	Correlation with video (= 0.99) and HOBO (= 0.93)
Steps (Swartz et al., 2016)	AfiTag II Afimilk Ltd., Kibbutz Afikim, Israel pedometer	N=5, 7 hours	15-minute intervals	Correlation with video (= 0.99)
Gait (walking, trotting, and galloping) (de Passillé et al., 2010)	HOBO Pendant G data logger Onset Computer Corp., Bourne, MA Attached to leg with wrap	N=7, 10 minutes	33 readings/second	Correlation between average number of peaks in acceleration data and number of steps observed from video in forward axis (=0.92), vertical axis (=0.93), and vector sum of forward and vertical axes (=0.88). distribution of interpeak intervals in forward axis differed among gait types ($P < 0.001$)

Table 1.1 Continued

Locomotory play (Luu et al., 2013)	HOBO Pendant G data logger Onset Computer Corp., Bourne, MA Attached to leg with wrap	N=30, 10 minutes	33 readings /second during recording simulated 11 readings and 1 reading/second during post-processing	Correlation between duration of peaks of acceleration in vertical axis and duration of locomotory play in video (=0.97 for 11 readings/second and 0.92 for 1 reading/second)
Inactive (Hill et al., 2017)	CowManager SensOor Agis, Harmelen, the Netherlands Ear tag	N=16, 12 hours per day for 4 days	1 reading/minute	Regression against live observations (R^2=0.97) Slope not different zero
Sleep (REM and NREM) (Hokkanen et al., 2011)	Purpose-built accelerometer; Attached to neck collar	N=10, 24 hours	25 readings/second	Sensitivity, lying awake (= 40%), REM (=66%) and NREM (=81%)
Rumination (Burfeind et al., 2011)	Hi-Tag Electronic Rumination Monitoring System SCR Engineers Lmtd., Netanya Israel Neck collar	N=35 grouped by age. 2-hour intervals 3 times in 1 day	1 summed reading/2 hours	Cohen's Kappa with live observations (r=0.99) Moderate correlation (r=0.89) Moderate regression (R^2=0.79) for calves at 8 weeks bias for over and under-estimation 20 min
Rumination (Eslamizad et al., 2018)	Rumiwatch System GmbH Feeding Technology, Liestal, Switzerland Neck collar	N=10, 12 hours for 1 day	1 reading/minute	Regression against video recordings (R^2=0.82), slope 0.87 Filtering out rumination events < 5 min (R^2=0.93), slope 0.93
Rumination (Hill et al., 2017)	CowManager SensOor Agis, Harmelen, the Netherlands Ear tag	N=16, 12 hours per day for 4 days	1 reading/minute	Regression against live observations (R^2=0.91) Slope not different zero

Table 1.1 Continued

Rumination (Rodrigues et al., 2019)	Hi-Tag Electronic Rumination Monitoring System SCR Engineers Lmtd., Netanya Israel Neck collar	32 calves 4 2-hour intervals pre and post weaning	1 summed reading/2 hour	Observer agreement correlation (r=0.96) Correlation pre and post weaning rumination time to live observation (r=0.70) Probability distribution different (*P* <0.001)
Rumination (Roland et al., 2018b)	SMARTBOW ear tag Smartbow GmbH, Weibern, Austria Attached to ear	N=15, 4 hours	10 readings / second	Cohen's kappa with video (=0.80). Sensitivity (89.4%), specificity (94.9%), accuracy (93.9%), and precision (78.5%).
Rumination (Reynolds et al., 2019b)	CowManager SensOor Agis, Harmelen, the Netherlands Attached to ear	24 weaned calves Two 2-hour observation periods per calf	1 reading/minute	Concordance correlations (ccc=0.55) and correlations (r=0.63) against visual observations Bias correction factor (=0.88)
Solid feed intake (Roland et al., 2018b)	SMARTBOW ear tag Smartbow GmbH, Weibern, Austria Attached to ear	N=15, 4 hours	10 readings / second	Cohen's kappa[115] with video (=0.52) Sensitivity (73.2%) Specificity (83.7%) Accuracy (81.2%) Precision (57.9%)
Water intake (Roland et al., 2018a)	SMARTBOW ear tag Smartbow GmbH, Weibern, Austria Attached to ear	N=15, 4 hours	10 readings / second	Cohen's kappa[115] with video (=0.04) Sensitivity (2.7%) Specificity (99.9%) Accuracy (99.4%) Precision (10.0%)

Table 1.1 Continued

Milk intake (teat-fed) (Roland et al., 2018a)	SMARTBOW ear tag Smartbow GmbH, Weibern, Austria Attached to ear	N=15, 4 hours	10 readings /second	Cohen's kappa[115] with video (=0.07). Sensitivity (4.2%), Specificity (99.7%), Accuracy (97.6%), Precision (27.1%).
Milk intake (bucket-fed) (Roland et al., 2018a)	SMARTBOW ear tag Smartbow GmbH, Weibern, Austria Attached to ear	N=3, 5 days 24 hours per day	10 readings /second	Cohen's kappa with video (=0.68). Sensitivity (82.9%), Specificity (96.9%), Accuracy (96.2%), Precision (60.4%).
Feeding time (Hill et al., 2017)	CowManager SensOor (Agis, Harmelen, the Netherlands) Attached to ear	16 calves (12 h/d for 4 d)	1 reading/minute	Regression against live observations (R^2=0.75) Slope not different zero
Feeding time (Reynolds et al., 2019)	CowManager SensOor (Agis, Harmelen, the Netherlands) Attached to ear	24 weaned calves (Two 2 h observations per calf)	1 reading/minute	Concordance correlations (ccc=0.72) and correlations (r=0.88) against visual observations Bias correction factor (=0.83)

[1]A comprehensive evaluation of the literature on validation of accelerometers to monitor feeding behavior and activity levels in calves. This table is published in J. Dairy Sci. as part of a literature review on the use of PDT for dairy calf management.

CHAPTER 2. DAILY FEEDING AND ACTIVITY BEHAVIORAL PATTERNS COLLECTED BY PRECISION TECHNOLOGY ARE ASSOCIATED WITH BOVINE RESPIRATORY DISEASE STATUS IN PREWEANED DAIRY CALVES

Introduction

Bovine Respiratory Disease (BRD) is a disease affecting the respiratory tract of dairy and beef calves. According to a USA-based producer survey, BRD affects 12% of preweaned dairy calves (USDA, 2018). This survey also reported that BRD was a major contributor to calf mortality, responsible for around a quarter of calf deaths (USDA, 2018). Similarly, a recent cross-sectional study in Germany suggested that BRD affected 9% of the calves (Dachrodt et al., 2021).

However, iHowever, it is likely that BRD prevalence in calves is under reported by producers. For example, a recent epidemiological study that regularly health scored over ten thousand calves found that BRD affected approximately a third of pre-weaned calves (Dubrovsky et al., 2019); this finding suggests that calfhood BRD may be higher than producer-reported prevalence. Disparities between studies reporting BRD prevalence might be due to the complexity of disease involvement, where a primary viral infection may be followed by opportunistic bacterial infection of the respiratory tract (Pardon and Buczinski, 2020), or due to different diagnostic criteria used to define BRD. Regardless, this body of evidence indicates that BRD is a prevalent disease in preweaned calves, and additional BRD identification devices are needed.

A systematic scoring system, such as the Wisconsin scoring system, is commonly used to detect BRD in calves (McGuirk and Peek, 2014). Use of this system involves assigning cumulative scoring from two abnormal categories (e.g., 0 normal to 3 severely abnormal) such as cloudy nasal discharge, colored eye discharge, presence of ear flick or head tilt, elevated rectal temperature (e.g. higher temperatures receive higher scores), and the presence of spontaneous, or repeated coughing to diagnose BRD (McGuirk and Peek, 2014). However, some authors have expressed concern that the Wisconsin scoring system is labor intensive (e.g., 20 hours per hundred health checks Aly et al., (2014)), or limited in finding sick calves. For example, the average sensitivity to detect BRD in calves using the Wisconsin scoring system was reported at 62%, while the average specificity was 74% when compared to the thoracic lung ultrasonography scoring system lung consolidation threshold of >1cm^2 (Buczinski et al., 2015). In comparison, Buczinski et al., (2015) observed that the validated thoracic lung ultrasonography scoring system increased sensitivity and specificity for diagnosing BRD in calves when the threshold of >1cm^2 of lung consolidation was used (e.g., average sensitivity was 79% and average specificity was 94%). Most recently, the thoracic lung ultrasonography scoring system was validated with no difference in average sensitivity (e.g., 84%) or average specificity (e.g., 74%) when compared to chest thoracic radiography to diagnose pneumonia in hospitalized calves (Berman et al., 2021). This suggests that the thoracic lung ultrasonography scoring system is a gold standard for diagnosing BRD in calves, and could also be used in combination with the Wisconsin scoring system to achieve a higher sensitivity in diagnosing BRD in preweaned calves (Buczinski et al., 2014). Thus, it is suggested that herds can assess the prevalence of BRD in calves by hiring a veterinarian to use the thoracic lung ultrasonography scoring system and the Wisconsin scoring system, collectively. However, though thoracic lung ultrasonography scoring system is sensitive to detect BRD in calves, we suggest that this is still not in common use on most dairy farms. Thus, additional BRD identification devices which limits the use of manual labor are needed.

The early diagnosis of BRD development is imperative to intervene sooner to avoid a negative impact on calf productivity; BRD was associated with less average daily gain in preweaned calves (Cramer and Ollivett, 2019). Similarly, BRD identified in calves at weaning was associated with less milk in the first lactation (Dunn et al., 2018). More recently, Buczinski et al., (2021) observed that BRD incurred preweaning in dairy calves was associated with a greater likelihood of reduced average daily gain, death, failure to complete the first lactation, and a greater likelihood of producing less milk in the first lactation compared to healthy counterparts. Therefore, detecting calves at risk for BRD in a timely manner for appropriate intervention may also potentially mitigate the effect of BRD on calf productivity.

One potential opportunity to improve BRD detection in calves is to use precision technology devices to monitor for changes in behavioral patterns. Research using precision technology devices encompasses two areas: (1) use of technology to increase farm efficiency and (2) use of wearable precision technology devices to manage production parameters, or monitor the behavior of livestock (Eckelkamp and Bewley, 2021). There is the potential for the incorporation of precision technology devices to monitor the behavior of dairy calves in relation to disease diagnosis. For example, automated feeders can record feeding behaviors such as drinking speed, milk intake, and visits, and these behaviors may change before BRD diagnosis in calves as reviewed by Morrison et al., 2021. For instance, diseased calves fed limited milk with automated milk feeders had fewer unrewarded visits prior to disease (Svensson and Jensen, 2007). Moreover, Borderas et al., (2009) identified that calves on higher milk allotments changed milk intake on the day of illness detection. However, both Borderas et al., (2009) and Svensson and Jensen (2007) defined disease as ill thrift, making comparisons to BRD difficult. More recently, Swartz et al., (2017) stratified feeding behavior by disease type using the Wisconsin Scoring System to diagnose BRD in calves and observed that milk intake declined on the day of BRD detection. Interestingly, when multiple farms were included, daily average drinking speed and unrewarded visits to an automated milk feeder changed on the day of antibiotic treatment for BRD calves diagnosed by the producer (Knauer et al., 2017). Moreover, calves diagnosed with BRD had slower drinking speeds for the 3 days prior to BRD diagnosis when compared to healthy calves using a thoracic lung ultrasonography scoring system and the Wisconsin Scoring System as disease diagnostic criteria (Cramer et al., 2020). Furthermore, Duthie et al., (2021) observed that calves had less unrewarded visits 3 days prior to BRD diagnosis (e.g., defined by the sum of days with the highest scores of abnormal signs on the Wisconsin Scoring System) and reduced milk intake on the day before BRD diagnosis compared to healthy calves. This work demonstrates that calves approaching a BRD diagnosis may have depressed feeding behavior in the days before diagnosis. However, it is unknown if individual calves have relative changes in their feeding behavior and this should be explored.

Another precision technology device to potentially detect BRD in calves is accelerometers which monitor behavioral activity levels, including lying time, lying bouts, step counts, and rate of acceleration in calves as reviewed by Costa et al., 2020. Some of these measures changed before diagnosis with BRD. For example, Swartz et al.

(2017) found that lying bouts decreased 2 days before and daily steps decreased 1 day before BRD diagnosis using the Wisconsin Scoring System. Belaid et al., (2020) observed that veal calves decreased lying time 10 days before diagnosis of ill thrift or breathing ailments/nasal discharge, although this effect was not observed on any other day leading up to diagnosis. Furthermore, Duthie et al., (2021) observed that calves increased their lying times and decreased their lying bouts prior to BRD diagnosis (e.g., defined by the sum of days with the highest scores of abnormal signs on the Wisconsin Scoring System) when compared to healthy calves. Interestingly, Belaid et al., (2020) also observed a change in feed bunk visits in calves prior to disease diagnosis, suggesting that behavioral activity surrounding solid feeding may be a sickness behavior. However, Belaid et al., (2020) did not record starter intake, thus this should be investigated. This work demonstrates that calves approaching a BRD diagnosis may have lethargic behavior such as decreased lying and solid feeding bouts, increased lying times, and decreased step counts in the days before diagnosis with BRD. However, it is unknown if individual calves have relative changes in their activity behavior and this should be explored.

This evidence suggests that differences in feeding behavior and activity levels between diseased and healthy calves are analyzed without identifying changes in an individual animal's behavior. However, precision technology devices are often designed to monitor for and alarm when there are relative changes from a behavioral baseline of the animal (Gargiulo et al., 2018). Indeed, a recent survey found that dairy producers were more likely to adopt precision technologies if it improved labor efficiency (e.g., especially larger herds), or could alert the producer for potential early intervention (e.g., detect relative changes from a cow's behavioral baseline; Gargiulo et al., 2018).

Precision technology devices have been used to evaluate relative changes in activity associated with estrus (e.g., Silper et al., 2015), relative changes in rumination patterns and activity to detect parturition (e.g., Borchers et al., 2017), and relative changes in feeding behavior and activity to detect metritis in cattle (e.g., Neave et al., 2018a). As of current, researchers have identified associations of feeding behavior and activity for the days prior to BRD diagnosis in calves. However, there remains a need to quantify the effect of BRD status on the relative changes in behaviors of individual calves using changes in an individual animal's behavior to alert for problems.

The objective of this study was to identify which feeding behaviors (e.g., milk intake, drinking speed, calf starter intake, rewarded and unrewarded visits) and activity behaviors (e.g., lying time, lying bouts, step count, and an acceleration activity index) were associated with BRD status in preweaned dairy calves before BRD diagnosis. The second objective of this study was to determine if BRD status was also associated with daily relative changes in feeding behavior and activity before BRD diagnosis. We predicted that feeding behavior and activity would change prior to BRD diagnosis in calves.

Materials and Methods

This study was conducted at the University of Kentucky Coldstream Research Dairy Farm in Lexington, KY, USA from 28 May, 2018 to 9 September, 2019. All calves enrolled were part of the Institutional Animal Care and Use Committee approval number 2018: 2864. This study and manuscript were conducted following the quality standards of

Strengthening the Reporting of Observational Studies in Epidemiology Veterinary Guidelines (Sargeant et al., 2016).

Enrollment criteria

On this case-control study we enrolled 66 calves (50 heifers and 16 bulls), where 33 BRD case calves incurring their first BRD diagnosis were matched to 33 healthy control calves. We pair matched BRD calves to healthy calves by gender, identical number of days in the group pen (e.g., exact age at time of BRD diagnosis), and birthdate within one month (i.e. pair matches could be from different groups). Healthy matched calves were negative for all diseases, never received antimicrobials, were negative on the lung ultrasonography scoring system for lung consolidation, and were negative for BRD on the Wisconsin Scoring System (McGuirk and Peek, 2014). The calves enrolled on this study were health scored daily (e.g., defined in health exam section) for signs of BRD, diarrhea and navel infection from birth to feeder day 50 (i.e., preweaning phase). We included BRD from the preweaning phase since weaning was associated with increased variation in feeding behavior in calves (Neave et al., 2019a). BRD calves met eligibility criteria for this study if they never received antibiotics for a pre-existing health event.

Enrolled calves in this study were part of a larger cohort of 120 calves (73 heifers, 47 bulls) who were health scored daily (e.g., defined in health exam section) for signs of BRD, diarrhea and navel health from birth until 14 d after weaning (87 ± 2.0 d of age; mean ± SD). Calves were excluded from cohort enrollment and sold if they were a twin, or had a serum BRIX of less than 8.0% at 48 h of life.

Management and feeding

Newborn calves were moved to individual pens (3 x 3 m) in the calf barn and were fed 4 L maternal colostrum (≥ 22% BRIX measured by digital refractometer) within 6 h of birth. Calves were weighed within 12 h of birth using an electronic scale (Brecknell PS1000, Avery Weigh-Tronix LLC, and Fairmont, MN). Calf birth weights were 39.50 ± 5.0 kg (mean ± SD). Each calf was fitted with a radiofrequency identification tag in the left ear for identification by the automated feeder, and a pedometer (IceQube, IceRobotics, and Edinburgh, Scotland) was attached above the metatarsal of the rear left leg using a Velcro band to track activity behaviors. Calves then received 6 L/d of 840 g milk replacer (Cow's Match; Land O' Lakes Animal Milk Products Co., Shoreview, MN) divided into two bottle feedings until calves showed a strong vigor by standing independently and suckling the bottle without pauses. Calves were then trained to suckle milk from the automated feeder (CF100, Forster Technik, Engen, Germany) and then moved from individual to group housing at an average age of 3.0 ± 1.5 d at the following morning feeding using techniques adapted from Neave et al., 2019. Calves were assisted at the automated feeder every 12 h until they visited the automated feeder independently. Calves no longer required assistance at the automated feeder at a maximum of 4 assisted feedings. There was one automated milk feeder located within a group pen (4.57 × 10.67 m) and the stocking density was 6 ± 3 calves (mean ± SD); calves were moved to an identical adjacent pen with the same stocking density using dynamic flow at an average age of 45.0 ± 3.0 d (mean ± SD) before weaning.

Calves were allotted up to 10 L (140 g/L) milk replacer/d from the automated milk feeder (Cow's Match Cold Front; Land O' Lakes Animal Milk Products Co., Shoreview, MN) for 50 d, reduced to 50% allotment for 14 d, and then reduced to 20% allotment for an additional 7 d until complete weaning at 70 d. Calves could consume milk in a minimum meal size of 0.5 L and a maximum meal size of 3 L. Calves were health scored daily for 14 d post-weaning, until the age of 87 ± 2.0 d of age (mean ± SD).

A separate automated starter feeder (Compact Smart, Förster-Technik, Engen, Germany) was present in each pen and contained calf starter (Special Calf Starter and Grower, Baghdad Feeds, Baghdad, KY); calves were also offered chopped alfalfa hay in a trough (1.83 × 0.33 × 0.16 m). Both the automated milk feeder and the calf starter feeder were calibrated weekly according to manufacturer instructions. All calves had ad libitum access to an automated waterer. Calf starter and chopped alfalfa hay were sampled weekly and immediately frozen at -20 °C. Later, the feed samples were weighed, dried in a forced air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) for 48 h at 55 °C, and weighed again to calculate % dry matter. Then, weekly samples were ground through a 1 mm sieve screen (Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA), composited into monthly samples, and sent to a laboratory (River Rock Lab, Watertown, WI) to determine nutrient composition (Appendix 1). Briefly, starch was analyzed using the acetate buffer only method as validated by Hall, 2009. Crude protein was analyzed according to the Dumas method as recently re-validated by Wiles et al., 2020 with an N analyzer (FP-528; LECO, St. Joseph, MI, USA). Crude fat, ash free NDF, and ADF were calculated using Ankom Technology (Macedon, NY, USA). Crude fat was analyzed using high temperature extraction (Ramos, 2005). The ash free NDF, and ADF were analyzed using the cell wall fractionation method as described in detail by Surendra et al., 2018. The ME kcal/kg was calculated according to the TDN x 0.04409 x 0.82 (NRC, 2001).

Health exams

Calves were health scored daily at approximately 8:30 h from birth until 2 weeks post-weaning (87 ± 2.0 d of age) by 1 of 3 observers (inter-observer agreement $\kappa > 0.90$) for the following signs of health events: BRD, diarrhea and sepsis as described in detail in Cantor et al., 2021. In brief, Fleiss' kappa was calculated every 4 mo. throughout the study by having all observers go to a commercial facility on the same day to score the health status of 40 calves where the health status of the calves was unknown to ensure unbiased agreement. The main observer health scoring the calves was not blind to the health status of the calves due to following calves daily. However, to minimize bias, only the farm staff manager administered antimicrobial treatments to the calves, and the other 2 observers were blind to the health status of the calves.

Bovine Respiratory Disease signs were recorded daily by trained observers using the Wisconsin Scoring System (McGuirk and Peek, 2014). The observer assigned a nasal discharge score, eye discharge score, ear tilt score, cough score, and temperature score to each calf every day. A trained observer performed the thoracic lung ultrasonography scoring system on all calves twice weekly (e.g., every Tuesday and Friday) using a portable linear rectal ultrasound (Ibex Pro, E.I. Medical, Loveland, CO) and 70% isopropyl alcohol as a transducing agent; lung lobes of each calf were evaluated by 1 of 2 observers (inter-observer agreement Cohens' kappa; $\kappa = 0.90$). The ultrasound was set to a depth of 9 cm, frequency of 6.2 MHz, and gain of 23 dB (near 13 dB; far 36 dB). The observers used the thoracic lung ultrasonography methodology first described by Ollivett et al., 2015. Briefly, both sides of the thorax were scanned beginning at the scapula of the calf with the probe held parallel to the rib. The observer first scanned the dorsal aspect of the tenth intercostal space, advancing cranially toward the ventral aspect of the first to second intercostal space (Ollivett et al., 2015).

The diagnosis and treatment of BRD of preweaned calves on this study required two criteria set by the herd veterinary protocol for this research station. One criteria required a calf to have a Wisconsin Scoring System score of ≥ 5, and two or more examination parameters that were moderately (score of 2) or severely (score of 3) abnormal (adapted from McGuirk and Peek, 2014). Also, this diagnosis was combined with a thoracic lung ultrasonography scoring diagnostic criteria. As described in Dunn et al., (2018), the consolidated lung appeared hypoechoic and both the bright white band at the pleural interface and the reverberation artifact were absent, $3cm^2$ was used for lung consolidation.

Thus, each calf had to meet both the Wisconsin Scoring System criteria and the thoracic lung ultrasonography scoring criteria. The calf received antimicrobials on that first day of both diagnostic criteria (hereafter day 0; **BRD bout**). Since the lung ultrasonography scoring system was performed twice weekly, the calf's most recent ultrasound score was used in combination with the current day's Wisconsin Score health checks to determine a diagnosis per the herd veterinarian protocol and recommendation by the responsible veterinarian team. On the same day of BRD bout diagnosis, calves received enrofloxacin subcutaneously with dosage calculated by BW (Baytril, Bayer, Leverkusen, Germany; 100 mg/15 kg) according to the herd veterinarian protocol. To avoid the severity of a BRD bout affecting later feeding behavior and activity levels, only calves who were diagnosed with their first BRD bout were enrolled in the study.

Body weights were recorded twice weekly using an electronic scale (Brecknell PS1000, Avery Weigh-Tronix LLC, and Fairmont, MN) from birth to 2 weeks post-weaning for all calves.

Study population

From the cohort of calves with a BRD bout, 33/49 calves were eligible (25 heifers and 8 bulls) who incurred their first BRD bout without ever receiving antibiotics for a pre-existing health event and these calves were matched to control calves with complete behavioral data. Calves with a BRD bout were diagnosed at an average age of 33.0 ± 9.0 d (mean ± SD) and weighed 56.1 ± 9.7 kg.

At the time of the study development, we did not have feeding behavior and activity level data available to estimate effect sizes for these parameters for BRD calves fed high allowances of milk replacer. Thus, for the power analysis, we wanted to assure we would capture the population prevalence of BRD in calves. Since we were following a cohort of animals for disease, we powered our study for the incidence of BRD bouts in calves. The national producer survey reported BRD preweaning prevalence at 14% (USDA, 2018). Thus, we made a priori calculation based our unpublished data and the reported prevalence of disease to calculate the number of BRD bouts necessary and thus the number of enrolled calves. Thus, we assumed a population incidence of 35% for BRD in preweaned dairy calves. We calculated the herd’s true BRD incidence preweaning at 60% by assigning 2 researchers to score calves for BRD (Cohen’s kappa= 0.95) twice weekly for 9 mo. prior to this study. Therefore, at 80% power, with half-widths of 0.05, we required 29 calves with a BRD bout. We also conducted a posteriori analysis with the variance found in this study for the variables of interest, and the number of calves used in the experiment was greater than the sample size required using the power analysis based on the variance of all the variables of interest.

Automated technology data

Accelerometer activity (IceQube, IceRobotics, Edinburgh, Scotland) was recorded at 16 Hz every min, and an automated summary was generated for each behavior for every calf every 15 min and transmitted to a data cloud wirelessly. Daily summaries were automatically generated by the software for each calf on the following behaviors: lying bouts, lying time, and total step count following Silper et al., 2015. Moreover, an acceleration activity index score was generated daily by an algorithm of this accelerometer's software (IceQube, Ice Robotics, Scotland). This algorithm evaluated each calf's average daily rate of acceleration on all three axes and included daily step counts to generate an activity index (Gladden et al., 2020).

The automated feeder's software (KalbManagerWIN, Förster-Technik, Engen Germany) summed milk intake, calf starter intake and milk feeder visits (rewarded and unrewarded visits) into daily summaries for each calf and transmitted the data to a data cloud associated with the automated feeder software.

The temperature and humidity were recorded every 15 minutes by a data logger (HOBO U23 Pro., Hobo, Onset Corp., Bourne, MA) placed in a pen in the calf barn and all data was automatically transmitted to a data cloud for the duration of the study. The mean temperatures and humidity for each month were calculated. Winter was defined as December through February and the mean temperature was 8.44 ± 5.07 °C (mean ± SD); the mean humidity was 76.52 ± 9.25 % (mean ± SD). Spring was defined as March through May and the mean temperature was 13.13 ± 7.99 °C (mean ± SD); the mean humidity was 68.04 ± 12.11 % (mean ± SD). Summer was defined as June through August and the mean temperature was 24.93 ± 3.74 °C (mean ± SD); the mean humidity was 75.38 ± 14.08 % (mean ± SD). Fall was defined as September through November and the mean temperature was 14.77 ± 9.20 °C (mean ± SD); the mean humidity was 81.51 ± 13.65 % (mean ± SD).

Statistical Analysis

All statistical analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive analyses were performed, and were these were also used to assess the data for completion. Data was verified for normality using the univariate procedure (Proc Univariate) and probability distribution plots. Normality was also investigated by visually examining the residuals from the linear mixed models (Proc Mixed) and by testing covariance structures for model fit. Only three outliers were detected in three healthy calves for all behaviors evaluated (i.e., data points beyond 3 SD from the mean) and these calves were excluded.

Calf starter intake and unrewarded visits were not normally distributed and were transformed accordingly (calf starter intake: common log with correction factor of 10g for calf starter intake; unrewarded visits: common log with a correction factor of 5 visits). The back-transformed geometric means minus the correction factors and the 95% CI are reported for both calf starter intake and unrewarded visits, with statistical significance reported on the modeled transformed values. For the activity behavior variables, one BRD calf did not have activity data recorded because of device malfunction, thus n=32 pairs were included in the activity behavior models.

For all linear mixed models in this study (Proc Mixed), multivariable models were reduced by manual stepwise backward elimination, where predictors with a P-value < 0.30 were retained in the models. Season was evaluated in all models as a fixed effect and birthweight, Brix % serum IgG at 48 h of age, and calf age were tested as quantitative covariates. Due to the collinearity observed during univariate analysis for the covariates BRIX % IgG at 48 h of age and age enrolled on the feeder, these were evaluated for model inclusion separately. If BRIX % IgG at 48 h of age was not significant, age enrolled on the automated feeder was evaluated. We pair matched BRD calves to healthy calves by gender, identical number of days in the group pen (e.g., exact age), and birthdate within one month (i.e. pair matches could be from different groups). Pair was a fixed effect in all models.

Effect of BRD status on average calf behavior

First, linear mixed models (Proc Mixed) were used to investigate the effect of BRD status, and the BRD status x day interaction, on the average feeding behavior and activity in preweaned calves for the 5 days prior to and including the day of diagnosis (day -5 to day 0; referred to as "**pre-diagnosis period"**). Data from up to 10 days prior to diagnosis were investigated, day -5 was selected due to model fit, and a lower Akaike's criterion. Models were designed with day as a repeated measure and calf as the subject with an autoregressive first order (AR1) covariance structure. BRD status x day interactions at $P < 0.20$ were explored using linear mixed models by day (Proc Mixed). This exploratory approach allowed us to describe the feeding behavior and activity levels of calves as they approached a BRD diagnosis, and how these behaviors change. These linear mixed models were used to investigate the daily behavioral patterns for each day within the pre-diagnosis period (day -5, day -4, day -3, day -2, day -1 and day 0; referred to as "**daily measures"**); calf was specified as a random effect in these models. Following the exploratory nature of our study, variables that did not have a BRD status x day interaction at $P < 0.20$ were still examined for daily behavioral patterns within the pre-diagnosis period.

Effect of BRD status on relative changes in calf behavior

Effects of BRD status and the BRD status x day interaction on the relative changes in feeding behavior and relative changes in activity over the pre-diagnosis period were created with an identical modeling structure to that described above (Proc Mixed). To determine the first day of unaffected behavior prior to BRD diagnosis, averages and variances of feeding and activity behaviors were tested by day and were deemed similar from day -5 to day -10 using Proc Npar1way. Thus, day -5 was set as the baseline, and deemed the 100% level for each behavior by calf; relative change was then calculated by dividing every calf's individual behavior on every daily measure (day -4, day -3, day -2, day -1 and day 0) by every calf's behavioral baseline at day -5. As above, following the exploratory nature of our study, BRD status x day interaction at $P < 0.20$ was examined using linear mixed (Proc Mixed) models by day. We conducted these linear mixed models for each day within the pre-diagnosis period (day -4 to day 0) to further explore any effects observed in the overall pre-diagnosis period; calf was a random effect in all models. Any variable which did not have a BRD status x day interaction at $P < 0.20$ were still examined for relative changes in daily behavioral patterns within the pre-diagnosis period.

Results

Effect of BRD status on average behavior over the pre-diagnosis period

The effect of BRD status, and the BRD status x day interaction on average feed intake, feeding and activity behaviors during the pre-diagnosis period are presented in **Table 2.1.** Briefly, BRD calves had lower milk intakes, greater lying times, and a lower step count and acceleration activity index compared to heathy calves for the pre-diagnosis period.

There was no effect of BRD status on unrewarded visits for the pre-diagnosis period ($F_{1, 31} = 4.72$, $P = 0.55$; healthy calves = 1.94 [95% CI: 1.70-2.18], BRD calves = 2.09 [95% CI: 1.85-2.34] unrewarded visits; geometric mean [95% CI]); there was an interaction of treatment and day ($F_{10, 314} = 1.84$, $P = 0.05$). For the interactions of treatment and day, BRD calves tended to decrease unrewarded visits when day -5 was compared to day -4 (P =0.08) day -3 (P =0.07), day -2 (P =0.09) and day -1 (P =0.09), but not day 0 ($P = 0.16$), but no other days were different for BRD or healthy calves. BRD calves consumed less calf starter during the pre-diagnosis period ($F_{1, 31} = 4.72$, P = 0.03; healthy calves = 66.55 g/d [95% CI: 27.39-105.72 g/d], BRD calves = 45.30 g/d [95% CI: 23.19-67.40 g/d]; there was no interaction between treatment and day ($F_{10, 314}$ =1.56, $P = 0.21$).

Effect of BRD status on daily behaviors within the pre-diagnosis period

Figure 2.1 shows differences in feeding behavior between BRD and healthy calves on each daily measure within the pre-diagnosis period. BRD calves drank less milk on day -4 ($F_{1,29}$ = 4.94, *P* = 0.03), day -3 ($F_{1,29}$ = 6.14, *P* = 0.02), day -1 ($F_{1,29}$ = 6.53, *P* = 0.02), and tended to drink less milk on day -2 ($F_{1,29}$ = 3.66, *P* = 0.07; **Figure 2.1a**), but the other daily measures were not different (day -5 and day 0; *P* > 0.10) compared to healthy calves. There was no effect of BRD status on any daily measure for drinking speed (days -5 to 0; *P* > 0.10; **Figure 2.1b**), or rewarded visits (days -5 to day 0; *P* > 0.10; **Figure 2.1c**).

There was no effect of BRD status on unrewarded visits for any daily measure (days -5 to day 0; *P* > 0.10); unrewarded visits on day 0 were ($F_{1,30}$ = 0.54, *P* = 0.47; healthy calves = 1.67 [95% CI: 1.05-3.18], BRD calves = 1.12 [95% CI: 0.56-2.34] unrewarded visits; geometric mean [95% CI]). BRD calves consumed less calf starter on the day of BRD diagnosis ($F_{1,30}$ = 5.23, *P* = 0.03; healthy calves = 70.88 g/d [95% CI: 55.65-110.53 g/d]; BRD calves = 40.30 g/d [95% CI: 30.00-70.40 g/d], but the other daily measures were not different (day -5 to day -1; *P* > 0.10) compared to healthy calves.

Figure 2.2 shows differences in activity behavior between BRD and healthy calves on each daily measure within the pre-diagnosis period. BRD calves had greater lying times on day -4 ($F_{1,29}$ = 4.61, *P* = 0.04) and day 0 ($F_{1,30}$ = 6.27, *P* = 0.02; **Figure 2.2a**), but the other daily measures were not different (day -5, day -3, day -2, day -1; *P* > 0.10) compared to healthy calves. BRD calves had fewer lying bouts on day -3 ($F_{1,29}$ = 4.09, *P* = 0.05), day -2 ($F_{1,29}$ = 4.02, *P* = 0.05), and tended to have lower lying bouts on day 0 ($F_{1, 30,} =$ 3.73, $P = 0.06$; **Figure 2.2b**), but the other daily measures were not different (day -5, day -4 day -1; $P \geq 0.10$) compared to healthy calves.

BRD calves had lower step counts on several daily measures (day -4, day -3, day -2, day -1 and day 0; $P \leq 0.01$) and tended to have lower step counts on day -5 ($F_{1,28}$ = 3.21, $P = 0.08$; **Figure 2.2c**) compared to healthy calves. Finally, BRD calves had lower activity indexes on several daily measures (day -4, day -3, day -2, day -1 and day 0; $P \leq 0.01$; **Figure 2.2d**), but day -5 was not different ($F_{1,29} = 1.90$, $P = 0.18$).

Effect of BRD status on relative changes in behavior over the pre-diagnosis period

The effect of BRD status and the BRD status x day interaction on relative changes in feed intake, feeding and activity behaviors of calves during the pre-diagnosis period are presented in **Table 2.2**. Briefly, there was an interaction with treatment and day for relative changes in the unrewarded visits during the pre-diagnosis period, suggesting BRD calves had lower relative changes in unrewarded visits compared to healthy calves. Moreover, there was a treatment and day interaction with calf starter intake, suggesting BRD calves had greater relative changes in starter intake over the pre-diagnosis period.

Effect of BRD status on daily relative changes in behavior over the pre-diagnosis period

Figure 2.3 shows relative changes in feed intake and feeding behavior between BRD and healthy calves on each day within the pre-diagnosis period. There was no effect of BRD status on any daily measure for relative changes in milk intake (days -4 to 0; $P >$ 0.10; **Figure 2.3a**), relative changes in drinking speed (days -4 to 0; $P > 0.10$; **Figure**

2.3b), or relative changes in rewarded visits (days -4 to 0; $P > 0.10$; **Figure 2.3c**) compared to healthy calves.

BRD calves had lower relative changes in calf starter intakes, suggesting a decline in their calf starter intake on day 0 ($F_{1,29} = 3.47$, $P = 0.04$; **Figure 2.3d**), but the other daily measures were not different (day -4, day -3, day -2 and day -1; $P > 0.10$) compared to healthy calves. BRD calves had lower relative changes in unrewarded visits, suggesting a decrease from their baseline unrewarded visits for day -4 ($F_{1, 29} = 3.93$, $P = 0.03$) and day -2 ($F_{1, 29} = 4.74$, $P = 0.04$; **Figure 2.3e**), but the other daily measures were not different (day -3 and day 0; $P > 0.10$) compared to healthy calves.

Figure 2.4 shows relative changes in activity behaviors between BRD and healthy calves on each day within the pre-diagnosis period. There was no effect of BRD status on any daily measure for relative changes in lying time (days -4 to 0; $P > 0.10$; **Figure 2.4a**) and relative changes in lying bouts (days -4 to 0; $P > 0.10$; **Figure 2.4b**) compared to healthy calves. BRD calves had lower relative changes in step counts on day -2, suggesting a decline in their activity ($F_{1, 28} = 1.02$, $P = 0.05$; **Figure 2.4c**) compared to healthy calves. There was no effect of BRD status on any daily measure for relative changes in the activity index (days -4 to 0; $P > 0.10$; **Figure 2.4d**) compared to healthy calves.

Discussion

The objective of this study was to identify which feeding and activity behaviors were associated with BRD status in preweaned dairy calves before BRD diagnosis, and if daily relative changes (i.e. demonstrating an increase or decrease in behavior) in these behaviors would be useful in the early detection of BRD. Overall, we observed that the earliest feeding behavioral changes for BRD calves was reduced milk intake over the 5-d before diagnosis, and a decline in unrewarded visits relative to baseline at 4 days prior to diagnosis. BRD calves also differed in activity before diagnosis; these calves had greater lying times, fewer lying bouts, lower step counts, and a lower activity index 4 days prior to diagnosis. We suggest that feeding behavior and activity change prior to BRD diagnosis in preweaned calves housed on automated feeders.

During onset of a BRD bout, calves in this study had lower milk intakes, and showed a decline in unrewarded visits relative to baseline as early as 4 days before BRD diagnosis compared to healthy calves. Changes in feeding behavior before diagnosis with BRD are expected during onset of illness and have been observed by others (Borderas et al., 2009, Johnston et al., 2016; Svensson and Jensen, 2007). Most recently, Duthie et al., (2020) identified that BRD calves offered milk at 16 L/d had fewer unrewarded visits at 3 days prior to BRD diagnosis, and had reduced milk intakes at 1 day before diagnosis, but drinking speed was not affected. Our findings broadly agree with the findings of Duthie et al., (2020), which may be due to a similar housing environment and a high milk allowance of milk.

Milk allowance influences feeding behavior of calves (Rosenberger et al., 2017). Thus, interpretation of behavioral changes in response to illness reported in other studies must consider the milk allowance offered to calves. Many studies have offered a limited daily allowance of milk to the calves (e.g., hereafter limited milk refers to 7 L/d or less), making comparisons with the current study challenging. Some of the studies offering calves limited milk allowances (7 or 8 L/d) found a decline in unrewarded visits before disease diagnosis, but not milk intake (Svensson and Jensen, 2007; Johnston et al., 2016; Knauer et al., 2017). These studies reported conflicting findings for drinking speed, with some reporting a difference in drinking speed in BRD calves on day of diagnosis (Knauer et al., 2017; Johnston et al., 2016) and another study reported no relationship (Swartz et al., 2017). Few studies evaluating the association of BRD with feeding behavior offered intermediate levels of milk to calves (i.e., at least 10 L/d; as reviewed by Morrison et al., 2021). Borderas et al., (2009) observed that sick calves decreased their milk intake, and decreased their unrewarded visits, but not drinking speed when calves were offered higher milk allowances, although these changes were detected only on the day of disease diagnosis. However, Borderas et al., (2009) included all diseases (e.g., BRD, diarrhea and ill thrift) in their analysis and offered *ad libitum* milk. Cramer et al., (2020) observed that BRD calves offered milk *ad libitum* decreased their drinking speed 3 days before diagnosis, but not milk intake or feeder visits.

The only differences observed in automated feeder studies which offered calves limited milk was on the day of diagnosis (Swartz et al., 2017, Borderas et al., 2009 and Johnston et al., 2016). One exception is that some studies have observed a decline in unrewarded visits prior to calves becoming sick (Svensson and Jensen 2007, and Johnston et al., 2016). Thus, we hypothesize that it is possible that feeding behavior differences among studies are partially explained by different milk feeding strategies. For example, the daily milk allotment (Rosenberger et al., 2017), meal size (Jensen et al., 2020), and stocking density per nipple (Jensen et al., 2004) are factors that influence milk feeding behavior of calves housed in automated feeding systems. The milk intake, calf starter intake and unrewarded visits in our study were similar to those reported in

Rosenberger et al., (2017) which offered a similar milk feeding strategy and stocking density before step-down weaning to calves. Thus, we suggest that the feeding behavior observed in our calves may be reflective of the BRD sickness behavior in calves offered 10 L/d milk replacer.

There was no association of BRD status with drinking speed in this study, which agrees with others (Duthie et al., 2020, Borderas et al., 2009 and Swartz et al., 2017). On the contrary, some have suggested that drinking speed was associated with disease development in calves (Knauer et al., 2017, Cramer et al., 2020; and Johnston et al., 2016). However, it is probable that differences between studies are also partially explained by different disease definitions for BRD status. Most studies diagnosed BRD according to the Wisconsin Scoring System, which has a lower sensitivity compared to the thoracic lung ultrasonography scoring system (Buczinski et al., 2015). However, Cramer et al., (2020) used both the Wisconsin Scoring System and thoracic lung ultrasonography scoring system ($\geq$1 cm^2) to diagnose BRD in calves fed ad libitum milk and observed slower drinking speeds, but no milk intake differences for the -3 days prior to BRD diagnosis when compared to healthy calves. Our study defined BRD with a more advanced lung consolidation ($\geq$ 3 cm^2) and our calves were offered less milk, which may have contributed to differences in drinking speed. As previously suggested by Morrison et al., (2021), we emphasize it is imperative for researchers to use consistent criterion for BRD diagnosis to determine which feeding behaviors are associated with a BRD bout in calves.

To our knowledge, there is very limited research investigating how calf starter intake is affected before diagnosis with BRD, which changed on the day of BRD diagnosis in our calves. However, calves are highly motivated to consume milk during the first few months of life (de Passillé, 2001), and feed motivation is dependent on milk availability (de Passillé and Rushen, 2006). We suspect we found calf starter intake differences prior to BRD since calves are less motivated to consume starter over milk. For example, calves offered higher milk allotments (e.g. 10 L/d) vary in the age in which they commence consuming 200 g/d of starter (e.g. 23 to 82 days of age; de Passillé et al., 2011) as well as how much starter they consume prior to step-down weaning (de Passillé and Rushen, 2016). Thus, we suspect our calves reduced their starter intake before reducing milk intake before BRD onset due to their lower motivation to consume starter over milk. Belaid et al., (2020) observed that veal calves decreased their starter bunk visits prior to becoming sick, but these authors did not measure starter intake, making comparisons with our study difficult. In dairy calves, Johnston et al., (2016) observed that starter intake did not differ between preweaned BRD and healthy calves, although these calves were offered limited milk allotments which may explain our study differences. It is important to note that on this study, calves were consuming small amounts of starter. Since the automated calf starter feeder must be calibrated weekly to reliably dispense grain by 10 g variation, we acknowledge that this is a limitation to this study. However, this work has a proof of concept that calves offered intermediate levels of milk may sacrifice their solid feed prior to becoming sick. Thus, future research should investigate the potential of the automated calf feeder to detect these small changes in starter intake in preweaned calves.

Calves offered limited milk allotments experience signs of hunger (De Paula Vieira et al., 2008), and calves offered similar milk allotments to our study reportedly consume less starter than limit-fed calves before weaning (Rosenberger et al., 2017). Previous work has reported individual differences in calf starter intake of preweaned calves (Neave et al., 2018b, Benetton et al., 2019), and thus individual differences in changes in intake in response to illness may also be expected. Future research should investigate if these changes in starter intake before BRD diagnosis are affected by milk feeding strategy.

We also observed differences in activity levels in BRD calves before diagnosis, in particular, increased lying time, less lying bouts, less step counts, and a smaller acceleration activity index. The activity levels of healthy calves in our study were similar to the lying times and lying bouts reported in healthy calves observed by Duthie et al., (2020), suggesting that our study findings may be reflective of calves housed in other automated feeding systems of similar stocking densities. Calves decreased step count relative to baseline at 2 days before BRD diagnosis on our study, which broadly agrees with Swartz et al., (2017) who observed that BRD calves had fewer lying bouts (i.e. up to -2 days prior to BRD detection) and less step counts (i.e. -1 days prior to BRD detection) compared to healthy matched calves. Belaid et al., (2020) also observed differences in step counts at -2 days prior to disease detection in veal calves. Similarly, we observed greater lying times and less lying bouts in our calves at 4 days before diagnosis, which broadly agrees with Duthie et al., (2020). Overall, our results agree with previous work, suggesting preweaned calves may increase their lying times, and decrease activity patterns such as steps and lying bouts prior to becoming sick.

These reported declines in activity levels before disease diagnosis may be an example of sickness behavior, where activity is typically reduced to conserve energy for fighting illness (as reviewed by Cramer and Ollivett 2020). For example, calves experimentally inoculated with *Mannheimia haemolytica* also expressed less activity patterns including increased lying time, less lying bouts and less steps than healthy calves (Hixson et al., 2018). Experimentally infected beef cattle also declined in their activity and had increased lying times (Toaff-Rosenstein et al., 2016), suggesting that cattle decrease behavioral activity prior to feeling sick. Collectively this research suggests that cattle likely decrease their activity levels prior to BRD diagnosis.

Reduced activity may also arise as a result of changes in exploratory behavior or due to self-isolating behavior. Calves diagnosed with BRD were less willing to approach a novel person and a novel object (Cramer and Stanton, 2015), which was interpreted as a change in exploration of the environment. Calves identified with BRD also had a greater odds of depression such as abnormal posture, self-isolation from the group, and a reluctance to stand (Cramer et al., 2019) compared to healthy calves. These measures are often observed in calves with little activity, and often considered to be classical sickness behavior. The acceleration activity index used in this study could also potentially be used as an indicator of activity level in calves, as it was previously validated as an indicator of locomotor play bouts (Gladden et al., 2020). Indeed, researchers remotely monitoring the frequency of activity patterns in beef cattle could automatically discriminate between healthy and sick animals (Smith et al., 2015). Future research should quantify if acceleration activity levels and step count is associated with exploratory behaviors in calves, which could then be automatically used to identify potential development of illness.

Our study together with previous work suggests there is potential to use precision technology devices on farm to detect early onset of disease. However, one limitation to this study is that we retrospectively evaluated calves classified with BRD status to determine associations with behavior before disease diagnosis. However, we suggest that future research needs to evaluate the potential of establishing baselines for calves using precision technology devices to detect deviations in feeding behaviors and activity levels in real-time. For example, automated calf feeders can be programmed to alarm for deviations in feeding behaviors. Cantor et al., (2021) was able to ameliorate a BRD bout in calves by administering colostrum replacer when an alarm on the automated feeder was triggered (e.g., deviations in a 12-day rolling average milk intake and drinking speed). While the use of alarms generated by precision technology devices could be useful to manage dairy cattle, future studies should investigate the use of early interventions based on precision technology data.

In this study, we found evidence that calves do deviate from baseline behaviors (relative changes) including unrewarded visits, and step activity. For this study, we were interested in exploring if relative changes in behavior were associated with BRD status prior to diagnosis. However, future research is needed to extrapolate the individual variability in these behaviors within a cohort and determine which deviations from baselines are representative of animals who may become sick. This methodology has identified a relationship between physiological measures and disease status in transition cattle (Wisnieski et al., 2019). Thus, observations in this study suggest that there is the potential to use relative changes in feeding behavior and activity to detect calves who may have a BRD bout.

One of the potential sources of bias in this study was the disease definition used to diagnose BRD bouts in calves. We only enrolled calves in this first BRD bout and compared them to healthy counterparts. We did this to limit variability in behavior that may be attributed to recovery from previous diseases (e.g., diarrhea). However, we may have influenced our findings by selecting only these calves. For example, including calves recovering from other diseases would likely increase the variation in feeding behavior and activity levels in the calves as they approached a BRD diagnosis. Thus, we may have biased our findings by standardizing our research to this group of calves that are experiencing the first bout of illness. Similarly, we required calves to have lung consolidation at $3cm^2$ and signs of BRD based on the Wisconsin Scoring System. If we had enrolled calves at an earlier BRD threshold, such as 1 cm^2 as reported by Cramer et al., (2020), we may have found different results. We used this 3 cm^2 threshold on the advice of the herd veterinarian and veterinarians on the IACUC team, who were using this threshold to recommend antimicrobial treatment. Similarly, this was the definition for BRD that was employed on the farm at the time of the IACUC approval and study design. Thus, we suggest there is external applicability for some producers.

Similarly, there may be bias in our findings for relative change in behaviors. It is currently unknown if the automated feeder and commercial accelerometer software can detect deviations in relative changes in behavior at the levels observed on this study. Thus, further work is needed to determine if these algorithms can be developed in real time to work with these technologies. This study was exploratory in nature, so we suggest that using deviations from an individual calf's baseline and relative changes in behavior may indicate a calf at risk for disease.

Thus, moving forward, we suggest it is imperative that future research evaluates the appropriate baselines to use to identify behavioral changes in calves. Furthermore, consideration should be given towards if the precision technology can measure the targeted differences between sick and healthy calves. For example, producers were highly likely to ignore alarms for relative changes in transition cow behavior over time due to poor predictive abilities of true disease and these alarms were generated by rumination collars and activity pedometers (Eckelkamp and Bewley, 2021). This demonstrates a need to develop more complex statistical techniques to determine which combination of feeding behaviors and activity in calves results in a high accuracy and positive predicting ability of true health events so that alarms are meaningful for producers. Machine learning has been successful at identifying disease in cattle. For example, machine learning identified dairy herds at risk for high prevalence of zoonotic diseases (e.g., *Tuberculosis*; Stański et al., 2021), outbreak patterns of disease such as mastitis (Hyde et al., 2020) and relative changes in cow behavior at parturition which were indicative of transition cow diseases (Sahar et al., 2020). Thus, we suggest that precision technology devices in concert with machine learning techniques hold the potential for monitoring a combination of behaviors for changes that may identify calves at risk for developing BRD.

Especially promising indicators, based on our study findings, may be milk intake, starter intake, lying time, lying bouts, step counts, relative changes in unrewarded visits, and relative changes in step counts. At present, it appears that behavioral changes might only be detectable 4 days before diagnosis of BRD in preweaned calves, but technique refinements may reveal subtle behavioral changes earlier. Thus, we suggest future research should incorporate more complex statistical modeling (i.e. machine learning) to determine which combination of feeding behaviors, activity behaviors, and farm generated variables can accurately identify calves at risk of developing BRD.

Conclusions

Feeding behavior recorded by an automated feeder for calves offered 10 L/d was associated with BRD status in preweaned dairy calves. Milk intake, starter intake, and relative changes in unrewarded visits were the most robust feeding behaviors, with BRD calves drinking less milk, and having greater relative changes in unrewarded visits as early as 4 days before BRD diagnosis. Similarly, activity behaviors recorded by a pedometer also changed in calves later diagnosed with BRD. Specifically, BRD calves increased their lying time as early as 4 days prior to disease diagnosis and decreased their step counts, lying bouts, and activity index at 4 days prior to disease diagnosis. This study offers early evidence that behavioral changes measured by precision technology devices may be associated with calves at risk for a BRD bout. Future advancements in the use of precision dairy technology for calf management should focus on integrating multiple systems to improve disease detection, and to develop alarms which identify at-risk calves for producers.

Table 2.1 Bovine Respiratory Disease and feeding behavior over the pre-diagnosis period

The association of Bovine Respiratory Disease (BRD) status with the daily least square means of feeding behaviors recorded by an automated milk feeder including average drinking speed, milk intake and number of rewarded visits and activity recorded by a pedometer including lying time, step count, acceleration index[1], and lying bouts for day -5 to BRD diagnosis (d 0) for preweaned, pair matched calves (n=33 pairs[2]) offered 10 L of milk replacer/d. An interaction with treatment day and BRD status was explored.

ITEM	HEALTHY	BRD	SEM	*F*-VALUE DEGREES FREEDOM	BRD STATUS[3]	TREAT * DAY
Milk intake (L/d)	9.41	8.58	0.16	$13.11_{1,31}$	**0.001**	0.66
Drinking speed (L/min)	0.89	0.96	0.04	$1.05_{1,31}$	0.31	0.57
Rewarded visits (visits/d)	3.58	3.70	0.10	$0.84_{1,31}$	0.37	0.54
Lying time (h/d)	17.20	17.70	0.15	$5.07_{1,30}$	**0.03**	*0.08*
Lying bouts (bout/d	20.49	18.72	0.68	$3.43_{1,30}$	*0.07*	0.71
Step count (step/d)	655.39	424.63	25.95	$40.34_{1,30}$	**< 0.001**	0.76
Acceleration activity index[1]	3491.97	2452.99	134.48	$28.92_{1,30}$	**< 0.001**	0.70

1 The acceleration activity index was generated by the commercial software algorithm (IceRobotics, Scotland) based on a calf's daily average acceleration rate and step count.
2 All calves were pair matched to healthy calves by age at diagnosis, birthdate, and gender. Bovine Respiratory Disease was defined as a clinical score of at least 5 (McGuirk and Peek, 2014) and lobar lung consolidation as described in Buczinski et al., 2016.
3 **Significance** P <0.05 and *tendencies* are reported at $P \geq 0.05 < 0.10$.

Table 2.2 Bovine Respiratory Disease and relative changes in behavior over the pre-diagnosis period

The association of Bovine Respiratory Disease (BRD) status with least square means of relative changes (relative change) in feeding behaviors recorded by an automated feeder including average drinking speed, milk intake, starter intake, number of rewarded and unrewarded visits and relative changes in activity behaviors recorded by a pedometer including daily lying time, lying bouts, total step count and an acceleration activity index for -5 d to the day of BRD diagnosis (d 0) of preweaned, pair matched calves (n=32 pairs) offered 10 L of milk replacer/d. Relative changes referred to d -5 as a baseline. An interaction with treatment day and BRD status was explored.

ITEM	HEALTHY	BRD	SEM	*F*-VALUE DEGREES FREEDOM	BRD STATUS	TREAT DAY	*
Relative change milk intake %	113.06	127.90	13.10	$0.66_{1,30}$	0.42	*0.07*	
Relative change speed %	113.91	109.11	5.58	$0.38_{1,31}$	0.54	0.10	
Relative change rewarded visits %	115.86	126.38	0.09	$0.77_{1,31}$	0.39	0.14	
Relative change unrewarded visits %	105.77	99.46	1.55	$8.50_{1,31}$	**0.001**	**0.04**	
Relative change starter intake %	105.31	111.56	4.80	$0.86_{1,30}$	0.36	**0.05**	
Relative change lying time%	108.00	102.96	3.24	$1.22_{1,30}$	0.27	*0.06*	
Relative change lying bouts%	103.50	97.81	3.33	$1.45_{1,30}$	0.24	0.97	
Relative change step count %	145.20	116.60	12.96	$2.51_{1,30}$	0.12	0.40	
Relative change index[1]%	136.37	112.21	9.26	$3.51_{1,30}$	*0.07*	0.48	

Figure 2.1. a-c Association of Bovine Respiratory Disease status with feeding behaviors in preweaned calves

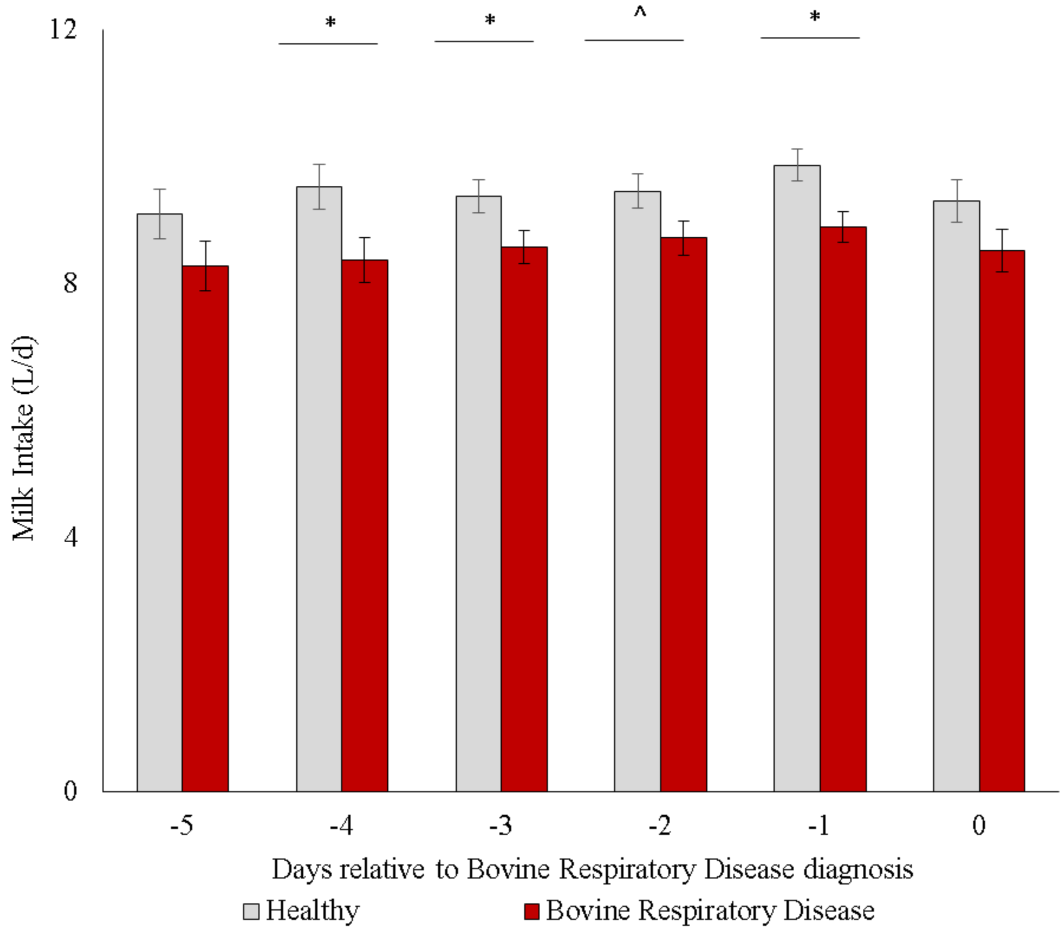
The association of Bovine Respiratory Disease (BRD) status[1] with milk intake (1a), drinking speed (1b), and rewarded visits (1c), (LSM ± SEM) by day for preweaned, pair matched calves (n=33 pairs[2]) offered 10 L of milk replacer/d by an automated feeder.

1Bovine respiratory disease was defined as a clinical score of ≥ 5 (McGuirk and Peek, 2014) and lobar lung consolidation ≥ 3 cm^2

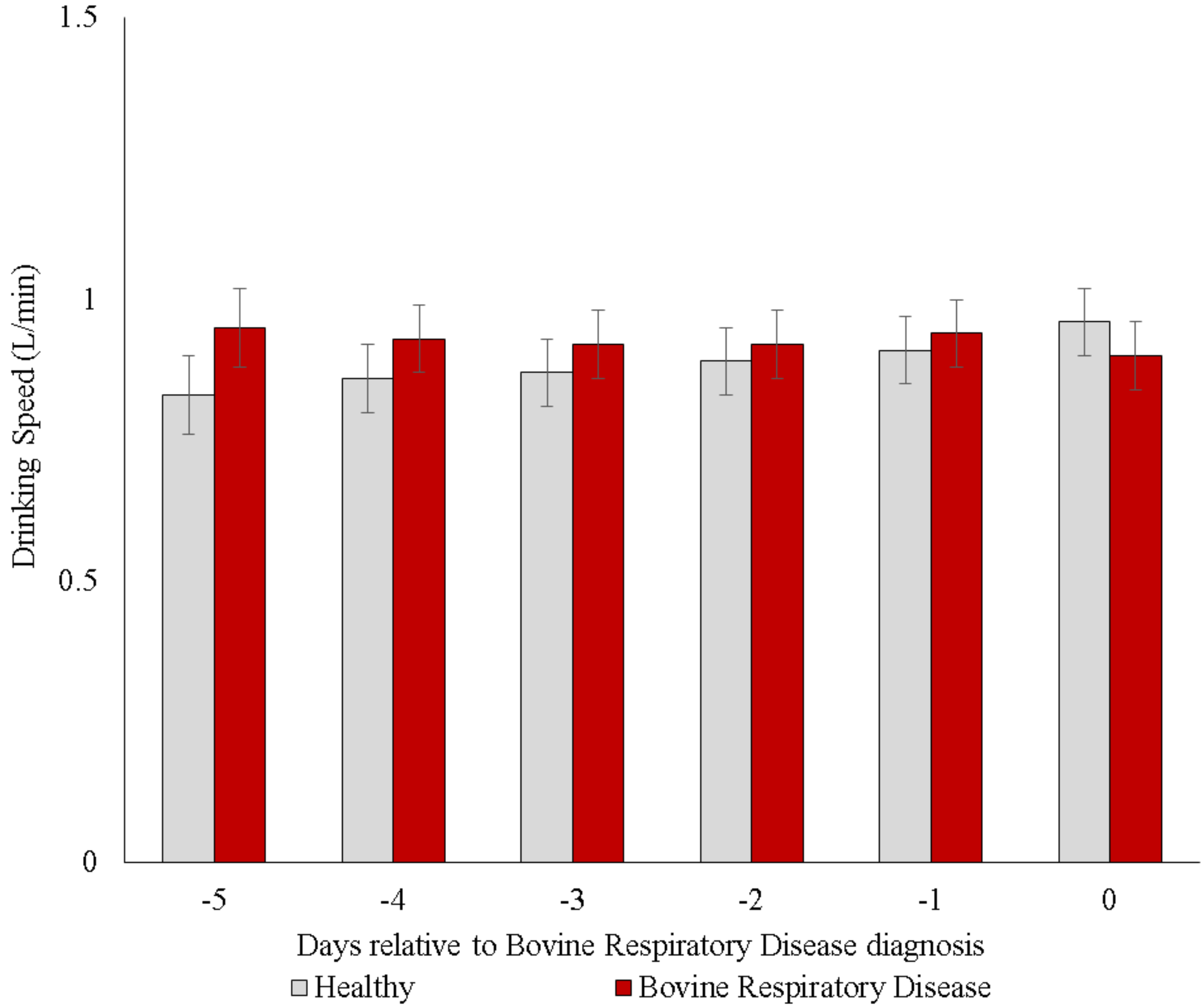
2Sick calves were matched to healthy calves as a pair by gender, age, and season as a fixed effect in a linear mixed model.

Significance*(*P* ≤ 0.05) and tendencies^ (*P* < 0.10) indicate differences by BRD status by day

a)


12
8
4
0
Milk Intake (L/d)
*
*
^
*
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

b)


1.5
1
0.5
0
Drinking Speed (L/min)
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

c)

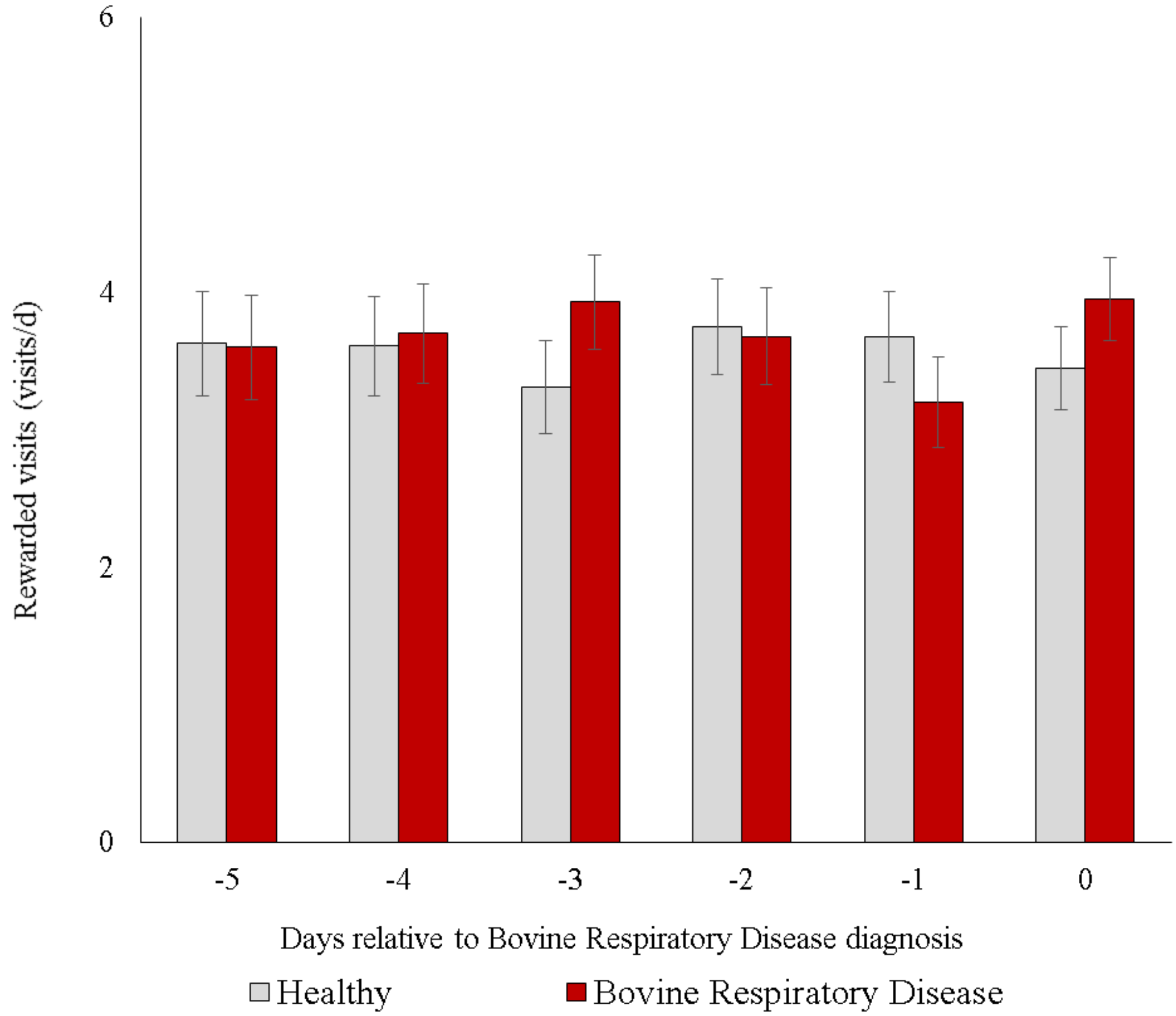

6
4
2
0
Rewarded visits (visits/d)
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

Figure 2.2. a-d Association of Bovine Respiratory Disease status with activity levels in preweaned calves

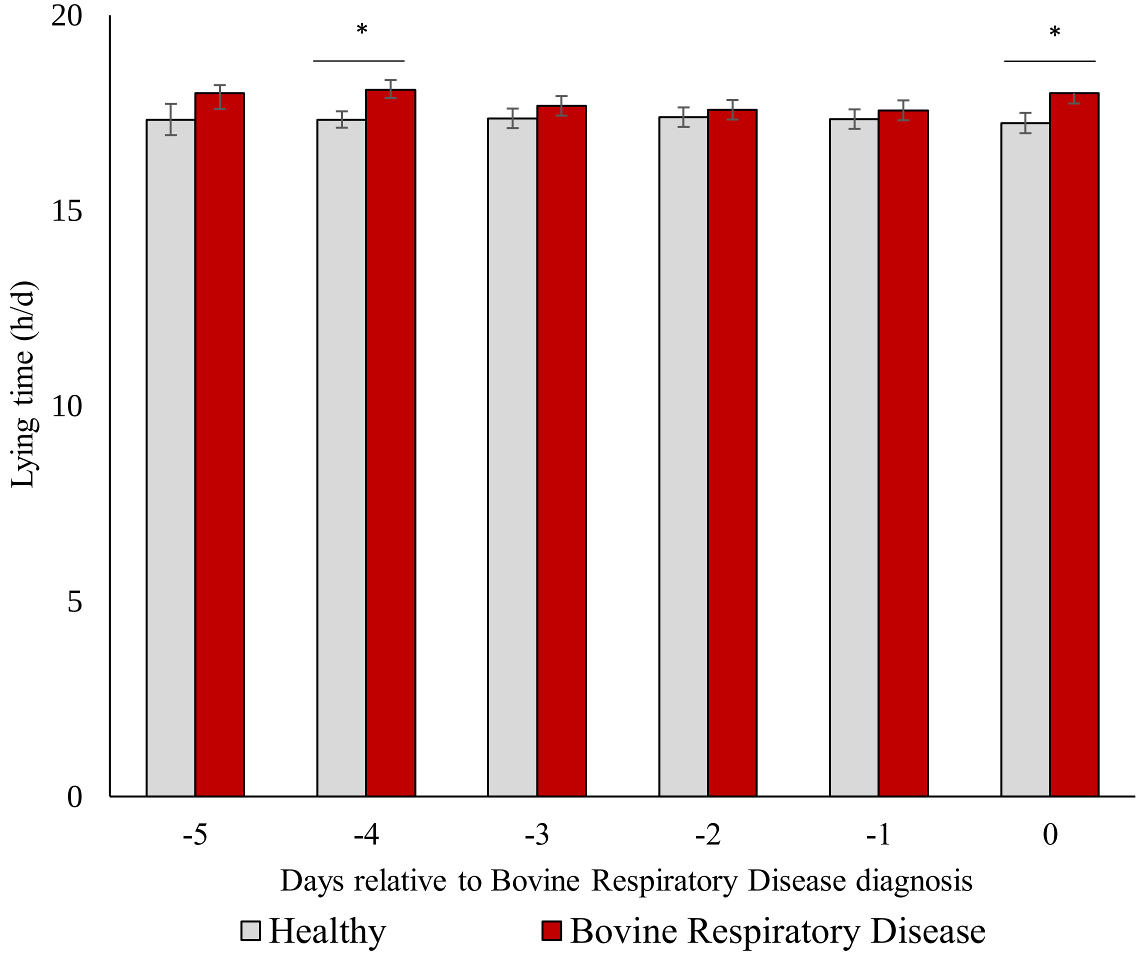
The association of Bovine Respiratory Disease (BRD) status[1] with lying time (2a), lying bouts (2b), step counts (2c), and the acceleration activity index[2] (2d) (LSM ± SEM) by day for preweaned, pair matched calves (n=32 pairs[3]) offered 10 L of milk replacer/d by an automated milk feeder.

1Bovine respiratory disease was defined as a clinical score of ≥ 5 (McGuirk and Peek, 2014) and lobar lung consolidation ≥ 3 cm^2

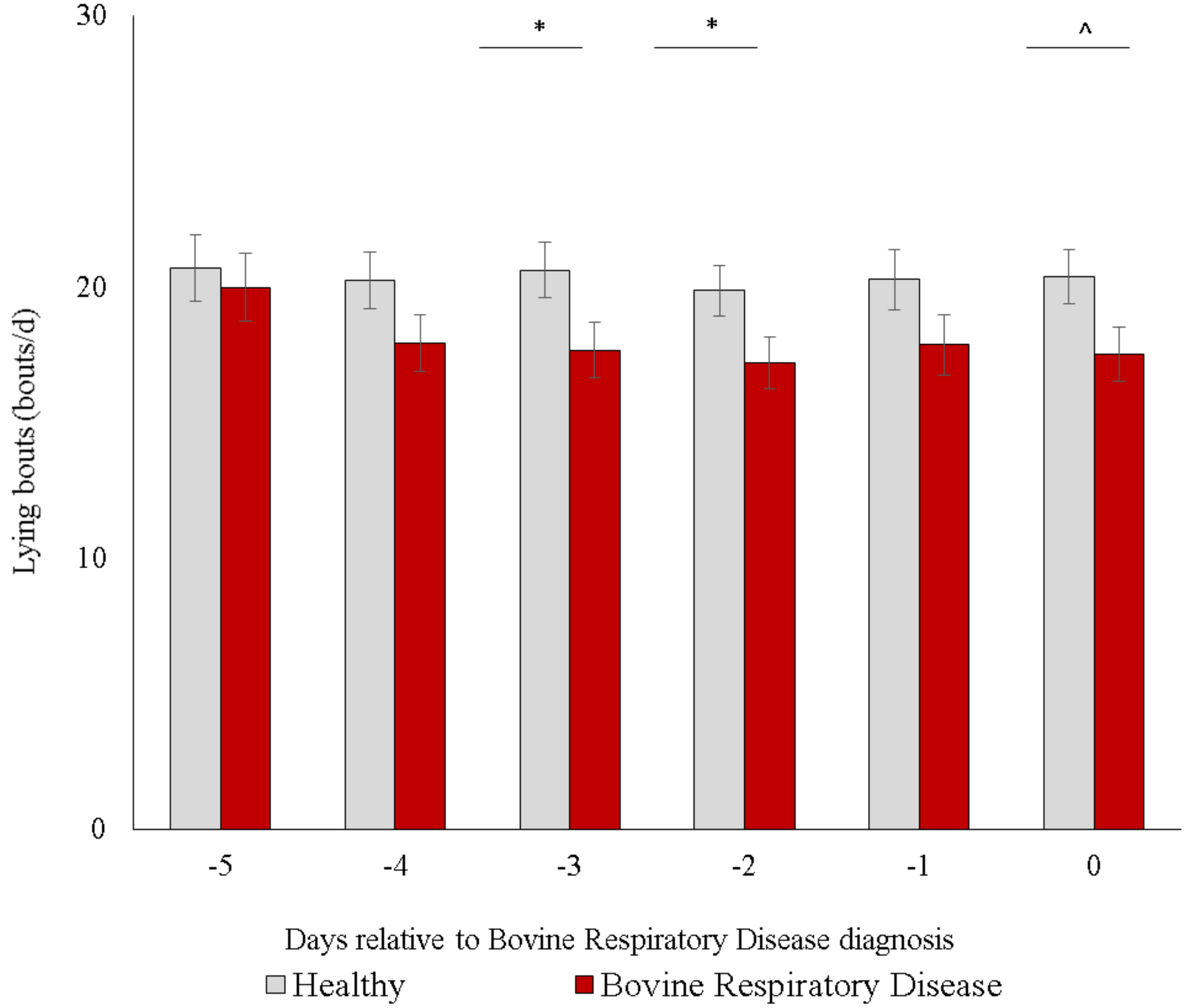
2The acceleration activity index was generated by a commercial algorithm (IceRobotics, Scotland) by recording the average daily rate of acceleration and step count

3 Sick calves were matched to healthy calves as a pair by gender, age, and season as a fixed effect in a linear mixed model. Significance*($P \leq 0.05$) and tendencies^ ($P < 0.10$) indicate differences by BRD status by day

a)

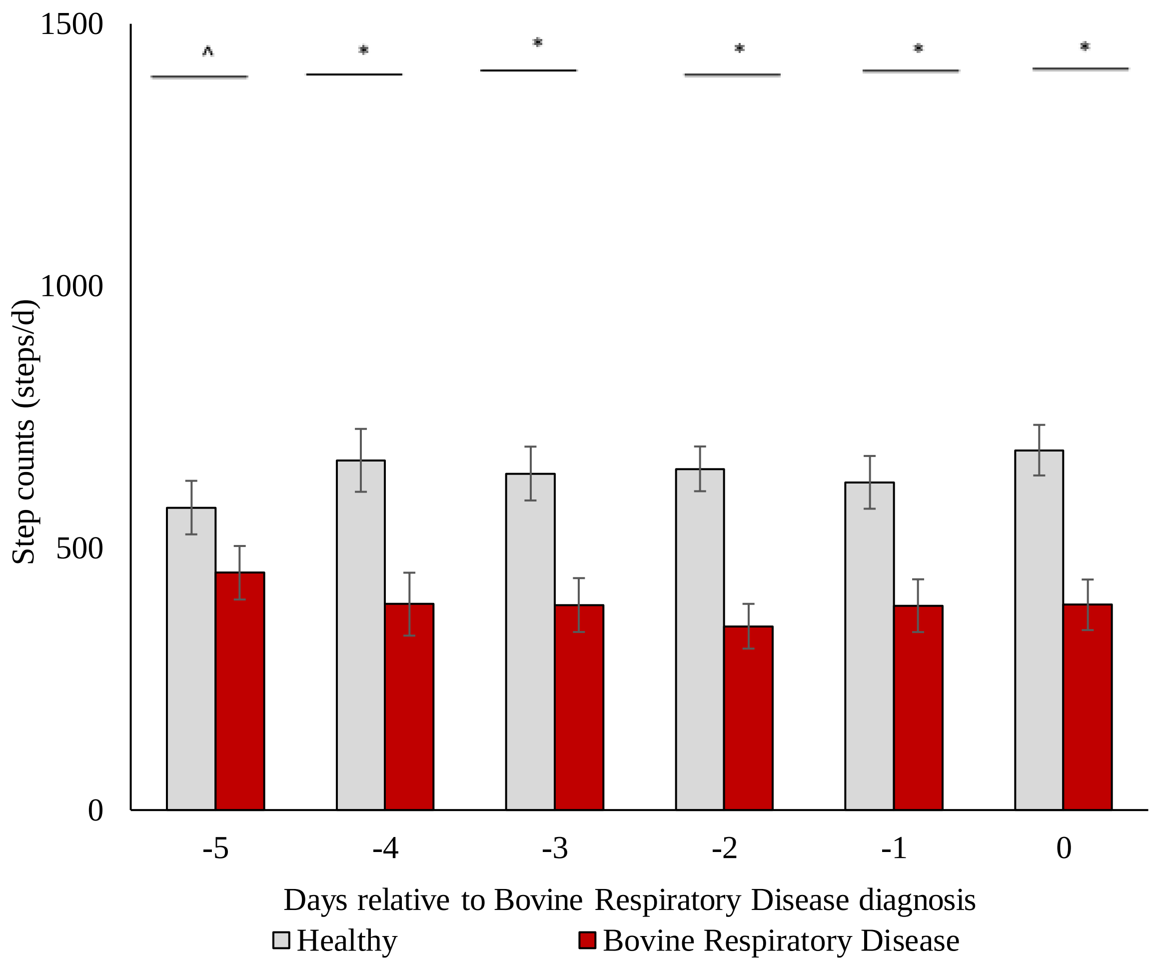

20
15
10
5
0
Lying time (h/d)
*
*
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

b)



30
20
10
0
Lying bouts (bouts/d)
*
*
^
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

c)



1500
1000
500
0
^
*
*
*
*
*
Step counts (steps/d)
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

d)

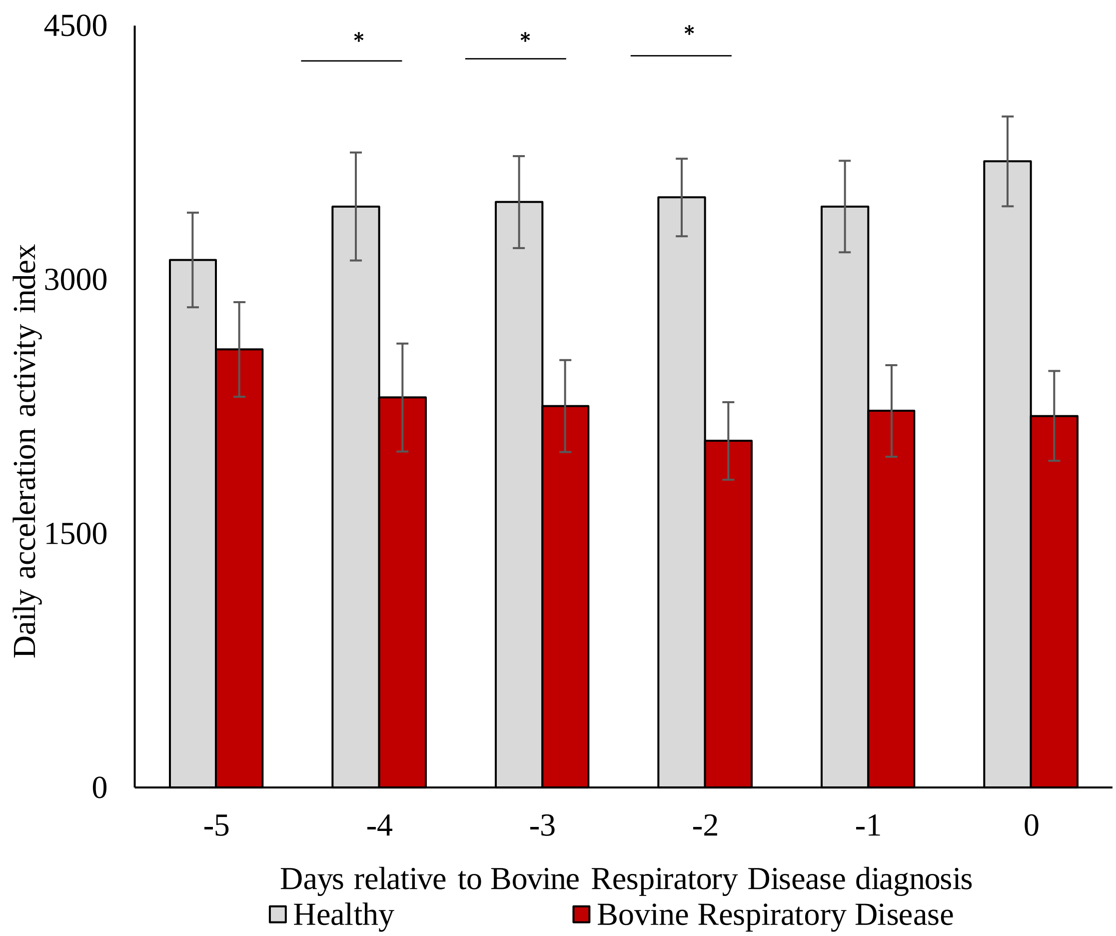

4500
3000
1500
0
Daily acceleration activity index
*
*
*
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

Figure 2.3. a-e Association of Bovine Respiratory Disease Status with relative changes in feeding behaviors in preweaned dairy calves

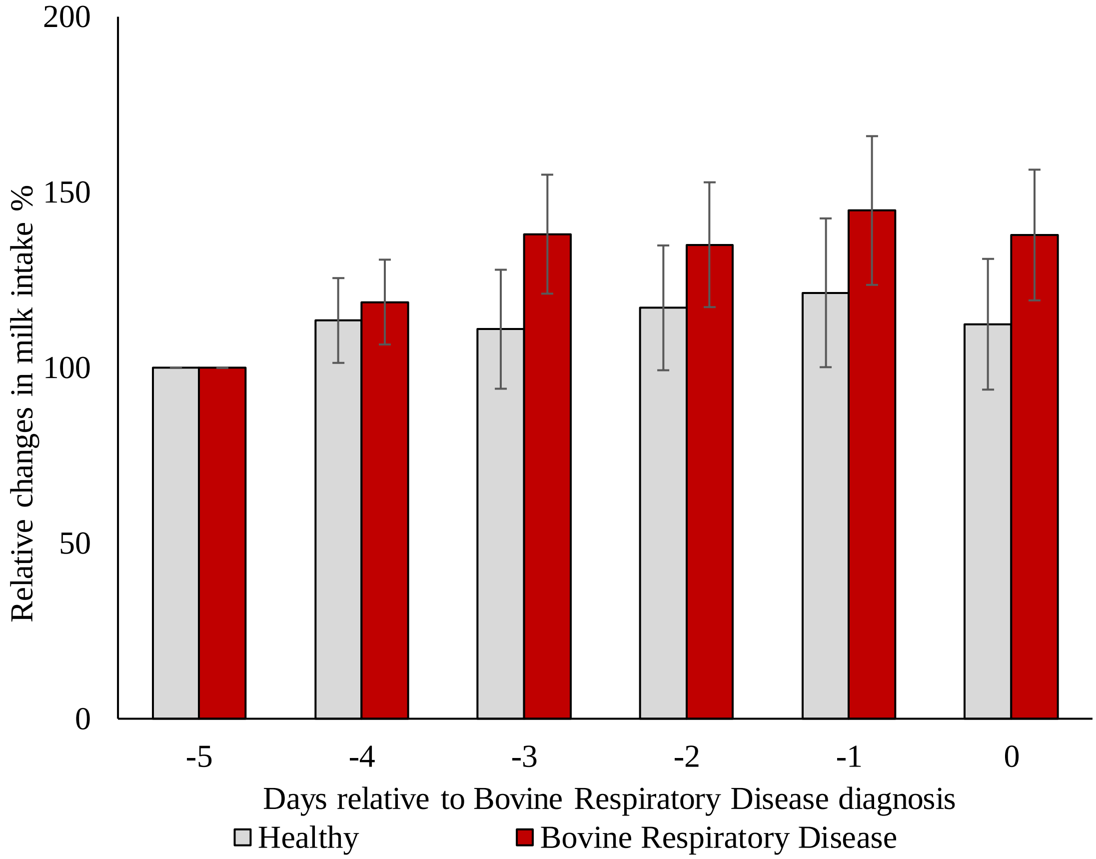
The association of Bovine Respiratory Disease (BRD) status[1] with relative changes in milk intake % (3a), relative changes in drinking speed % (3b), relative changes in rewarded visits % (3c), relative changes[2] in starter intake (3d), and relative changes in unrewarded visits (3e) (LSM ± SEM) by day for preweaned, pair matched calves (n=33 pairs[3]) offered 10 L of milk replacer/d, and starter, *ad libitum* by an automated feeder.

1Bovine respiratory disease was defined as a clinical score of ≥ 5 (McGuirk and Peek, 2014) and lobar lung consolidation ≥ 3 cm^2

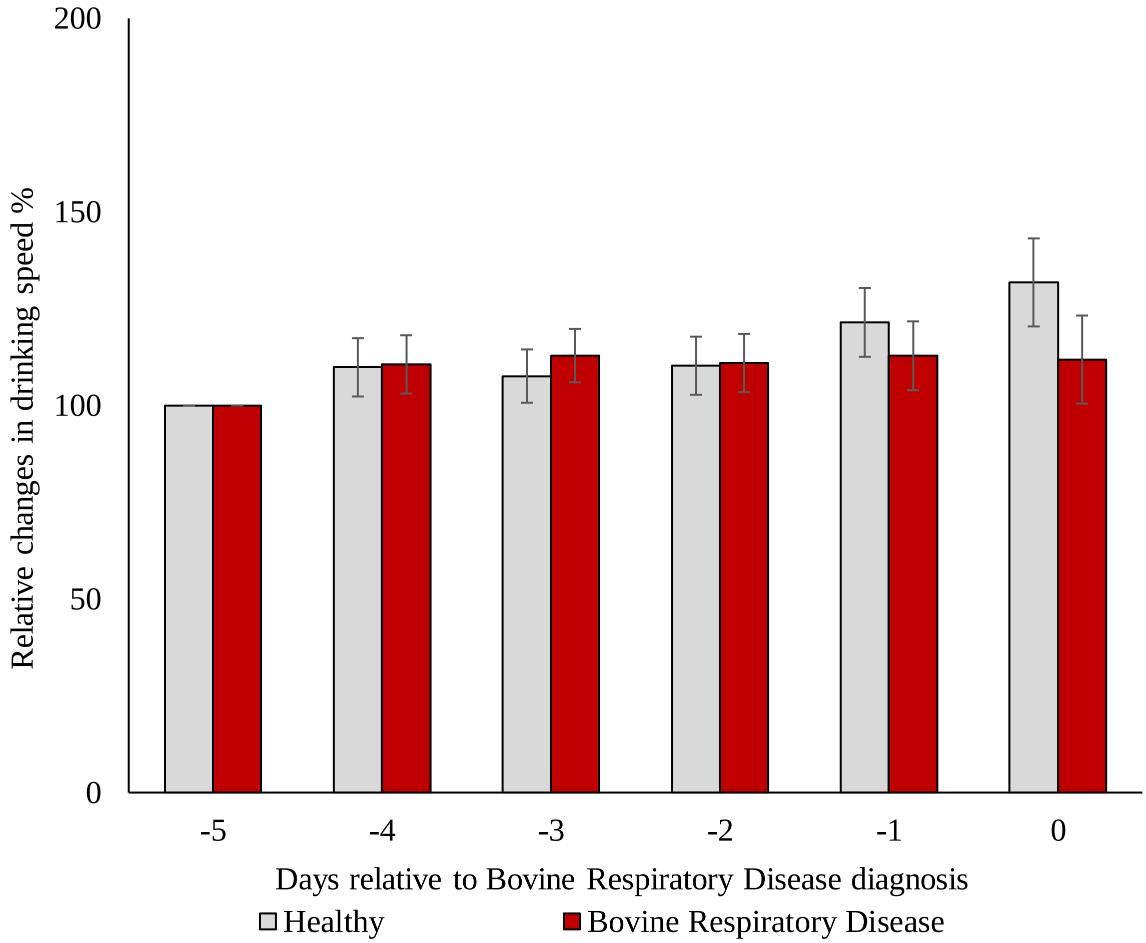
2Relative changes were calculated with day -5 as a baseline (100%); relative changes were generated by dividing each day before diagnosis (d -4 to d -1) and the day of diagnosis (d 0) by the baseline (day -5)

3Sick calves were matched to healthy calves as a pair by gender, age, and season as a fixed effect in a linear mixed model. Significance*($P \leq 0.05$) and tendency^ ($P < 0.10$) indicate differences by BRD status by day

a)

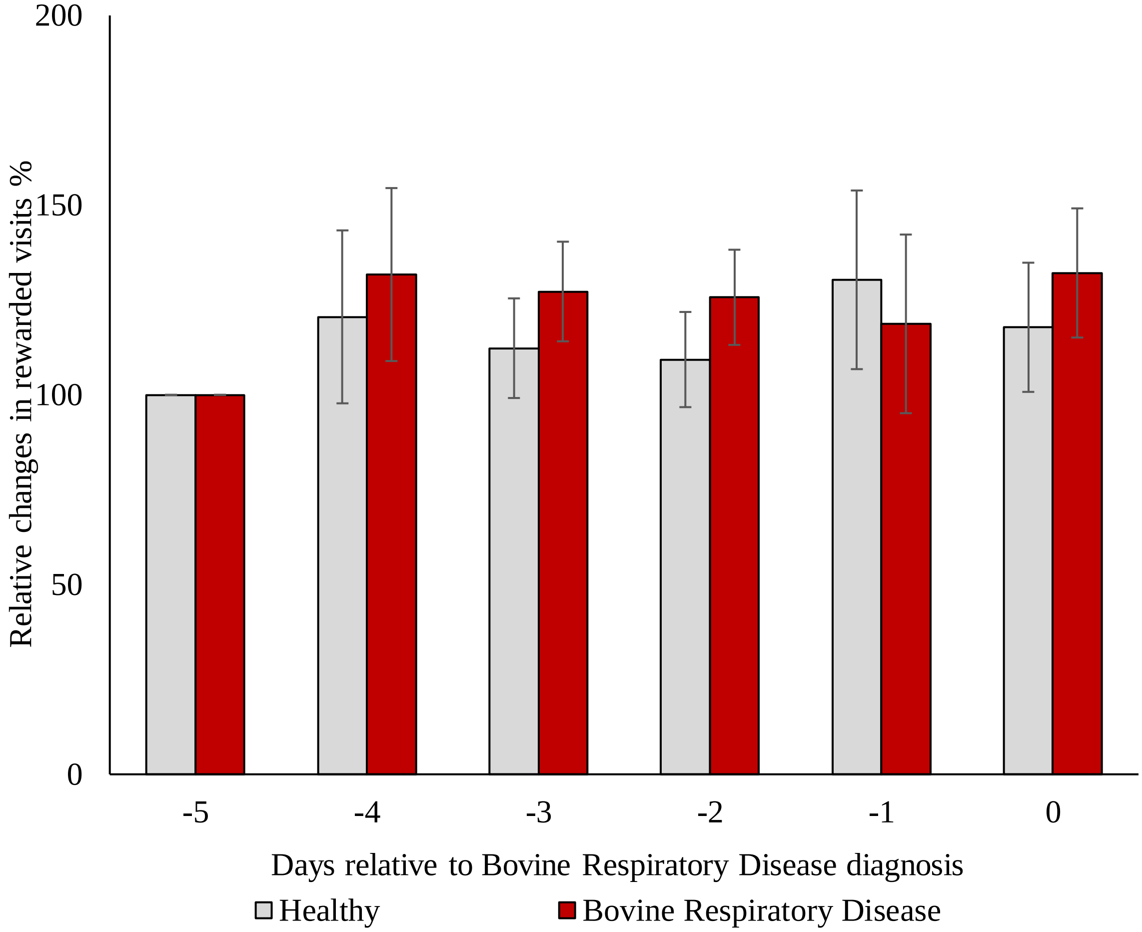

200
150
100
50
0
Relative changes in milk intake %
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

b)

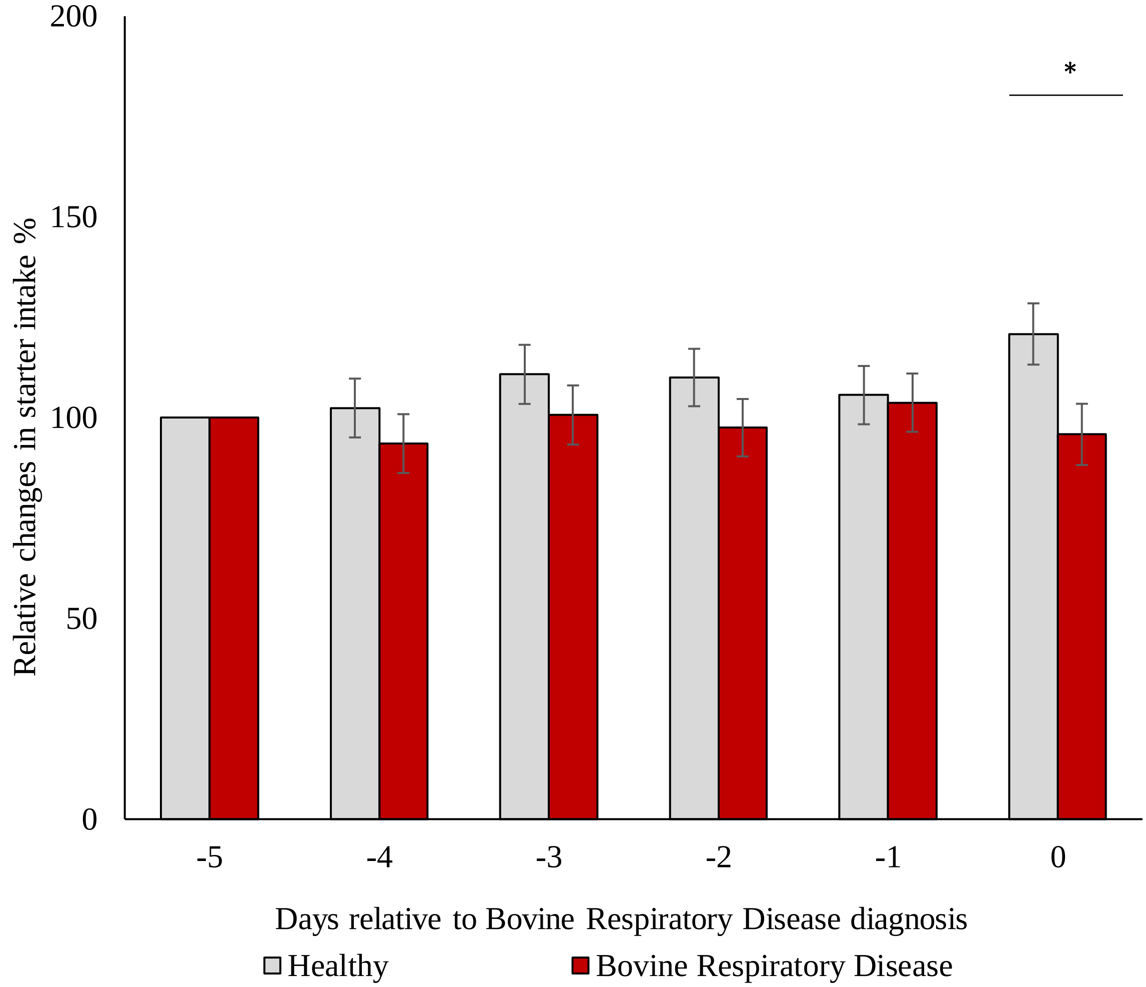


200
150
100
50
0
Relative changes in drinking speed %
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

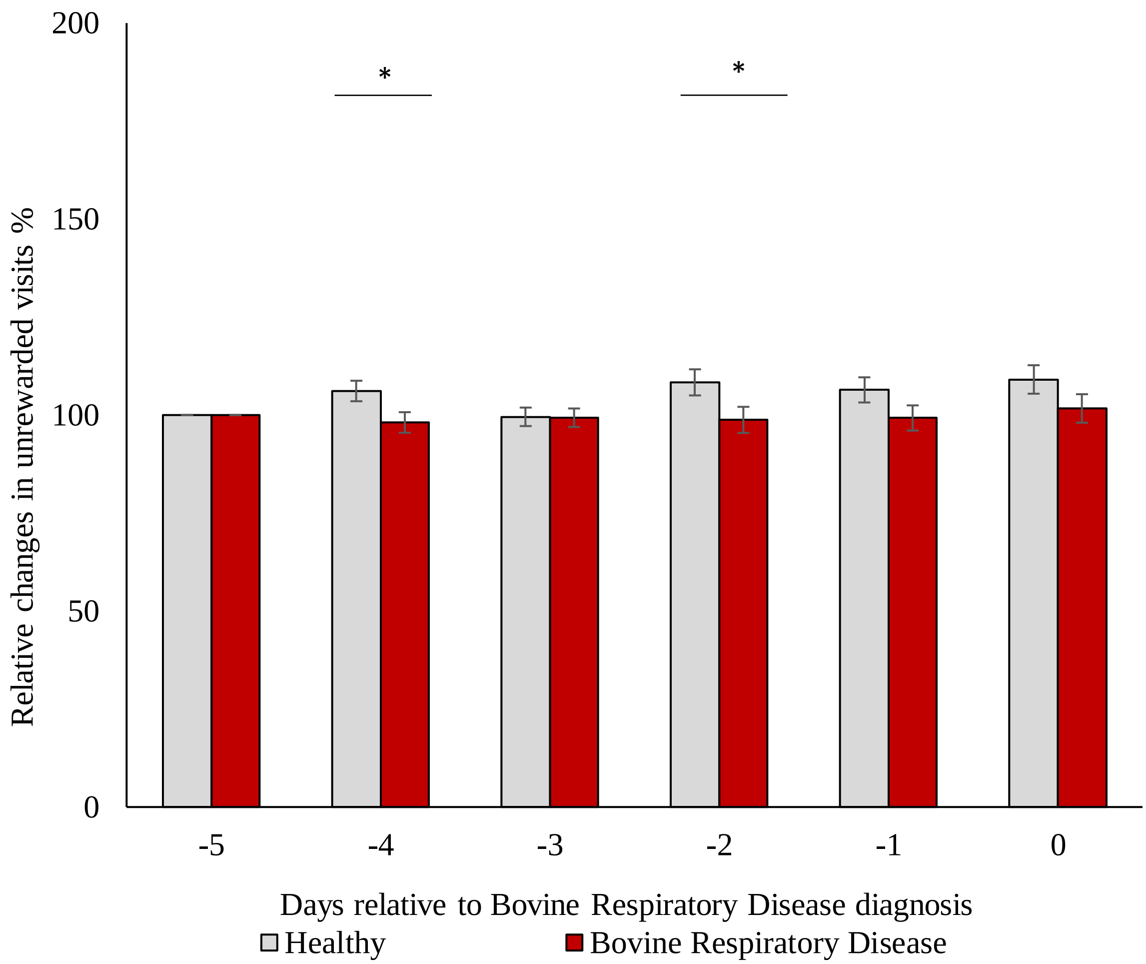
c)


Relative changes in rewarded visits %
200
150
100
50
0
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

d)


200
150
100
50
0
Relative changes in starter intake %
*
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

e)



200
150
100
50
0
Relative changes in unrewarded visits %
*
*
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

Figure 2.4. a-d Association of Bovine Respiratory Disease Status with relative changes in activity levels in preweaned calves

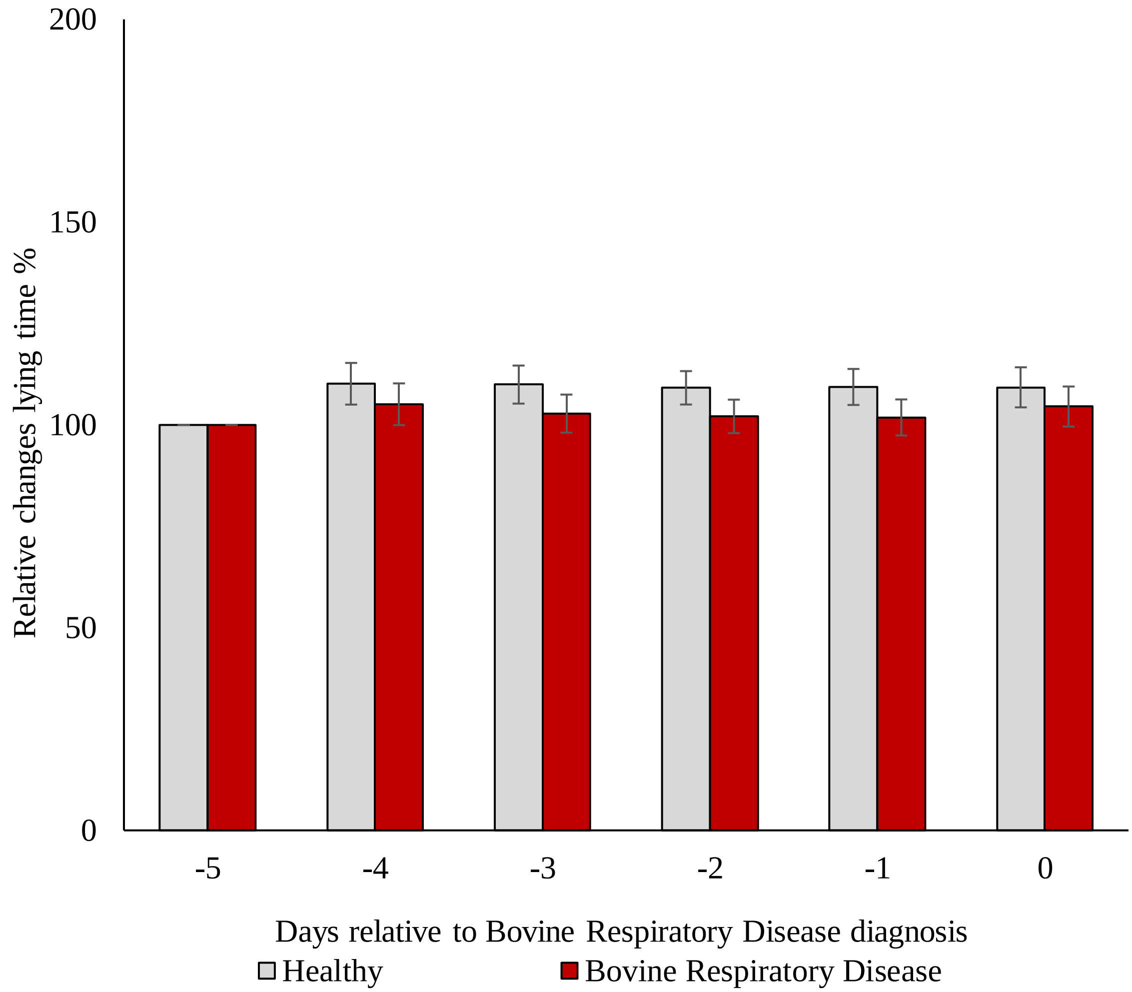
The association of Bovine Respiratory Disease (BRD) status with relative changes in lying time (4a), relative changes in lying bouts (4b), relative changes in step counts (4c), and relative changes in the acceleration activity index % (4d) (LSM ± SEM) by day for preweaned, pair matched calves (n=32 pairs) offered 10 L of milk replacer/d, and starter, *ad libitum* by an automated feeder.

[1]Bovine respiratory disease was defined as a clinical score of ≥ 5 (McGuirk and Peek, 2014) and lobar lung consolidation ≥ 3 cm^2

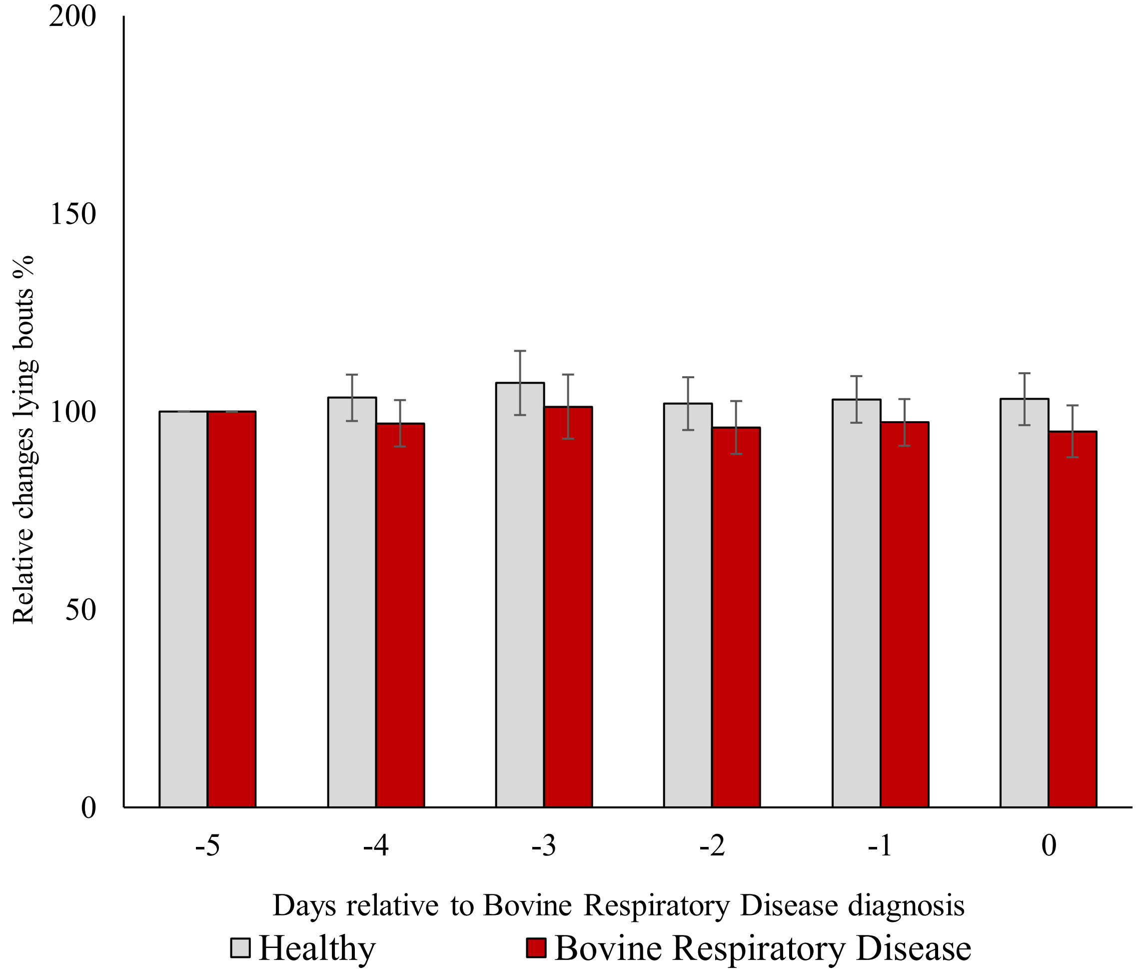
[2]Relative changes were calculated with day -5 as a baseline (100%); relative changes were generated by dividing each day before diagnosis (d -4 to d -1) and the day of diagnosis (d 0) by the baseline (day -5)

[3]Sick calves were matched to healthy calves as a pair by gender, age, and season as a fixed effect in a linear mixed model. Significance*($P \leq 0.05$) and tendency^ ($P < 0.10$) indicate differences by BRD status by day

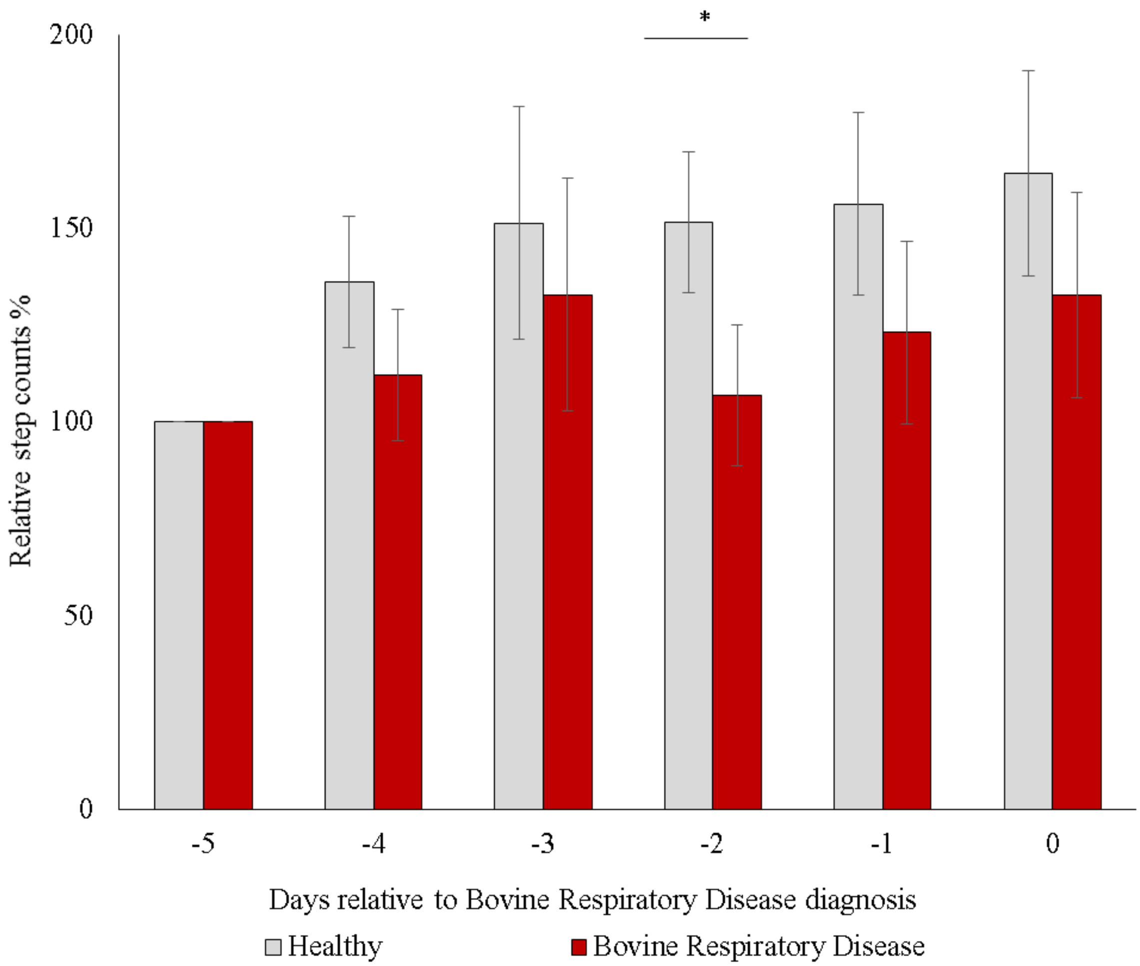
a)


200
150
100
50
0
Relative changes lying time %
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

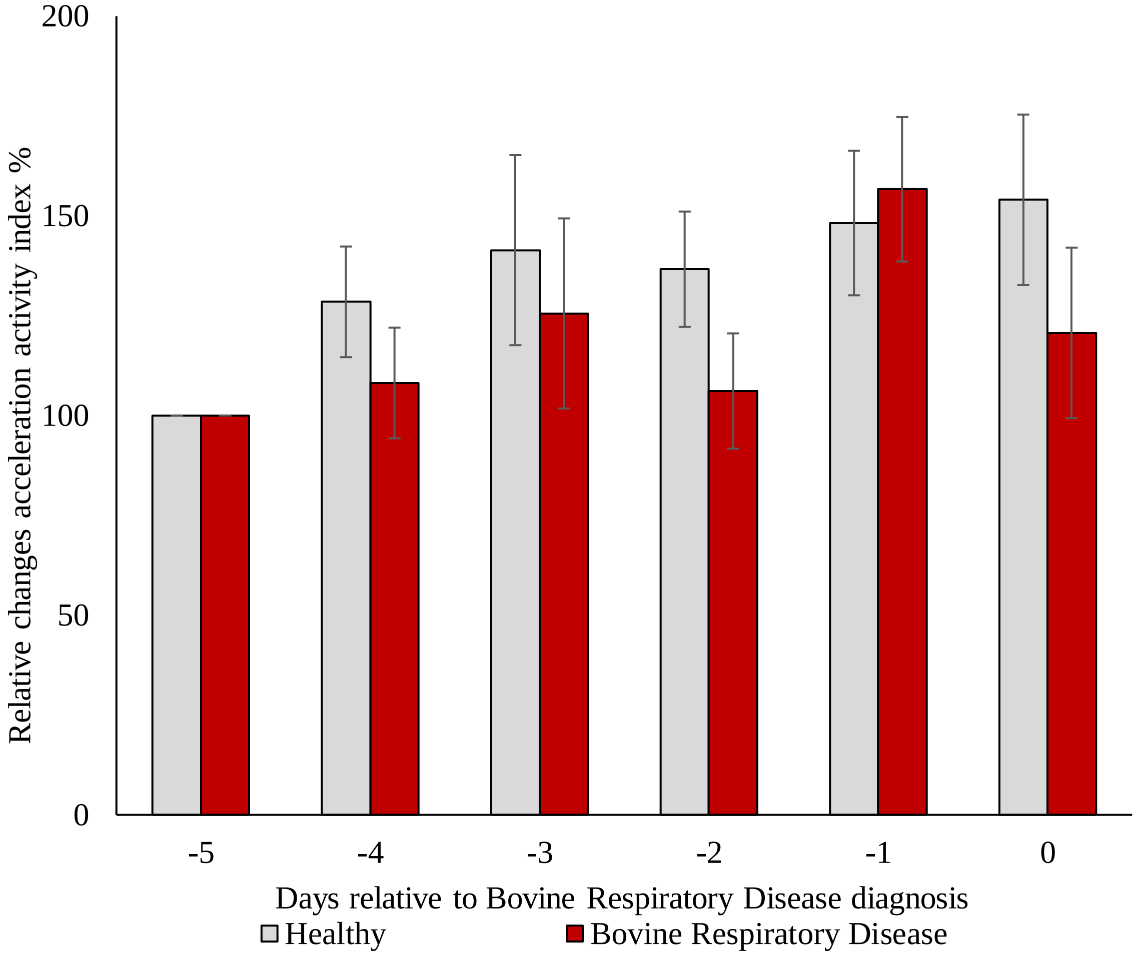
b)


200
150
100
50
0
Relative changes lying bouts %
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

c)


*
200
150
100
50
0
Relative step counts %
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

d)


200
150
100
50
0
Relative changes acceleration activity index %
-5
-4
-3
-2
-1
0
Days relative to Bovine Respiratory Disease diagnosis
Healthy
Bovine Respiratory Disease

CHAPTER 3. NUTRACEUTICAL INTERVENTION WITH COLOSTRUM REPLACER: CAN WE REDUCE DISEASE HAZARD, AMELIORATE DISEASE SEVERITY, AND IMPROVE PERFORMANCE IN PREWEANED DAIRY CALVES?

Introduction

Bovine Respiratory Disease (**BRD**) and diarrhea are significant causes of morbidity in dairy calf operations and disease mitigation may increase farm efficiency. According to a national producer survey, BRD and diarrhea affected 33% of preweaned dairy calves, with a high majority of affected calves receiving antibiotics (BRD 95% and diarrhea 76%; USDA 2018). It is essential to mitigate disease as the preweaning period is the most economically intensive for heifer raising operations (Hawkins et al., 2019). For example, BRD was estimated to cost $42 per calf (Dubrovsky et al., 2020) and treatment for diarrhea was estimated to cost a median of $56 per calf (Goodell et al., 2012). Furthermore, in the short term BRD (i.e. lung consolidation on ultrasound) was associated with less preweaning average daily gain (Cramer and Ollivett, 2019). In the long-term, BRD at weaning was associated with less milk production during the first lactation (Dunn et al., 2018), and a reduced hazard of pregnancy, coupled with a reduced hazard of producing milk in the first lactation (Teixeira et al., 2017). Similarly, diarrhea identified preweaning was associated with a reduced hazard of pregnancy (Aghakeshmiri et al., 2017). Therefore, it is of interest to mitigate incidence of BRD and diarrhea. Moreover, lowering the duration of disease will improve calf welfare since disease is metabolically costly and potentially painful for calves.

The dairy industry is under pressure to lower reliance on antibiotics, particularly for sub-therapeutic use as reviewed by Placzek et al., 2020; thus, alternative therapies should be considered for mitigating disease risk in calves. One potential alternative therapy is the use of nutraceuticals, which are nutritional interventions thought to have positive effects on physiological status and health outcomes (Nasri et al., 2014). For example, colostrum may be a nutraceutical intervention for calves as it has a high immunoglobulin and lactoferrin content (Francesco et al., 2016), and has anti-inflammatory properties (Lee et al., 2019). Interestingly, first-milking bovine colostrum has been used as a preventative nutraceutical to ameliorate disease in human medicine (Alexieva et al., 2011, Bagwe et al., 2015). Similar immune improvement has been observed in parallel in other species: immunocompromised mice (Menchetti et al., 2020), weaned piglets (Boudry et al., 2007), and dogs vaccinated for distemper (Satyaraj et al., 2013). Moreover, 100 young Thoroughbred horses fed a colostrum nutraceutical (50 g/d) for 4 months ameliorated disease duration (2 weeks) versus soy flour controls (4 weeks: Fenger et al., 2016). Thus, bovine colostrum as a nutraceutical may improve immune status in other species. To date, there is no work that has investigated the efficacy of colostrum nutraceuticals as an intervention strategy for dairy calves at risk for disease. However, there is literature which suggests feeding colostrum to healthy dairy calves as a longer-term preventative feeding strategy may improve calf health outcomes.

There is preliminary evidence that healthy dairy calves benefit from the preventative feeding, or extended feeding, of colostrum as a nutraceutical. For example, calves fed colostrum replacer (150 g/d) during the first 14 d of life had a reduced likelihood of abnormal feces, reduced likelihood of abnormal respiration, and a reduced likelihood of receiving antibiotics (Chamorro et al., 2017). Different results were observed at a lower dosage, where 30 calves receiving a colostrum nutraceutical at 70 g/d had a lower likelihood of diarrhea, and a decreased likelihood of receiving antibiotics, but the likelihood of developing BRD were not different (Berge et al., 2009a). Moreover, a recent study observed that partially replacing the milk diet with different levels of colostrum at up to 700 g/d reduced the likelihood of diarrhea, improved performance, and tended to reduce the risk of Bovine Respiratory Disease (Kargar et al., 2020). Similarly, there is evidence that extended colostrum feeding for the first 21 days of life decreased BRD morbidity for the first year of life and decreased diarrhea risk, while milk production was higher for extended colostrum-fed calves during the first lactation (Armengol and Fraile, 2020). Hence, there is the potential for colostrum to ameliorate disease in dairy calves when fed as a preventative therapy. However, it is unknown if colostrum can be used as an intervention feeding strategy to mitigate disease risk in calves.

One limitation to feeding a colostrum nutraceutical across an extended period is cost. Therefore, it is of interest to determine if an intervention provided to a calf during early signs of disease development can reduce disease likelihood and ameliorate disease bouts. Automated milk feeding systems which measure and record feeding behavior of dairy calves are able to detect deviations in feeding behavior that have been associated with disease development (Knauer et al., 2017; Sutherland et al., 2018). Therefore, the objective of this study was to determine if intervention with one dose of a colostrum replacer nutraceutical fed across three days (125 g/d in one L by bottle) versus a control milk replacer (125 g/d in one L by bottle), offered to calves that triggered an automated feeder alarm detected by the default automated feeder algorithm could ameliorate disease bouts (BRD and diarrhea), reduce the likelihood of disease, and improve performance (ADG) in calves offered intermediate levels of milk (10 L/d) for 50 days. We chose deviations in feeding behavior as an intervention point since an epidemiological study found an association between negative deviations in feeding behavior up to 4 days prior to disease in calves fed by automated feeders (Knauer et al., 2017). We hypothesized that colostrum replacer would ameliorate disease bouts (BRD and diarrhea), and reduce the likelihood of disease in calves, but would not impact performance.

Materials and Methods

This study was conducted at the University of Kentucky Coldstream Research Dairy Farm in Lexington, KY from July 2018 to August 2019. This facility was selected for the availability of dairy calves with a high prevalence of passive transfer in the herd (90%), and due to the ability to blind farm staff to treatment assignments for this study. A total of 120 calves were selected for this trial, of which 84 calves (42 placebo; 42 colostrum replacer) met the following enrollment criteria: removed from dam within 6 h and fed maternal colostrum, had a serum BRIX of 8.4% at 48 h of life as a passive transfer threshold (Deelen et al., 2014), were not a twin, and triggered an alarm on the automated milk feeder between feeder d 14 to 50. All calves enrolled were part of the Institutional Animal Care and Use Committee approval number 2018: 2864.

Management and feeding

Calves were randomly assigned (www.random.org) by due date in groups of 10 to receive an intervention with either a daily dose (125g) of 50 g IgG colostrum replacer fed for 3 days (**CR**; Premolac Plus, Zinpro, MN; % DM basis minimum 70% CP, and maximum 8% fat, 0.2% crude fiber, 10% ash, and contained 4.65 Mcal of ME/kg) or a daily dose (125 g) of placebo milk replacer (Milk replacer on a percentage DM basis was a minimum 28% CP, 20% fat, a maximum 15% crude fiber, a minimum 1.0% calcium and 0.70% phosphorus, and contained 4.87 Mcal of ME/kg, Cows Match, Land O Lakes, Shoreview, MN). The treatment dose was fed at 11:00 by bottle to calves that received an alarm that morning detected by an automated milk feeder (**AMF**; Forster-Technik, Engen, Germany); the two researchers were not blind to treatment as the bottles needed to be prepared across 3 d (1L 125g/d). Similarly, the calves received the placebo dose milk replacer by bottle for 3 d (1L 125g/d).

No study has investigated the ability of a colostrum intervention to reduce BRD incidence in calves. Thus, we powered this study based on true herd BRD incidence (60%) calculated prior to the study with a 10% reduction in disease; we considered 10% to be the minimum disease reduction required for colostrum to be considered as a potential nutraceutical by producers. A power analysis showed that a total of 84 calves (n = 42 per treatment) was required at 80% power to detect a 10% BRD incidence difference, with half-widths at 0.05, and true herd BRD incidence at 60%. Our power analysis for ADG showed that a total of 62 calves (n=31 per treatment) were required for calves offered 10 L/d in an AMF at an average ADG 0.67 kg/d, with a variation of 0.07 kg/d as was observed in the literature (Rosenberger et al., 2017). The power analysis for ADG projected a targeted difference of 0.05 kg/d between treatments in ADG for feeding CR at 80% power, and half-widths at 0.05. The true herd incidence for BRD was calculated by one trained researcher health scoring all newborn calves from birth until 2 weeks post-weaning twice weekly for 9 months using the UW Calf Health Scoring System to define a new BRD case (48/80 BRD calves(McGuirk and Peek, 2014) prior to this study. The AMF algorithm that was used was the default manufacturer alarm programmed into the automated feeder. The AMF algorithm generated a baseline feeding behavior average for milk intake, and for drinking speed, based on the rolling 12 d average for each calf. The AMF triggered an alarm when a calf deviated in milk intake (20% reduction) or drinking speed (30% reduction) from this rolling baseline average. Calves could trigger an AMF alarm from feeder d 14 to d 50.

Calves were removed from the dam within 6 hours after birth and placed in individual pens (3 x 3 m) bedded with sawdust and were weighed using an electronic scale (Brecknell PS1000, Avery Weigh-Tronix LLC, Fairmont, MN). Calves weighed 40.08 ± 5.46 kg (mean ± SD) at birth. Calves were fed 6 L/d of 840 g milk replacer (Cow's Match; Land O' Lakes Animal Milk Products Co., Shoreview, MN) by bottle divided into two feedings when housed individually. Calves were enrolled on the AMF when a strong suckle reflex was present at an average age of 4.0 ± 2.0 d and placed in a group pen (4.57 × 10.67 m) containing 6 ± 3 other calves and bedded with sawdust shavings. Pens were cleaned, emptied, and bedding was removed and sanitized every two weeks. To ensure calves in a pen were of similar size and age, calves were moved to an identical adjacent pen with the same stocking density using dynamic flow at an average age of 45.0 ± 3.0 d before weaning.

Calves were fed the first meal with human assistance on the AMF and received human assistance every 12 h (maximum learning duration 4 feedings) until independent milk consumption was observed. Calves were allotted up to 10 L (140 g/L) milk replacer/d from the AMF (Cow's Match; Land O' Lakes Animal Milk Products Co., Shoreview, MN) for 50 days, reduced to 50% allotment for 14 days, and then reduced to

20% allotment for an additional 7 days until weaning at 70 days. Calves were followed for 14 d post-weaning.

The automated starter feeder contained a 22% CP starter (Calf Starter; Baghdad Feeds, Shelby, KY). Calves were also offered chopped alfalfa in a trough (1.83 × 0.33 × 0.16 m). Both the AMF and the starter feeder were calibrated weekly according to manufacturer instructions. All calves had ad libitum access to water, starter, and chopped alfalfa hay during the study period.

Health exams

Calves were scored daily beginning at birth to 2 weeks post-weaning at 88 ± 2.0 d of age by 1 of 3 observers (inter-observer agreement $\kappa > 0.90$) for BRD using the UW Calf Health Chart (McGuirk and Peek, 2014) which assigns and sums the nasal discharge score, eye discharge score, ear tilt score, cough score, and temperature score. The inter-observer agreement for health scoring was measured using Fleiss' kappa every 4 months throughout the study by having all observers go to a commercial facility on the same d to score 40 calves where the health status of the calves was unknown to ensure unbiased agreement. Similarly, 12 calves enrolled on a separate study and kept in a separate pen raised at this facility (Woodrum Setser et al., 2020) were evaluated for inter-observer agreement twice. Moreover, these 12 calves were scored using lung ultrasonography by all 3 observers on one morning, then euthanized in the afternoon for another study; lungs were retrieved and confirmed that the 3 observers diagnosed lung lobe status accurately.

Calves were rectally stimulated daily for fecal fluidity assessment, where a score of 0 (solid no spread), 1 (solid spreads), 2 (semi-solid sits on bedding) were considered normal, whereas a score of 3 (fluid and sifts through bedding) indicated diarrhea (Renaud et al. 2020). Weights were collected twice weekly using the same scale used to weigh calves at birth. Lung ultrasounds were collected twice weekly using 70% isopropyl alcohol as a transducing agent and by evaluating 5 lung lobes in each calf with 1 of 2 observers (inter-observer agreement Cohens' kappa; $\kappa = 0.90$) with a portable linear rectal ultrasound (Buczinski et al., 2018). In brief the ultrasound was set to a depth of 9 cm, frequency of 6.2 MHz, and gain of 23 dB (near 13 dB; far 36 dB; Ibex Pro, E.I. Medical, Loveland, CO). A BRD score ≥ 5 and lobar consolidation in any one lung lobe ≥ 3.0 cm^2 was considered a BRD bout, as described in (Buczinski et al., 2018). All calves with a positive BRD bout received enrofloxacin subcutaneously with dosage calculated by body weight (Baytril, Bayer, Leverkusen, Germany; 1 cc/15 kg) according to the herd veterinarian protocol. Calves who failed to respond to BRD bout antibiotic treatment were not re-treated until 14 d after the first treatment. Failure-to-respond to antibiotic calves were classified as a new BRD bout on d 15 if calves had an average BRD score ≥ 5 from d 10 to 14 after antibiotic treatment and at least one lung lobe consolidation at ≥ 3.0 cm^2. Failure-to-respond calves were treated with tulathromycin (Draxxin, Zoetis Animal Health, USA; 1 cc/45 kg, once at diagnosis, subcutaneously). Every health exam also included palpation of the umbilical site and was graded as a score of zero (closed), 1 (open, normal), or 2 (swelling, clouded discharge infection) according to (Cantor et al., 2019b). Only one calf presented with an umbilical infection and was eliminated from this study and not enrolled.

Statistical analysis

All statistical methods were carried out in SAS version 9.4 (SAS Institute, Cary, NC, USA). Initially, descriptive statistics and residual diagnostics assessed the relationship with all outcomes reported at the univariable level, using a cutoff of $P < 0.20$ for inclusion in the model. Normality was confirmed by visual assessment of residuals in the model using quartile plots.

T-tests (Proc Ttest) were used to confirm there was no difference between treatments for health status, weight, and feeding intake (milk and starter) on the day prior to triggering the alarm, and differences in feed intake in the week after the alarm were also assessed.

Efficacy of colostrum replacer on ameliorating disease

The effect of CR to ameliorate a BRD bout was calculated with a logistic model (Proc Logistic) utilizing day for the 7 d prior to and after an alarm as fixed effects, with milk intake as a covariate. The effect of CR to ameliorate a diarrhea bout was calculated with a logistic model (Proc Logistic) utilizing day for the 7 d prior to and after an alarm as fixed effects, with milk intake as a covariate.

Effect of colostrum replacer on survival from a disease bout

A survival analysis using Breslow methods (Proc Lifetest) was performed to determine if treatment intervention affected the hazard of being positive for a BRD bout in the 14-d following treatment. Calves were classified as healthy at 15 d if they were never diagnosed with a BRD bout. A survival analysis using Breslow methods was also performed to determine if treatment intervention affected the hazard of being positive for a diarrhea bout in the 14-d following treatment. Calves were classified healthy at 15 d if they were never diagnosed with a diarrhea bout.

Effect of colostrum replacer on likelihood of a disease bout

A Cox-Proportional Hazards model (Proc Hazard) evaluated the effect of CR on the hazard of a BRD bout using the Nelson-Aalen Cumulative Hazard method. Nelsen-Aalen cumulative hazards were used since the probability of survival from a disease bout generated in these calves was for a short duration of 14 days. A Cox-Proportional Hazards model also evaluated the effect of CR on the hazard of a diarrhea bout with sex as a fixed effect using the Nelson-Aalen Cumulative Hazard method. In the Cox proportional hazards model, proportionality assumption was tested graphically using log-log plots. No outliers were found in any of the models.

Effect of colostrum replacer on improving performance

The effect of CR on the ADG during the 7 d after the intervention was determined using a mixed linear model (Proc Mixed) with dam parity, feed intake, and BRD status as covariates, repeated by day, calf as a subject, and birth date as a random effect. Sex was evaluated for its association with ADG, but it was not significant and was removed from the model. The effect of CR on ADG for the 2 weeks post weaning was also evaluated with a mixed linear model, with BRD status and starter intake as covariates, repeated by day and calf. To ensure outliers did not have leverage, standardized residuals were plotted against predicted outcomes to assess for model leverage, though no extreme outliers were identified.

Results

Descriptive Statistics

A total of 120 calves were followed, but 2 calves did not meet enrollment criteria (failed transfer of passive immunity), 1 calf had an umbilical infection prior to enrollment, and 27 calves never triggered an alarm from day 14 to 50. There were 6 calves (3 placebo and 3 CR) which refused the bottle on one of the three days of intervention, and thus, these 6 calves were not enrolled in the analysis due to failure to complete the treatment. A total of 84 calves with 14 bulls and 28 heifers per treatment (42 colostrum and 42 placebo) triggered an alarm from the AMF (112 alarms), drank the bottle on all 3 d, and were assigned to a treatment group (n = 59 alarms placebo, n = 53 CR alarms). Only the first alarm was considered for all health outcomes (42 placebo and 42 CR calves). Treatment groups did not differ on the d before alarm for health status (BRD: 4 placebo, 3 CR; diarrhea: 14 placebos, 13 CR), or body weight (56.34 ± 2.31 kg placebo and 57.19 ± 2.20 kg CR, mean ± SE; $P > 0.10$). Similarly, health status was not different on the day the alarm was triggered and no new cases of BRD or diarrhea were present. Calf age was also not different on the day the alarm was triggered (31.63 ± 1.67 d placebo and 32.51 ± 1.67 d CR; $P > 0.10$). Similarly, average milk intake on the day prior to the alarm was not different between treatments (7.91 ± 0.23 L placebo and 7.87 ± 0.29 L CR; $P > 0.10$). Starter intake was also not different between treatments on the day prior to the alarm (0.15 ± 0.06 kg placebo and 0.18 ± 0.04 CR; $P > 0.10$).

For the week after treatment intervention, milk intake was not different between treatments (8.17 ± 0.11 L/d placebo and 8.18 ± 0.10 L CR; $P > 0.10$) but starter intake was greater for CR calves (0.16 ± 0.02 kg/d placebo and 0.28 ± 0.02 kg/d CR; $P <$ 0.0001). Similarly, starter intake post-weaning was different between treatments (3.52 ± 0.09 kg/d placebo and 4.08 ± 0.10 kg/d CR; P <0.0001).

Efficacy of colostrum replacer on ameliorating disease

Placebo calves had a 1.64 (95% CI: 1.11-2.43; P =0.01) times greater likelihood of being positive for BRD compared to CR calves for the 7-d following intervention (18 placebo calves vs. 11 CR calves). Moreover, placebo calves had a 1.50 (95% CI: 1.15-2.08; $P = 0.01$) times greater likelihood of having lobar lung consolidation than CR calves for the 7-d following intervention (21 placebo calves vs 14 CR calves). No difference was found in the likelihood of diarrhea between groups for the 7-d following treatment intervention (OR 0.91; 95% CI: 0.71-1.16; $P > 0.10$; 11 placebo calves and 12 CR calves).

Effect of colostrum replacer on survival from a disease bout

Bovine Respiratory Disease

A BRD bout was defined as a calf developing at least lobar lung consolidation ≥ 3.0 cm^2 and a BRD score of > 5. Placebo calves had 2.38 times (95% CI: 1.30-4.33) greater hazard of incurring a BRD bout in the 14 days post-intervention compared to CR calves ($P = 0.001$). Treatment intervention evaluated by Breslow survival curves for the 14 days post-treatment were significantly different (**Figure 3.1**; P =0.001). For example, the first quartile (25%) of placebo calves were already positive for BRD by day 3 (95%

CI: 1-5), and placebo calves were positive for BRD on average at 7.50 ± 0.82 days (mean ± SE) post-treatment. In contrast, the first quartile of CR calves was positive for BRD later at day 7 (95% CI: 5-8) and CR calves were positive for BRD on average at 10.51 ± 0.63 days post-treatment. Moreover, a placebo calf's Nelson-Aalen Cumulative Hazard of having BRD by day 14 was 1.09 ± 0.22 (± SE), and the Breslow survival probability of remaining healthy on day 13 was 0.33. Alternatively, a CR calf's Nelson-Aalen Cumulative Hazard of having BRD by day 13 was 0.50 ± 0.12, and the Breslow survival probability of remaining healthy was 0.60. The proportion of calves who were positive for BRD in the 14 days after intervention is summarized in **Table 3.1.**

Diarrhea

The hazard of incurring diarrhea in the 14 days post-intervention was not different by treatment intervention (*P* =0.18). Similarly, the treatment intervention survival curves for the post-treatment intervention had overlapping Hall-Welner confidence interval bands and were not significantly different (**Figure 3.2**; *P* = 0.16). For example, the first quartile of calves was positive for diarrhea by day 3 for placebo (95% CI: 2-5), and day 4 for CR (95%: CI 3-7). Nearly all calves had a diarrhea event (**Table 3.2**); the Nelson-Aalen cumulative hazard of a diarrhea bout by day 9 was placebo 0.84 ± 0.18 (± SE), and CR 0.70 ± 0.15.

Effect of colostrum replacer on improving performance

The final weight of calves 2 weeks-post weaning was (mean ± SD) 104.1 ± 18.17 kg for placebo and 104.3 ± 18.78 kg for CR. Average daily gain was not affected by treatment intervention for the 7-d following intervention (placebo 0.73 ± 0.07 kg/d and

CR 0.70 ± 0.08 kg/d; *P* >0.10 LSM ± SEM). The ADG for calves from birth to 90 days of age (2 weeks post-weaning) was also not different by treatment (placebo 1.12 ± 0.11 kg/d and CR 0.96 ± 0.11 kg/d; *P* > 0.10). Similarly, ADG was not associated with treatment intervention for the 2 weeks-post weaning (placebo 1.26 ± 0.08 kg/d and CR 1.17 ± 0.08 kg/d; *P* >0.10).

Discussion

The objective of this study was to determine if we could ameliorate BRD or diarrhea bouts, reduce the likelihood of these diseases, and improve performance by feeding a CR intervention to preweaned calves who triggered a feeding behavior alarm. We found that CR may serve as a nutraceutical for preweaned calves, as it reduced the hazard of a BRD bout, and increased the probability of remaining healthy 15 d after intervention. However, we did not observe any effect of CR intervention on ameliorating diarrhea or improving performance. To our knowledge, this is the first study to evaluate the effect of early intervention of a colostrum replacer nutraceutical in response to a change in feeding behavior alarm. Early intervention with CR may improve calf welfare as BRD is one of the leading causes of mortality in preweaned calves (Dubrovsky et al., 2019a). Similarly, BRD is economically costly to treat (Dubrovsky et al., 2020), and negatively affects ADG (Cramer and Ollivett, 2019) and future milk production (Dunn et al., 2018). However, we acknowledge that one limitation to this study is that some of our researchers were not blind to treatment assignments after the colostrum or placebo feedings. Also, the AMF alarm utilized in this trial has not been previously used as a proxy for intervention in dairy calves. Nonetheless, preventing BRD by intervening with CR when an AMF alarm is detected may improve the welfare of calves.

There was a preventative benefit of reducing the likelihood of BRD in calves treated with colostrum replacer. While we fed our calves for a short duration, our results agree with the literature which fed bovine colostrum for an extended period of several days. For example, colostrum as a nutraceutical decreased the likelihood and duration of respiratory disease in calves (Chamorro et al., 2017), and ameliorated duration of respiratory infection in juvenile racehorses (Fenger et al., 2016). Specifically in calves, research has shown feeding 150 g/d colostrum to younger calves from day 0 to 14 days of age reduced the likelihood of BRD preweaning (Chamorro et al., 2017). Moreover, a more recent, long-term study observed that extended feeding of colostrum for 21 days reduced BRD risk for the first year of life, and increased first lactation milk production (Armengol and Fraile, 2020). However, a recent study which partially replaced the milk diet with colostrum from birth until 14 d (doses included 0, 350 or 700 g/d), found only a tendency for BRD to be lower in calves (Kargar et al., 2020). We suspect Kargar et al., (2020) only found a tendency due to a low observed prevalence of BRD in their population of calves. In general, our results agree with the literature in calves and in other species that colostrum may reduce the risk of respiratory ailments. However, in this study, we did not evaluate the effect of colostrum replacer on the severity of lobar consolidation as we were not powered for magnitude effects. Future research should determine which component in colostrum is responsible for lowering BRD risk, and the effect of colostrum replacer on the severity of lobar consolidation in calves.

While the intervention of colostrum replacer did not reduce the likelihood of diarrhea in our calves, preventative feeding of extended colostrum to calves during the first 14 d of life has been found to reduce the risk of diarrhea (Chamorro et al., 2017)

Specifically, colostrum fed for 14 d reduced likelihood of diarrhea, number of days sick with diarrhea and number of antibiotic treatment days received compared to controls (Berge et al., 2009). Similarly, feeding 750 ml of lipolyzed bovine colostrum fed at 24 hour of life reduced incidence of diarrhea in calves, although this was not seen in older calves at 48 h or 72 h of life (Dezfouli et al., 2007). Moreover, others found that preventative feeding with colostrum for 21 d reduced the likelihood of diarrhea for 180 days of life (Armengol and Fraile, 2020). Our results probably differed from other studies because we did not feed CR for a long duration. Instead, we fed our calves one dose (50 IgG for 3 days) of CR at a shorter interval and our calves were much older (i.e., a month old at the time of intervention). Calves are most at-risk for diarrhea from birth until approximately 14 days of age, when passive immunity provided from colostrum is low (Cortese, 2009), or until 21 days of age when innate immunity is naive against endemic pathogens such as protozoans as reviewed by Cho and Yoon, 2014. Since we chose to provide the AMF a 12-d acclimation period for an algorithm to establish an average, it is possible we missed the high-risk period for diarrhea in our calves. Future research should determine if younger calves receiving an AMF-based colostrum intervention would lower the risk of diarrhea.

We did not observe an effect of colostrum replacer on ADG in this study. Research has shown that extended feeding of colostrum by partially replacing the milk fed diet for 14 days with either 0 g, 350 g, or 700 g/d colostrum improved performance in calves at 81 days of life (Kargar et al., 2020). Similarly feeding calves by partially replacing the milk meal with colostrum (2.3 L) for the first 21 days of life, resulted in greater first lactation body weights, though early life weights were not recorded

(Armengol and Fraile, 2020). Thus, the literature suggests that calves fed colostrum for extended periods had positive average daily gain effects. Our results may have disagreed with these studies because we fed a much lower dose of colostrum for a shorter duration (i.e., 125 g for 3 days). Alternatively, it is possible that we did not find an effect of CR on ADG because the placebo calves received milk replacer that contained more energy (i.e., 0.22 ME/kg) than the colostrum replacer. Thus, in our study, ADG was not affected in CR fed calves when the placebo calves received a high energy milk replacer. It is possible that if we had offered the placebo calves a negative control, or even a milk replacer with a lower ME/kg, CR at this dosage may have positively impacted average daily gain. Future research should investigate if CR at this dosage compared to a negative control positively affects ADG in calves.

In summary, we observed that colostrum supplementation during a time characterized by negative deviation in average milk intake and drinking speed resulted in reduced likelihood for the calf to develop BRD in the following 15 days, but there was no effect on development of diarrhea or performance over this period. While colostrum in our study did not reduce the likelihood of diarrhea, our calves triggered alarms at a month of age and were past the at-risk period for pathogenic diarrhea. Future research needs to identify whether protein, specific amino acids, IgG, lactoferrin or another component in colostrum is responsible for improved respiratory health in older calves. Similarly, a lack of effect of colostrum on performance was likely a result of a high plane of nutrition being offered to the calves. Colostrum many ameliorate BRD in preweaning calves, and the use of an AMF negative deviation in feed intake may be a potential intervention tool.

Conclusions

Colostrum offered as a nutraceutical at 125 g per day for three days (50 IgG per day) ameliorated BRD bouts and reduced the likelihood of BRD. However, there was no effect of colostrum treatment on amelioration of diarrhea, which may be related to the older age at which calves were treated (i.e., at one month of age). There also was no effect of colostrum treatment on performance, perhaps because calves were only offered colostrum replacer for three days. This study offers early evidence that colostrum intervention can improve calf health. Future work is required to determine which components in colostrum are most effective, and whether colostrum intervention at younger ages may also reduce diarrhea risk.

Table 3.1 Proportion of preweaned dairy calves (n=84) with a Bovine Respiratory Disease bout for 14 days following intervention with either 1 L of 125 g milk replacer or colostrum replacer for 3 days

Proportion of preweaned group-housed calves (n=84) with a Bovine Respiratory Disease (BRD) bout 14 days following intervention by a treatment of 1 L 125 g for 3 d of milk replacer (Placebo) or colostrum replacer (Colostrum Replacer) after triggering a health alarm on automated feed system for negative deviations in milk intake or drinking speed. Censored calves remained healthy at 15 d and failed calves were diagnosed with a Bovine Respiratory Disease bout within 14 days following intervention.

Summary of the Number of Censored and Uncensored Values

Stratum	Treatment	Total	Failed	Censored	Percent Censored
1	Placebo	42	29	13	31.0
2	Colostrum Replacer	42	17	25	59.5
Total		84	46	38	45.2

Table 3.2 Proportion of preweaned dairy calves (n=84) with a diarrheal bout for 14 days following intervention with either 1 L of 125 g milk replacer or colostrum replacer for 3 days

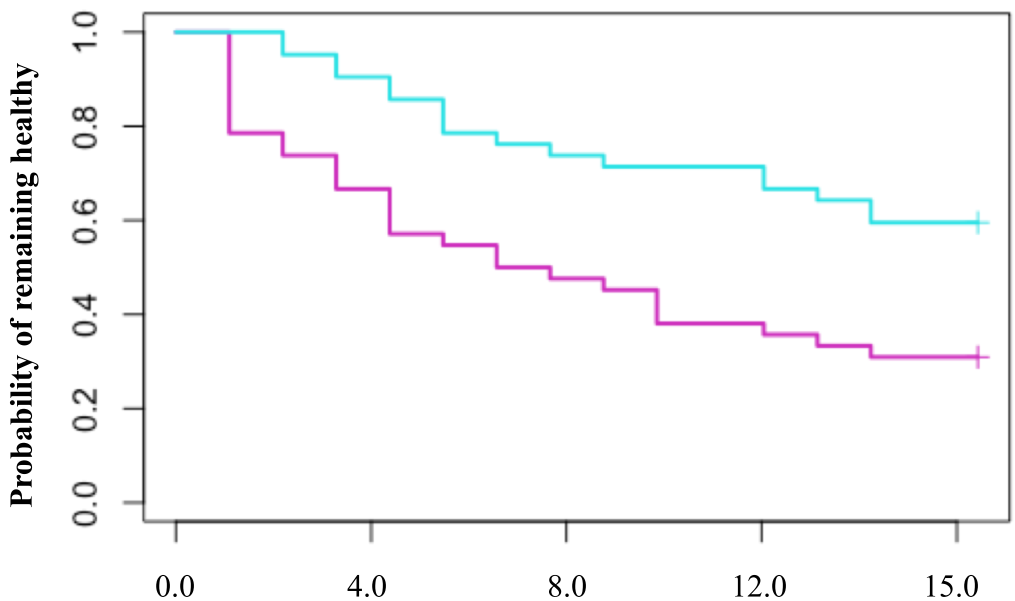
Proportion of calves with a diarrhea bout after triggering an alarm on an automated feeder for negative deviations in milk intake or drinking speed 14 days following intervention by a treatment of 1 L 125 g for 3 d of milk replacer (Placebo) or colostrum replacer (Colostrum Replacer). Censored calves remained healthy at 15 d and failed calves were diagnosed with a diarrhea bout within 14 days following intervention.

Summary of the Number of Censored and Uncensored Values

Stratum	Treatment	Total	Failed	Censored	Percent Censored
1	Placebo	42	27	15	35.7
2	Colostrum Replacer	42	22	20	47.6
Total		84	49	35	41.7

Figure 3.1. Probability of calves remaining negative for Bovine Respiratory Disease for the 14 days after receiving either 1 L of 125 g milk replacer or colostrum replacer for three days

Breslow survival probability estimates (*P* < 0.01) with number of subjects (n=84; 42 colostrum replacer, 42 placebo) at risk for a Bovine Respiratory Disease bout. Day 0 represents when an automated feeder alarm (negative deviation in baseline milk intake or drinking speed) was detected for a calf. All calves received 125 g in 1 L for 3 d of either milk replacer, or colostrum replacer. Day 15 represents calves who remained healthy.



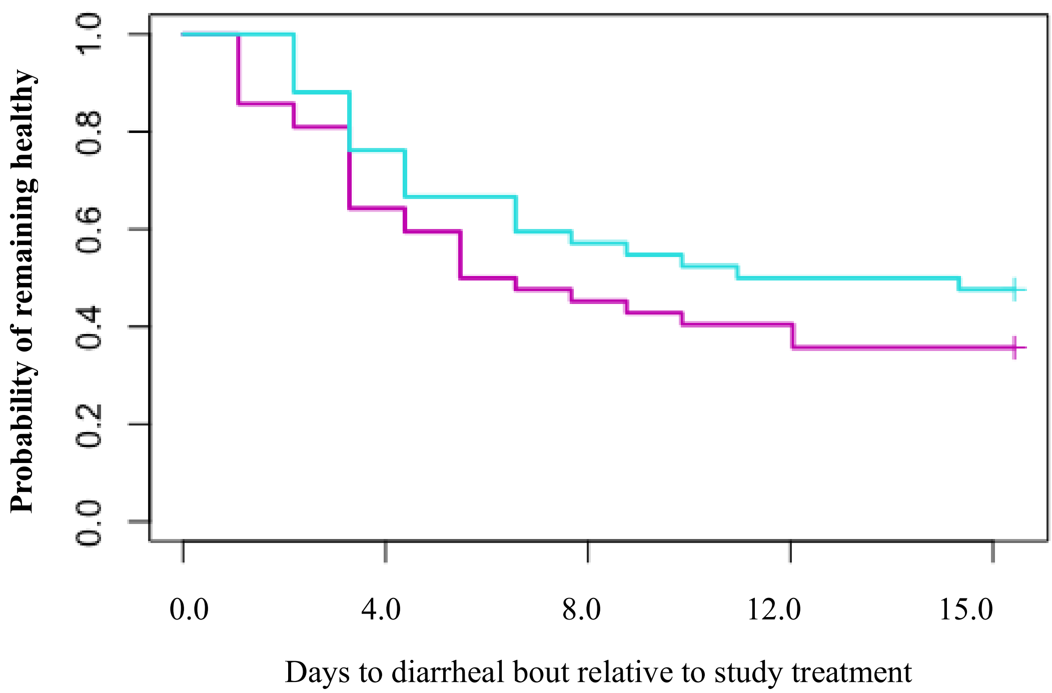
Days to Bovine Respiratory Disease bout relative to study treatment

Treatments ----- Placebo (Milk Replacer)

----- Colostrum Replacer

Figure 3.2. Probability of calves remaining negative for a diarrheal bout for the 14 days after receiving either 1 L of 125 g milk replacer or colostrum replacer for three days

Breslow survival probability estimates ($P < 0.01$) with number of subjects (n=84; 42 colostrum, 42 placebo) at risk for a diarrheal bout. Day 0 represents when an automated feeder alarm (negative deviation in baseline milk intake or drinking speed) was detected for a calf. All calves received 125 g in 1 L for 3 d of either milk replacer, or colostrum replacer. Day 15 represents calves who remained healthy.



Treatments -----_Placebo (Milk Replacer)

-----_Colostrum Replacer

CHAPTER 4. BEHAVIORAL CHANGES ASSOCIATED WITH RECOVERY IN DAIRY CALVES TREATED WITH ANTIMICROBIALS FOR BOVINE RESPIRATORY DISEASE

Introduction

Bovine Respiratory Disease (BRD), a disease of the upper or lower respiratory tract in cattle, is a welfare challenge to manage; it also is the leading cause for antimicrobial use on calf raising operations (Urie et al., 2018). This disease causes physiological changes such as inflammation of the respiratory tract (Bednarek et al., 1999), which leads to coughing, pain, a febrile response, and activation of the HPA-immune axis, resulting in the display of sickness behaviors by the inflicted animals (Hart and Hart, 2019). Some examples of sickness behavior related to BRD bouts in dairy calves can be passively recorded by precision technology devices such as depressed feeding behavior with automated feeders as reviewed by Morrison et al., 2020, and reduced activity levels with accelerometers, as reviewed by Costa et al., 2020. Other sickness behaviors in calves were directly observed (i.e., video recordings) such as self-isolation behavior (Cramer and Stanton, 2016), depressive behaviors with signs of physical pain (Cramer et al., 2019), labored breathing (Love et al., 2016), and lateral lying (Hixson et al., 2018). Thus, a change in a calf's affective state likely has welfare consequences when a calf experiences a BRD bout and the time a calf experiences disease should be minimized (Bull et al., 2021).

Bovine respiratory disease (BRD) also presents a sustainability issue due to the possibility of antimicrobial resistance; calves often require multiple antimicrobial interventions to treat the disease and the symptoms. For example, BRD calves often have poor response to additional antimicrobial interventions (Welling et al., 2020), leading to chronic pneumonia (Heins et al., 2014, Welling et al., 2020), and potentially death (Heins et al., 2014, Welling et al., 2020). Therefore, identifying recovery status in cattle with BRD is a welfare challenge and a sustainability issue.

Effectiveness of antimicrobial intervention is less than optimal for BRD bouts in calves. For example, nearly a fifth of initial BRD bouts treated were relapsed cases, and some of these calves died (Welling et al., 2020). Similarly, the pathogens involved, and the class of antimicrobials used can affect the cure rate suggesting a need to quantify recovery status in these calves promptly. For example, multiple in vitro studies demonstrated some of the most common pathogens associated with BRD had a high minimum inhibitory concentration for most of the commercially available antimicrobials used to treat BRD in beef calves as reviewed by Lysnyansky and Ayling, 2016. This may partially explain why relapsed calves are common and are at a much greater likelihood for chronic pulmonary disease and mortality (Heins et al., 2014, Welling et al., 2020). However, there is also a lag between antimicrobial intervention and resolution of clinical symptoms in recovered BRD calves, complicating the detection of compromised calves as reviewed by Cramer and Ollivett, 2020. One potential solution is to use behavioral changes (i.e., sickness behaviors) to identify recovery status in these calves and provide prompt interventions.

Sickness behavior in relapsed cattle is like that associated with the initial BRD diagnosis, where decreased feed intakes, signs of depression, lethargy, and labored breathing are observed prior to clinical re-presentation (Skogerboe et al., 2005). Indeed, researchers determined that a reticulorumen bolus was more sensitive at detecting recovery status than using only clinical symptoms in beef cattle who recently received an antimicrobial intervention for BRD (Lhermie et al., 2017). However, research is limited since precision technology devices were initially developed to alert for deviations in behavior before the initial diagnosis of clinical disease (Eckelkamp and Bewley, 2020). Calves who respond to the initial antimicrobial intervention should resume behavioral baseline behavior as convalescence approaches. In contrast, it is likely that BRD calves who relapse from the initial antimicrobial intervention experience depressed feeding behavior and reduced activity, but this research is still needed.

Sickness behaviors often occur prior to clinical presentation since the immune system activates the HPA-axis upon initial detection of a pathogen. Depressed feed intake was identified as the most accurate and sensitive sickness behavior in cattle, with changes several days before BRD diagnosis (Kayser et al., 2020). However, lethargic behavioral changes such as increased lying times and fewer lying bouts were also indicators of BRD, though more individual variation was present in sick animals (Kayser et al., 2020). Depressed feeding behavior and reduced activity levels were also observed a few days before diagnosis of metabolic diseases in lactating dairy cattle (Dittrich et al., 2021) and before the initial BRD diagnosis in calves (Swartz et al., 2017, Duthie et al., 2021). Furthermore, precision livestock researchers suggested that a combination of precision technology devices (automated feeder, accelerometer, and a reticulorumen bolus) could detect BRD status in cattle several days earlier than staff (Kayser et al., 2018). Thus, we suggest that there is real potential to use precision technology devices to monitor for sickness behaviors to detect recovery status in calves. The use of sickness behavior to indicate recovery status in livestock may be a new frontier for precision technology devices that requires investigation.

The objective of this study was to evaluate if recovery status was associated with daily changes in feeding behavior and activity in dairy calves for the 10 days after antimicrobial intervention. We also evaluated if recovery status was associated with relative changes in feeding behavior and activity on an individual basis (i.e., a calf increases or decreases its behavior in response to initial BRD diagnosis and antimicrobial therapy). We hypothesized that relapsed calves would show depressed feeding behavior and reduced activity levels compared to recovered calves.

Materials and Methods

This study was conducted at the University of Kentucky Coldstream Research Dairy Farm in Lexington, KY from 28 May 2018 to 9 September 2019. All calves enrolled were part of the Institutional Animal Care and Use Committee approval number 2018: 2864. This study and manuscript were conducted following the quality standards of Strengthening the Reporting of Observational Studies in Epidemiology Veterinary Guidelines (Sargeant et al., 2016).

Management and feeding

Detailed information on calf management and feeding are outlined in Chapter 3. Briefly, calves were trained to drink milk from an automated feeder (CF100, Forster Technik, Engen, Germany) at 3 days of age and were fitted with a leg-based accelerometer (IceQube, IceRobotics, Edinburgh, Scotland). Calves were housed in group pens of 6 ± 3 calves (mean ± SD) and offered up to 10 L (140 g/L) milk replacer per day from the automated milk feeder (Cow's Match Cold Front; Land O' Lakes Animal Milk Products Co., Shoreview, MN) until 50 d of age after which weaning occurred. Calves were also offered calf starter (Special Calf Starter and Grower, Baghdad Feeds, Baghdad, KY) from an automated dispenser (Compact Smart, Förster-Technik, Engen, Germany), chopped alfalfa hay in a trough, and water from an automated waterer.

Health exams

Calves were health scored daily at approximately 08:30 h from birth until 2 weeks post-weaning (87 ± 2.0 d of age) by 1 of 3 observers (inter-observer agreement $\kappa > 0.90$) for Bovine Respiratory Disease (BRD), diarrhea and sepsis as described in detail in chapter 3. Fleiss' kappa was calculated every 4 mo. throughout the study by having all observers go to a commercial facility on the same day to score the health status of 40 calves where the health status of the calves was unknown to ensure unbiased agreement. Since the calves were followed daily for health events, the main observer was not blind to disease outcomes. However, to limit observational bias, the other 2 researchers were blind to antimicrobial interventions, and the farm staff administered all antimicrobial treatments to calves.

Bovine respiratory disease symptoms were recorded using the Wisconsin scoring system (McGuirk and Peek, 2014) with a trained observer who assigns scoring (0 normal to 3 severely abnormal) on: abnormal presentation of cloudy nasal discharge and eye discharge, degree of ear tilt, degree of coughing, and degree of elevated rectal temperature in calves. Lung consolidation was recorded twice weekly for all calves using a portable linear rectal ultrasound (Ibex Pro, E.I. Medical, Loveland, CO) and 70% isopropyl alcohol as a transducing agent; lung lobes of each calf were evaluated by 1 of 2 observers (inter-observer agreement Cohens' kappa; $\kappa = 0.90$). The ultrasound was set to a depth of 9 cm, frequency of 6.2 MHz, and gain of 23 dB (near 13 dB; far 36 dB).

A BRD score measured by the Wisconsin scoring system of ≥ 5 and lobar consolidation in any one lung lobe ≥ 3.0 cm^2 was considered a BRD bout on day 0 (**"initial diagnosis")** as described in Buczinski et al., 2018. All calves with a positive BRD bout received antimicrobial intervention on initial diagnosis; enrofloxacin was administered subcutaneously with dosage calculated by BW (Baytril, Bayer, Leverkusen, Germany; 1 ml/15 kg) according to the herd veterinarian protocol. From day 1 to day 9 after initial diagnosis, calves were health scored, but no clinical diagnosis was made (**"post-treatment period"**). From day 10 to day 14 after the initial diagnosis, all calves were assessed for recovery status from their initial BRD bout (**"recovery classification period"**). Calves who had an average BRD score ≥ 5 and at least one lobe of lobar consolidation at ≥ 3.0 cm^2 during the recovery classification period were classified as failures (**"relapsed"**) and received a new antimicrobial intervention on day 15 as per the veterinary protocol for this research station. Calves negative for the clinical symptoms described above during the recovery classification period were considered responsive (**"recovered"**). Relapsed calves were treated with tulathromycin on day 15 (Draxxin, Zoetis Animal Health, USA; 1 cc/45 kg, once at second diagnosis, subcutaneously), following the veterinary protocol of the research farm.

Body weights were recorded twice weekly using an electronic scale (Brecknell PS1000, Avery Weigh-Tronix LLC, and Fairmont, MN) from birth to 2 weeks post-weaning for all calves.

Automated data recording

The automated data recording for the automated feeder, accelerometer, and the seasonal average temperature and humidity in the calf barn are presented in chapter 2.

Enrollment Criteria

This observational case-control study was a subpopulation of an observational cohort study of 120 calves (73 heifers, 47 bulls) which were health scored daily for symptoms of Bovine Respiratory Disease (BRD), diarrhea and navel health from birth until 14 d after weaning, with the final health exam performed on calves at 87 ± 2.0 d of age (mean ± SD). All calves born at this facility were weighed within 12 hours after birth with an electronic scale (Brecknell PS1000, Avery Weigh-Tronix LLC, Fairmont, MN). Calf birth weights were 39.42 ± 5.31 kg (mean ± SD). Only calves with successful transfer of passive immunity (> 8.0% BRIX) at 48 h of age, and those that were not a twin were enrolled. Any calf not meeting these requirements were excluded from the study.

All calves enrolled in this case-control study (38 of 120 calves) had an initial BRD bout diagnosis, defined as a BRD score ≥ 5 measured by the Wisconsin scoring system lobar consolidation in any one lung lobe ≥ 3.0 cm^2, and had antimicrobial intervention (e.g., enrofloxacin) administered on the day of initial diagnosis. Initial diagnosis of BRD occurred between 3 ± 2 days of age (age of training to drink milk from an automated feeder) and 53 ± 2 days of age (age before weaning). We chose the preweaning phase since individual variation in feeding behavior during weaning might have introduced bias to our findings (Neave et al., 2018b).

Case-control classification and study population

Of the original cohort of 120 calves, 49 calves had BRD bouts and of these BRD bouts, we enrolled [(n=38) pair matched cases to controls; 19 recovered cases and 19 relapsed cases]. The calves on this study had an initial BRD bout diagnosis at an average age of 32.0 ± 13.0 d (mean ± SD) and weighed 54.4 ± 9.7 kg. To limit bias, only clinical BRD symptoms (not behavior) were used to classify recovery status from day 10 to day 14 in these calves. Criteria for a case relapsed calf required the average BRD score ≥ 5, positive clinical lobar pneumonia on ultrasound for the recovery classification period, and follow-up antimicrobial intervention. Criteria for a control recovered calf required the average clinical BRD score to be < 5, plus resolution of lobar pneumonia during the recovery classification period, complete technology data, and meeting pair matching criteria. The pair matching criteria was same gender, age of the initial antimicrobial intervention no more than 2 days different, and a birthdate within 6 weeks.

For the power analysis, we assessed the literature for the most conservative relapse-to-antimicrobial-intervention-rate when treating non-specified BRD bouts in calves as an estimate of the incidence rate of BRD relapse in the dairy calf population. The most conservative literature report was that of Welling et al. (2020) who observed 16% (19/117) of BRD calves relapsed from their first antimicrobial intervention for Bovine Respiratory Disease. To calculate the incidence of relapse-to-antimicrobial-intervention-rate for the herd in this study, 2 researchers (Cohen's kappa= 0.95) scored calves twice weekly for BRD (32/80 BRD calves) for the 9 months prior to this study; farm staff administered antimicrobial interventions based on veterinary protocol and clinical signs of BRD (McGuirk and Peek, 2014, Buczinski et al., 2018). The relapse-to-antimicrobial-intervention-rate was 43.75% (14/32). Therefore, at 80% power, with half-widths of 0.05, we required 17 relapsed calves to detect behavioral differences from recovered calves.

Statistical analysis

All statistical analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive analyses were performed, and data was verified for normality using the univariate procedure and probability distribution plots. Normality was also investigated by visually examining the residuals from the linear mixed models, and by testing covariance structures for model fit. One outlier relapsed calf was detected for abnormally low drinking speeds that were out of the biological range (greater than 3 SD of the mean); this pair was excluded from the drinking speed analyses.

The residuals of the modeled values for calf starter intake and unrewarded visits were not normally distributed and were transformed accordingly (calf starter intake: common log with correction factor of 10g for calf starter intake; unrewarded visits: common log with a correction factor of 5 visits). The back-transformed geometric means minus the correction factors and the 95% CI are reported for both calf starter intake and unrewarded visits, with statistical significance reported on the modeled transformed values.

For all linear mixed models in this study, multivariable models were reduced by manual stepwise backward elimination, where predictors with a P-value < 0.30 were retained in the models. Season was evaluated in all models as a fixed effect and birthweight, BRIX % serum IgG at 48 h of age, and calf age were tested as quantitative covariates. Due to the collinearity observed during univariate analysis for the covariates BRIX % IgG at 48 h of age and age enrolled on the feeder, these were evaluated for model inclusion separately. If BRIX % IgG at 48 h of age was not significant, age enrolled on the automated feeder was evaluated. Pair was a fixed effect in all models.

Effect of recovery status on average calf behavior

First, linear mixed models were used to investigate the effect of recovery status, and the recovery status x day interaction, on the average feeding behavior and activity in preweaned calves for the 10 days after and including the day of BRD diagnosis (day 0 to day 10; referred to as "**post-treatment period"**). The post-treatment period was selected based on model fit, and a lower Akaike's criterion. The recovery classification period was not included to limit bias of finding behavioral differences during clinical presentation of symptoms in relapsed calves. Models were designed with day as a repeated measure and calf as the subject with an autoregressive first order (AR1) covariance structure. For the reasoning of the investigative nature of the study each behavioral pattern was modeled for each day. Linear mixed models were used to investigate the daily behavioral patterns for each day within the post-treatment period (day 0, day 1, day 2, day 3, day 4, day 5, day 6, day 7, day 8, day 9 and day 10; referred to as "**daily measures"**); calf was specified as a random effect in these models.

Effect of recovery status on relative changes in calf behavior

Effects of recovery status and the recovery status x day interaction on the relative changes in feeding behavior and relative changes in activity over the post-treatment period were created with an identical modeling structure to that described above. We used non-parametric analyses (PROC NPAR1WAY) to confirm that averages and variances of feeding and activity behaviors were deemed similar on the day of BRD diagnosis in all calves (e.g., recovery status was not yet evident). Since no differences were evident, the day of diagnosis (day 0) was set as the baseline and deemed the 100% level for each behavior by calf. Relative change was then calculated by dividing every calf's individual behavior on every daily measure (day 1, day 2, day 3, day 4, day 5, day 6, day 7, day 8, day 9 and day 10) by every calf's behavioral baseline at day 0. We also conducted linear mixed models for each day within the post-treatment period (day 1 to day 10) to further explore any effects observed in the overall post-treatment period; calf was a random effect in all models.

Results

Study population

All recovered calves (19/19) resolved symptoms at 7.0 ± 3.0 (mean ± SD) days after the first intervention. Relapsed calves who resolved symptoms (15/19) took an average 22.0 ± 5.0 days (mean ± SD) after the first antimicrobial intervention. Four relapsed calves did not resolve the BRD bout and were euthanized by veterinary recommendation. All euthanized calves were sent to a diagnostic lab (University of Kentucky Diagnostic Lab, Lexington, KY, USA); the preliminary cause of death was chronic pneumonia.

Effect of recovery status on behavior over the post-treatment period

The effect of recovery status, and the recovery status x day interaction on average feed intake, feeding and activity behaviors during the post-treatment period are presented in **Table 4.1.** Briefly, relapsed calves had less milk intakes and fewer lying bouts compared to recovered calves during the post-treatment period. There was also a treatment x day interaction for relapsed calves to have greater lying times, less step counts, and reduced acceleration activity indexes compared to recovered calves for the post-treatment period.

Relapsed calves had less unrewarded visits during the post-treatment period ($F_{1,18} = 4.77$, $P = 0.04$; geometric mean [95% CI]: recovered calves = 2.40 [95% CI: 2.21-2.59], relapsed calves = 1.53 [95% CI: 1.35-1.55]) and there was no interaction of antimicrobial intervention and day ($F_{20,359} = 0.63$, $P = 0.24$). Relapsed calves also consumed less calf starter during the post-treatment period ($F_{1, 18} = 5.07$, $P = 0.03$; recovered calves = 137.50 g/d [95% CI: 111.25-263.74 g/d], relapsed calves = 51.54 g/d [95% CI: 23.74-126.24 g/d]), and there was no interaction between antimicrobial intervention and day ($F_{20,355} = 1.22$, $P = 0.22$).

Effect of recovery status on daily behaviors within the post-treatment period

Figure 4.1 shows differences in feeding behavior between relapsed and recovered calves on each daily measure within the post-treatment period. Relapsed calves drank less milk on day 6 ($F_{1,16} = 3.32$, $P = 0.05$) and tended to drink less milk on day 9 ($F_{1,16} = 3.39$, $P = 0.08$; Figure 4.1a), but the other daily measures were not different (day 0 to day 5, day 7, day 8 and day 10; $P > 0.10$) compared to recovered calves. Relapsed calves tended to have slower drinking speeds on day 9 ($F_{1,14} = 2.81$, $P = 0.08$; Figure 4.1b), but the other daily measures were not different (day 0 through day 8, day 10; $P > 0.10$) compared to recovered calves. Relapsed calves also had less rewarded visits to the feeder on day 9 ($F_{1,15} = 5.45$, $P = 0.03$; Figure 4.1c), but the other daily measures were not different (day 0 to day 8, day 10; $P > 0.10$) compared to recovered calves.

Unrewarded visits to the feeder were less for relapsed calves on day 5 ($F_{1,16} = 3.28$, $P = 0.05$; geometric mean [95% CI]: recovered calves = 3.73 [95% CI: 2.82-4.65], relapsed calves = 2.27 [95% CI: 1.62-2.91]) but the other daily measures were not different (day 0 to day 4, and day 6 to day 10; $P > 0.10$). Starter intake for relapsed calves was less on day 3 ($F_{1,13}$ = 7.74, P = 0.02; recovered calves = 122.50 g/d [95% CI: 16.47-252.34 g/d], relapsed calves = 33.54 g/d [95% CI: 19.74-136.20 g/d]. Starter intake was also less for relapsed calves on day 9 ($F_{1,13}$ = 4.79, P = 0.05; recovered calves = 132.50 g/d [95% CI: 56.47-286.34 g/d], relapsed calves = 43.51 g/d [95% CI: 19.21-126.10 g/d] but the other daily measures were not different (day 0 to day 4, and day 6 to day 8, day 10; P > 0.10).

Figure 4.2 shows differences in activity levels between relapsed calves and recovered calves for each daily measure within the post-treatment period. Relapsed calves had greater lying times on several daily measures (day 5, day 6, day 8, day 9, day 10, $P \leq 0.05$; **Figure 4.2a**), but the other daily measures were not different (day 0 to day 4, day 7; P > 0.10) compared to recovered calves. Similarly, relapsed calves had fewer lying bouts on several daily measures (day 4, day 5, day 7, day 8, day 9, day 10, $P \leq 0.05$; **Figure 4.2b).** In addition, lying bouts tended to be less on day 0 ($F_{1,16}$ = 3.55, P = 0.08), but the other daily measures were not different (day 1 to day 3, day 6; P > 0.10) compared to recovered calves. Relapsed calves also had less step counts on most daily measures (day 1, day 3, day 4, day 5, day 6, day 7, day 8, day 9, day 10, $P \leq 0.05$; **Figure 4.2c**) and step counts tended to be less on day 0 ($F_{1,11}$ =3.51, P = 0.09), but the other daily measures were not different (day 2 to day 4; P > 0.10) compared to recovered calves. Finally, relapsed calves had reduced activity indexes on several daily measures (day 4, day 6, day 7, day 8, day 9, day 10 $P \leq 0.05$; **Figure 4.2d**), but the other daily measures were not different (day 0 to day 3, day 5; P > 0.10) compared to response calves.

Effect of recovery status on relative changes in behavior over the post-treatment period

The effect of recovery status and the recovery status x day interaction on relative changes in feed intake, feeding and activity behaviors of calves during the post-treatment period are presented in **Table 4.2**. Briefly, for relapse calves, there was a tendency for greater relative changes in unrewarded visits, and a treatment by day interaction for less relative changes in calf starter intake compared to recovered calves for the post-diagnosis period. There was also a treatment by day interaction for greater relative changes in lying time, less relative changes in step counts, and less relative changes in the acceleration activity index, suggesting suppressed activity levels compared to recovered calves for the post-treatment period.

Effect of recovery status on daily relative changes in behavior within the post-treatment period.

Figure 4.3 shows relative changes in feed intake and feeding behavior between relapsed and recovered calves on each day within the post-treatment period. There was no effect of recovery status on any daily measure for relative changes in milk intake (day 1 to day 10; $P > 0.10$; **Figure 4.3a**). Relapsed calves had fewer relative changes in drinking speed, suggesting slower drinking speeds on day 9 and day 10 ($P \leq 0.03$; **Figure 4.3b**), and there was a tendency for less relative changes in drinking speeds on day 8 ($F_{1,15} = 1.20$, $P = 0.08$) compared to recovered calves. There was no effect of recovery status on any daily measure for relative changes in rewarded visits (day 1 to day 10; $P > 0.10$; **Figure 4.3c**) compared to recovered calves.

BRD calves had fewer relative changes in calf starter intake, suggesting a decline in their solid feed intake on day 3 ($F_{1,17}$ = 7.01, P = 0.02) and day 9 ($F_{1,17}$ = 7.24, P = 0.02. **Figure 4.3d**), but the other daily measures were not different (day 0 to day 2, day 4 to day 8, day 10; $P > 0.10$) compared to recovered calves. There was no association of recovery status on any daily measure with relative changes in unrewarded visits (day 1 to day 10; $P > 0.10$; **Figure 4.3e**)

Figure 4.4 shows relative changes in activity behaviors between relapsed and recovered calves on each day within the post-treatment period. Relapsed calves had greater relative changes in lying time, suggesting greater lying times on day 8 ($F_{1,11}$ = 6.73, P =0.02) and there was a tendency for greater relative changes in lying time on day 9 ($F_{1,12}$ = 3.36, P = 0.09; **Figure 4.4a**), but the other daily measures were not different (day 0 to day 7, day 10; $P > 0.10$) compared to recovered calves. Relapsed calves tended to have fewer relative changes in lying bouts, suggesting a potential decline in lying bouts on day 5 ($F_{1,16}$ = 2.85, P = 0.08; **Figure 4.4b**), but the other daily measures were not different (day 1 to day 4, day 6 to day 10; $P > 0.10$) compared to recovered calves. Relapsed calves also tended to have fewer relative changes in step count, suggesting potential less activity on day 8 ($F_{1,17}$ = 2.87, P =0.08; **Figure 4.4c**), but the other daily measures were not different (day 1 to day 7, day 9, day 10; $P > 0.10$) compared to recovered calves. Relapsed calves also had fewer relative changes in the acceleration activity index, suggesting a potential decline in activeness on day 8 ($F_{1,17}$ = 2.70, P =0.08; **Figure 4.4d**, but the other daily measures were not different (day 1 to day 7, day 9, day 10; $P > 0.10$) compared to recovered calves.

Discussion

This study found an association between recovery status (recover vs relapse), and the daily behavioral patterns in dairy calves for the 10 days after antimicrobial intervention (post-treatment period). Calves that relapsed and required a second antimicrobial intervention had clear behavioral changes before the recovery classification period. These behavioral changes included depressed feed intakes, fewer unrewarded visits, and a reduction in all activity measures (e.g., further referred to as lethargic behavior) when compared to recovered calves. Furthermore, within this post-treatment period, relapsed calves had fewer relative changes in starter intake a few days after the first intervention, suggesting relapsed calves deviate from their baseline behaviors before clinical re-diagnosis. We suggest that relapsed calves show signs of sickness behavioral changes that could be detected automatically by precision technology devices, while recovered calves resolved these abnormal behavioral patterns. This research supports a new opportunity to passively monitor calves in recovery from BRD using precision technology devices.

Calves that recovered from BRD showed a response to the initial antimicrobial intervention much earlier than the time required for recovery classification. These recovered calves appeared to no longer experience the antigenic innate immune response associated with induced sickness behaviors (Hart and Hart, 2019), including the reduced feed intake and lethargic behavior observed in relapsed calves in this study. Cytokine-induced sickness behavior is an antigenic response (Hart and Hart, 2019) and a motivational state. The motivational state during a disease bout is an active response to infection (Johnson, 2002) to meet the metabolic demands of the febrile response, increasing the chance of a calf's recovery. In contrast, calves that relapsed from the initial antimicrobial intervention for BRD may have had the inflammatory pathway re-activated, leading to sickness behavior. Thus, a relapsed calf would lack motivation to seek feed by being less active, so that more energy resources are allocated to fighting an immune response. Moreover, the veterinary definition of recovery status following a disease response in livestock includes the absence of sickness behaviors such as depression, lethargy, and anorexia in conjunction with symptom resolution (Radostits et al., 2006). In this study, we only classified recovery status with resolution of clinical pulmonary symptoms, to test the hypothesis that recovery status was associated with sickness behavior. Evidence from our study suggests that the absence of depressive states such as less feed intake, slower drinking speeds, and lethargic behavioral activity may indicate a calf in recovery from a BRD bout.

Recurrent BRD is a major welfare challenge for the cattle industry. It is painful for the calf and reduced the likelihood of survival past the first lactation (Buczinski et al., 2021), depresses normal calf behavior (Cramer et al., 2019) and relapsed BRD calves require additional antimicrobial interventions (Rooney et al., 2005, Welling et al., 2020). We suspect the antimicrobial response rate for relapsed BRD bouts is poor (Welling et al., 2020) due to the lag between recurrence of disease and re-emergence of clinical symptoms (Cramer and Ollivett, 2020). This agrees with our findings, that calves who relapsed to the initial antimicrobial intervention have behavioral changes prior to the re-classification of disease, and recovered calves have positive relative changes in their behaviors following initial diagnosis and antimicrobial intervention. The welfare issue in recurrent BRD is its effects on the calf's biological functioning, expression of natural behaviors, and a calf's affective state, all components of good animal welfare and a life worth living (Mellor, 2016). Relapsed BRD also increases the likelihood of mortality (Holland et al., 2010). Thus, we suggest that the use of sickness behavior as a screening tool for assessing recovery status in calves may permit earlier antimicrobial interventions, improving calf welfare and overall health. This agrees with veterinarian protocols for BRD which suggests that minimizing the duration of BRD status in calves is essential to promote calf well-being (Bull et al., 2021).

Relapsed BRD is a sustainability problem as re-treatment of BRD promotes antimicrobial resistance. For example, nearly half of the bacteria isolated from recurrent BRD calves were resistant to commercial antimicrobials in a Belgian study (Thomas et al., 2002). This may be due to the most dominant pathogen associated with BRD in calves; it was identified as having a high minimum inhibitory concentration to most of the commercial antimicrobials available (Lysnyansky and Ayling, 2016). For our study, we had a 14-day delay between initial BRD diagnosis and re-treatment with antimicrobials following herd veterinary protocol, which was like other veterinary protocols (Rooney et al., 2005, Holland et al., 2010, Heins et al., 2014, Welling et al., 2020). However, earlier antimicrobial intervention for a BRD bout results in greater initial antimicrobial response, and more positive outcomes for the calf (Binversie et al., 2020). Thus, we suggest precision technology devices may provide a bridge to detect sickness behavior in relapsed calves before re-emergence of clinical symptoms.

Our relapsed calves had depressed feeding behavior for each variable measured in this study (e.g., milk intake, drinking speed, starter intake, rewarded visits, unrewarded visits), and for multiple daily measures within the post-treatment period. Furthermore, relapsed calves had fewer relative changes for starter intake within a few days after the initial antimicrobial intervention. Depressed feeding times, and presence of lethargic activity levels were classified as classical sickness behaviors in dairy cattle (Dittrich et al., 2021). Furthermore, there is a myriad of evidence which suggests that declines in feed intake, slower drinking speed, and less unrewarded visits to the automated feeder were associated with sickness behaviors in dairy calves as reviewed by Morrison et al., 2021. Our results are in broad agreement with observations of relapsed beef cattle; depressed feed intake and lethargy were indicative of poor response to antimicrobial treatments (Skogerboe et al., 2005). Moreover, one study challenged beef cattle with BRD associated pathogens and found that dry matter intake (i.e., feed intake) was the most robust sickness behavior to indicate recovery status (e.g., declined after disease and resolved earlier in cattle given an NSAID; Toaff-Rosenstein et al., 2016). This agrees with our findings, that solid feeding behavior in calves was one of the earlier indicators of recovery status. We suggest that less solid feeding behavior is a sickness behavior in calves, as has been observed in dairy calves in BRD challenge studies (Hixson et al., 2018) and naturally occurring BRD in veal calves (Belaid et al., 2020) and beef cattle (Jackson et al., 2016). Future research should investigate the potential of calf starter intake, which can be automatically recorded, as an alarm for recovery status in calves.

We hypothesize that our relapsed calves prioritized milk intake over starter intake in this study. It has been suggested by many others that calves offered higher milk allotments (e.g., 10 L/d or greater) prefer milk over starter (de Passillé and Rushen, 2016, Neave et al., 2018a, Neave et al., 2018b). Indeed, on one study which weaned calves by their voluntary starter intake, multiple calves never approached the target grain consumption rate (e.g., 200 g/d) when they were offered high milk allotments(Benetton et al., 2019). This would explain why rewarded visits (e.g., nutritive visits) were only less for our relapsed calves on day 9, as milk intake was prioritized by the calf. Furthermore, unrewarded visits were less across the post-treatment period for relapsed calves compared to those who recovered. We suggest that recovered calves, who were more active in general, were motivated to try to receive more milk than the 10 L/d offered compared to relapsed calves. Fighting for automated feeder access is common in healthy calves, even when stocking densities are limited to smaller group sizes (Jensen, 2004). Thus, it is possible that relapsed calves had low motivation to access solid feed and used their limited energy sources to access milk. Unrewarded visits have also been suggested by researchers as a sign of poor satiety in calves (De Paula Vieira et al., 2008). Thus, we suspect recovered calves may have had post-compensatory feeding motivation and this should be explored.

There were fewer relative changes in drinking speed for relapsed calves, contrary to our hypothesis and other trials. We hypothesize that relapsed calves had depressed relative changes in drinking speed due to the labored breathing associated with Bovine Respiratory Disease (Love et al., 2016). It is not uncommon for sick dairy calves to take multiple pauses at the automated milk feeder during suckling. Indeed, studies observed a relationship with a higher milk meal duration in a BRD challenge study (Hixson et al., 2018), and naturally occurring BRD bouts in calves (Borderas et al., 2009). We suggest that perhaps calves who relapse with BRD have difficulty in drinking their milk meal without pauses due to the inflammation of the airways and coughing associated with a relapsed BRD bout (Love et al., 2016). Thus, relative changes in drinking speed may be a useful indicator of recovery status in calves.

In this study, we observed that relapsed calves had much fewer relative changes in activity patterns (step and activity index) within daily measures and across the post-treatment period compared to relapsed calves. Lethargy is one of the most documented changes associated with the sickness behavioral response in cattle. For example, sickness behavior post-inoculation with BRD associated pathogens in beef cattle has been reflected in increased lying times and lying bouts (Toaff-Rosenstein et al., 2016). For naturally occurring BRD, lower activity index scores in cattle were observed (Marchesini et al., 2018). There is also a myriad of literature which suggests that calves become lethargic for the days prior to BRD diagnosis such as increased lying times, decreased lying bouts, and declines in step activity as reviewed by Costa et al., 2020. Thus, we suggest that lethargy, such as greater lying times, and less step counts is associated with the sickness response in relapsed calves. In contrast, we suggested that the greater relative changes in activity behavior that was observed in recovered calves on our study might be indicative of calves who appropriately responded to antimicrobial intervention.

We also observed fewer lying bouts in our relapsed calves starting at day 4 within the post-treatment period. We suspect altered lying bouts in our relapsed calves suggests a poor affective state in these calves. For example, there is evidence which suggests BRD is a painful disease for calves (Bednarek et al., 1999, Bednarek et al., 2012). Furthermore, a shift in lying behavior, including increased lying times and decreased lying bouts in mature dairy cattle has been associated with painful diseases such as lameness (Ito et al., 2010) and metabolic diseases in dairy cattle (Sepúlveda-Varas et al., 2014). Thus, declined behavioral activity levels in dairy calves may be indicative of BRD recovery status; development of alerts to deviations in behavioral patterns that could be indicators of failed recovery from BRD is warranted.

In this study we observed that feeding behaviors measured by an automated feeder and activity measured by an accelerometer can indicate recovery in calves for the 10 days post-treatment. Precision technology devices that are used for calf management, such as the automated feeder, may allow for feeding more milk without increasing management costs (Kung et al., 1997), or tailoring individual weaning strategies to a calf in a herd setting to encourage calf starter intake to promote rumen development (as reviewed by Khan et al., 2011. Since sickness behavior indicates a motivational state, automated feeders and accelerometers may be used to detect BRD calves failing to respond to antimicrobial interventions. Similarly, accelerometers are a precision technology device used to detect cattle in estrus and are one of the most common precision technologies considered for adoption on a dairy farm (Dolecheck et al., 2016); this is primarily due to reduced labor costs associated with observing cattle for mounting behavior (Silper et al., 2015b). However, in recent years, precision technology devices were observed as an opportunity to also monitor the health of calves in a herd setting, such as the use of automated feeders to detect BRD and the use of accelerometers to detect BRD. Our study suggests there is the potential to use these precision technology devices for a new frontier in animal health: to detect recovery status in BRD calves. To our knowledge, only one study has used precision technology devices as an indicator of BRD relapse status in cattle (Lhermie et al., 2017). For beef cattle, the use of a reticulorumen bolus in real-time was successful at detecting an elevated temperature (e.g., febrile response) in cattle that relapsed from the initial BRD antimicrobial intervention (Lhermie et al., 2017). Moving forward, researchers should incorporate precision technology devices into a collective working system to detect diseases.

Machine learning techniques should be explored to evaluate the potential of an automated feeder and accelerometer to detect recovery status in calves. Machine learning techniques using a precision technology device (e.g., recorded feed intake) improved the accuracy and timing of BRD detection in beef cattle compared to clinical symptoms (Kayser et al., 2018, Kayser et al., 2020). Furthermore, the use of sickness behavior (e.g., feed intake) promoted the detection of BRD, and BRD was detected one to 14 days before clinical diagnosis by the veterinarian (Jackson et al., 2016). Incorporating multiple precision technology devices (e.g., feeding stations, accelerometers, and temperature bolus) further improved the accuracy of BRD detection (compared to using clinical symptoms alone (Kayser et al., 2020) and this occurred sooner than diagnosis by veterinarians. Collectively, we suggest that there is potential to detect recovery status by use of machine learning techniques to detect sickness behaviors in dairy calves, and possibly to have a support therapy to improve the outcome of these animals. Future research should quantify the capability of multiple behaviors recorded by precision technology devices to detect recovery status in calves in real-time, and develop early intervention strategies to these at-risk animals..

Conclusions

The results of our study suggest that recovery status in calves can be monitored with precision technology devices. Recovered calves showed signs of improved behavioral responses after their initial antimicrobial intervention for BRD; these calves showed increased relative changes in their starter intakes and were generally more active compared to relapsed calves. In contrast, relapsed calves expressed sickness behavior over the 10 period after initial antimicrobial intervention, including depressed feeding behaviors (fewer feed intakes and less unrewarded visits) and signs of lethargy. We suggest that relative changes in feeding behavior (starter intake and drinking speed), and activity (lying time, lying bouts, step count, activity index) may be indicative of recovery status in dairy calves. Future research should explore the use of machine learning techniques to develop algorithms from precision technologies to evaluate recovery status from a BRD bout in dairy calves.

Table 4.1 The association of recovery status with feeding behavior and activity levels in preweaned calves treated for the 10 days after antimicrobial intervention for Bovine Respiratory Disease

The association of recovery status with the daily least square means of feeding behaviors recorded by an automated milk feeder including average drinking speed, milk intake, and number of rewarded visits and activity recorded by a pedometer including lying time, step count, acceleration activity index and lying bouts for day of BRD diagnosis (day 0) to day 10 after antimicrobial intervention for Bovine Respiratory Disease in preweaned pair matched calves (n=19 matched pairs) offered 10 L of milk replacer/d. An interaction with treatment and day with recovery status was explored.

ITEM	RECOVER	RELAPSE	SEM	*F*-VALUE DEGREES FREEDOM	RECOVERY STATUS	TREAT* DAY
Milk intake (L/d)	8.89	7.75	0.25	$9.07_{1,18}$	**0.01**	0.19
Drinking speed (L/min)	0.93	0.91	0.06	$0.04_{1,16}$	0.85	0.40
Rewarded visits (visits/d)	4.02	3.73	0.12	$2.71_{1,18}$	0.12	0.48
Lying time (h/d)	16.74	17.93	0.18	$28.21_{1,17}$	< **0.001**	**0.02**
Lying bouts (bouts/d)	18.86	17.10	0.51	$6.08_{1,17}$	**0.02**	0.80
Step count (steps/d)	560.44	322.25	27.64	$48.56_{1,17}$	< **0.001**	< **0.001**
Acceleration activity index[2]	2903.61	1856.96	135.71	$34.86_{1,17}$	< **0.001**	< **0.001**

Table 4.2 The association of recovery status with relative changes in feeding behavior and activity levels in preweaned calves treated for the 10 days after antimicrobial intervention for Bovine Respiratory Disease

The association of recovery status with least square means of relative changes (relative change) in feeding behaviors recorded by an automated feeder including average drinking speed, milk intake, starter intake, number of rewarded and unrewarded visits and relative changes in activity behaviors recorded by a pedometer including the day of BRD diagnosis (day 0) to day 10 after antimicrobial intervention for Bovine Respiratory Disease in preweaned pair matched calves (n=19 matched pairs) offered 10 L of milk replacer/d. Relative changes referred to day 0 as a baseline. An interaction with treatment and day with recovery status was explored.

ITEM	RECOVER	RELAPSE	SEM	*F*-VALUE DEGREES FREEDOM	RECOVERY STATUS	TREAT* DAY
Relative change milk intake %	96.66	101.13	3.01	$1.00_{1,17}$	0.33	0.91
Relative change drinking speed %	111.83	112.72	8.54	$0.01_{1,18}$	0.95	0.18
Relative change rewarded visits %	100.49	105.62	4.28	$0.73_{1,17}$	0.41	0.44
Relative change unrewarded visits %	100.38	104.97	1.75	$3.11_{1,18}$	*0.09*	0.93
Relative change starter intake %	115.82	112.57	3.1	$0.57_{1,17}$	0.46	0.05
Relative change lying time %	94.79	98.67	0.86	$9.14_{1,17}$	0.01	0.04
Relative change lying bouts %	112.21	107.82	3.10	$0.91_{1,18}$	0.35	0.63
Relative change step count %	255.91	141.78	29.77	$5.77_{1,17}$	0.03	0.01
Relative change index[2] %	235.21	150.05	25.33	$5.06_{1,17}$	0.04	0.02

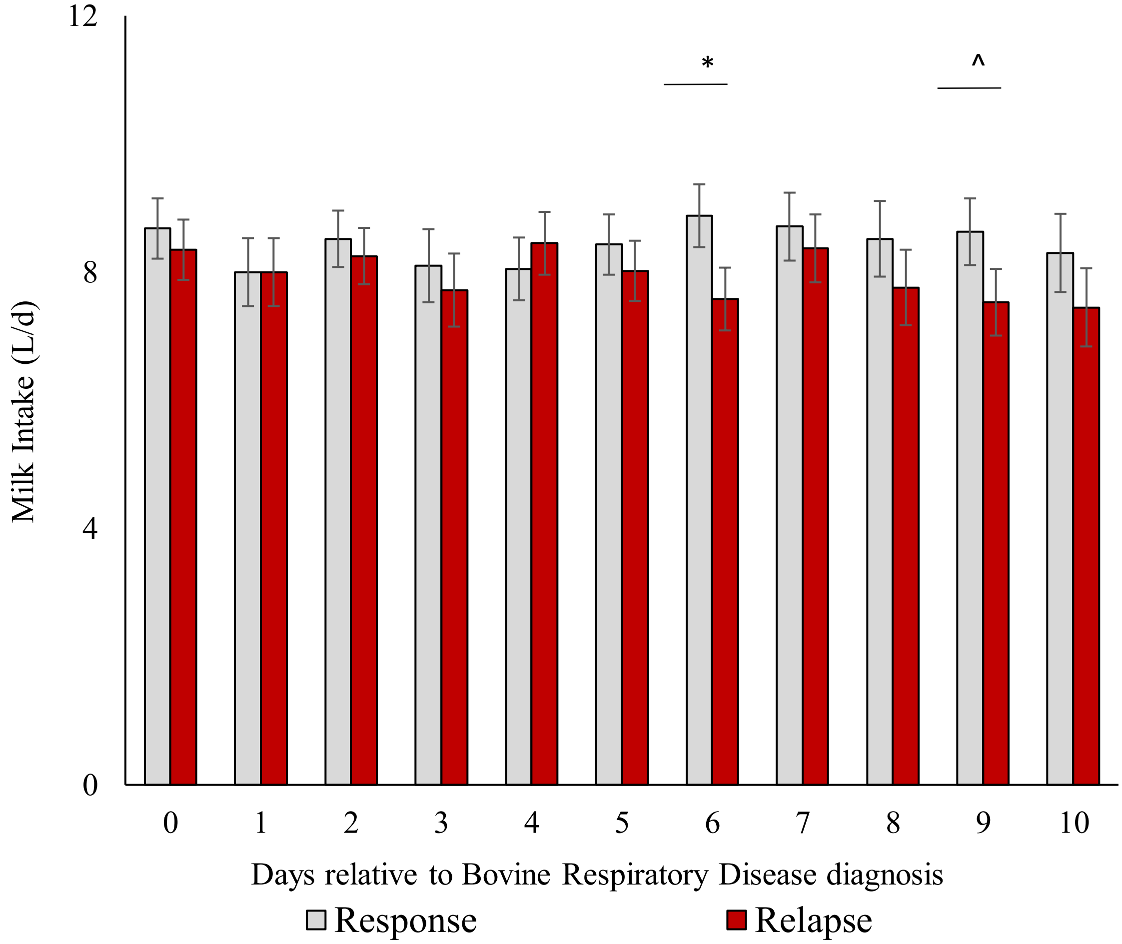
Figure 4.1. a-c The association of recovery status with feeding behavior in dairy calves within a 10-day period after antimicrobial intervention for Bovine Respiratory Disease

The association of recovery status[1] with milk intake **(1a)**, drinking speed **(1b)**, and rewarded visits **(1c)** (LSM ± SEM) by day for preweaned, pair-matched calves (n=19 pairs) treated for Bovine Respiratory Disease and offered 10 L of milk replacer/d by an automated feeder.

1Bovine respiratory disease (BRD) status was defined as a clinical score of ≥ 5 and lobar lung consolidation ≥ 3 cm^2 and all calves were treated for BRD on day 0. Recovery status was defined as calves who either resolved symptoms of BRD within 10 days post-antimicrobial treatment (recovered) or had clinical BRD status from days 10 to 14 (relapsed)

Significance*($P \leq 0.05$) and tendency^ ($P < 0.10$) indicate differences by BRD status by day

(a)



12
8
4
0
Milk Intake (L/d)
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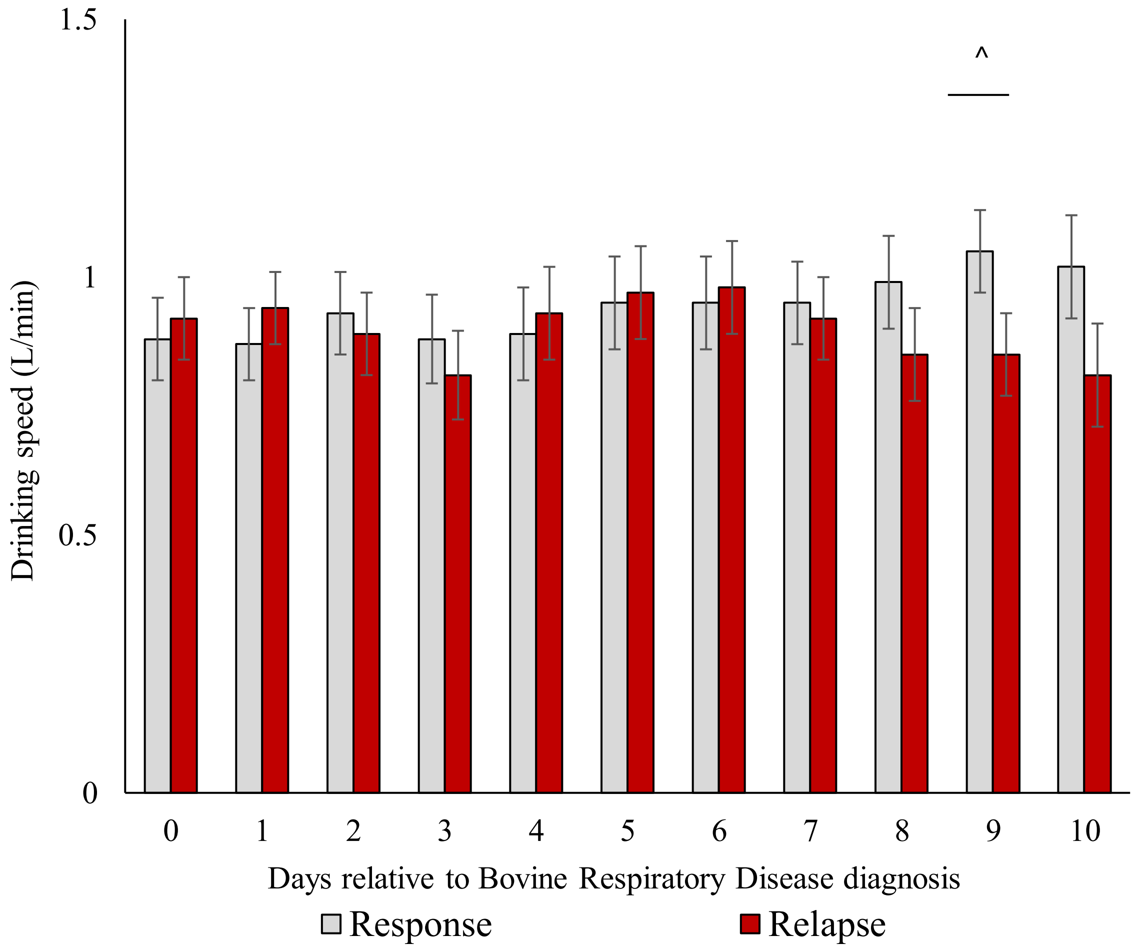
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(b)



1.5
1
0.5
0
Drinking speed (L/min)
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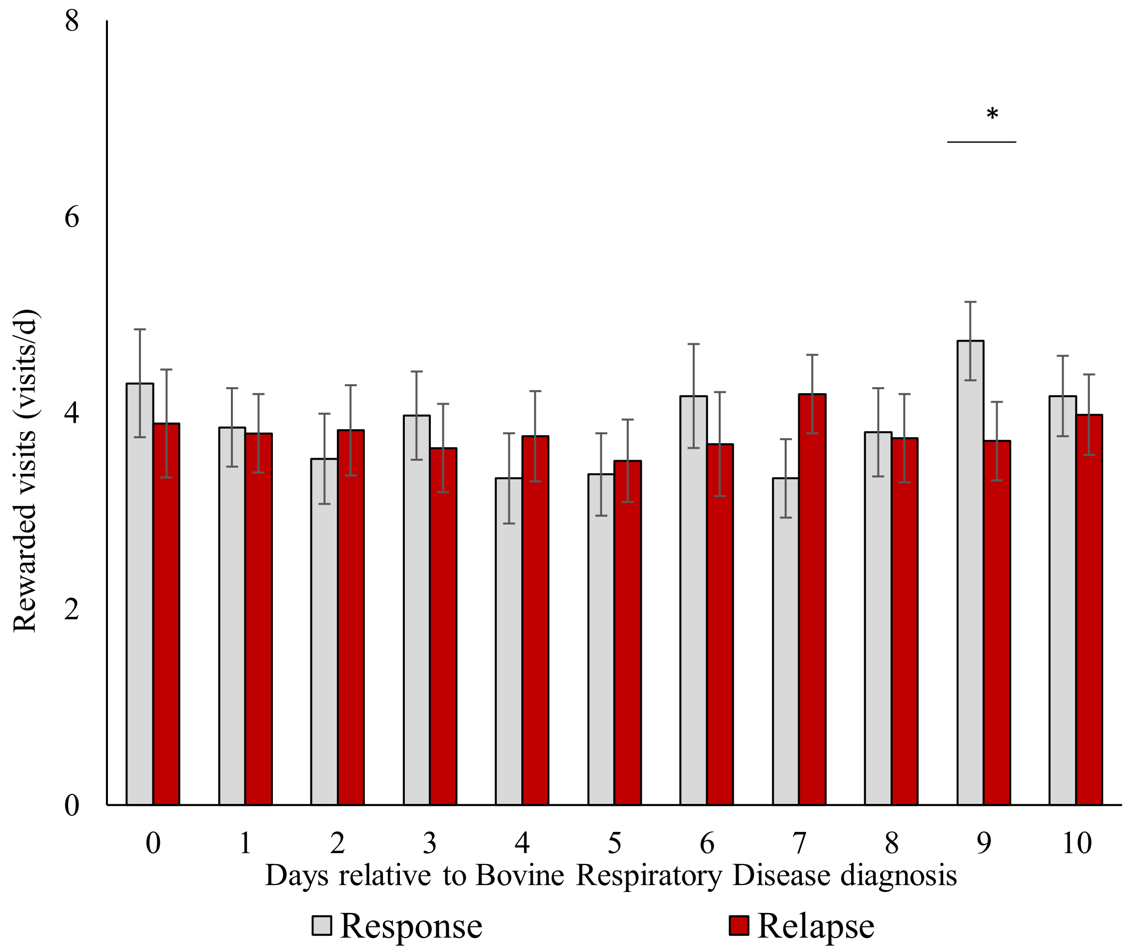
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(c)



8
6
4
2
0
Rewarded visits (visits/d)
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0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

Figure 4.2. a-d The association of recovery status with activity levels in dairy calves within a 10-day period after antimicrobial intervention for Bovine Respiratory Disease

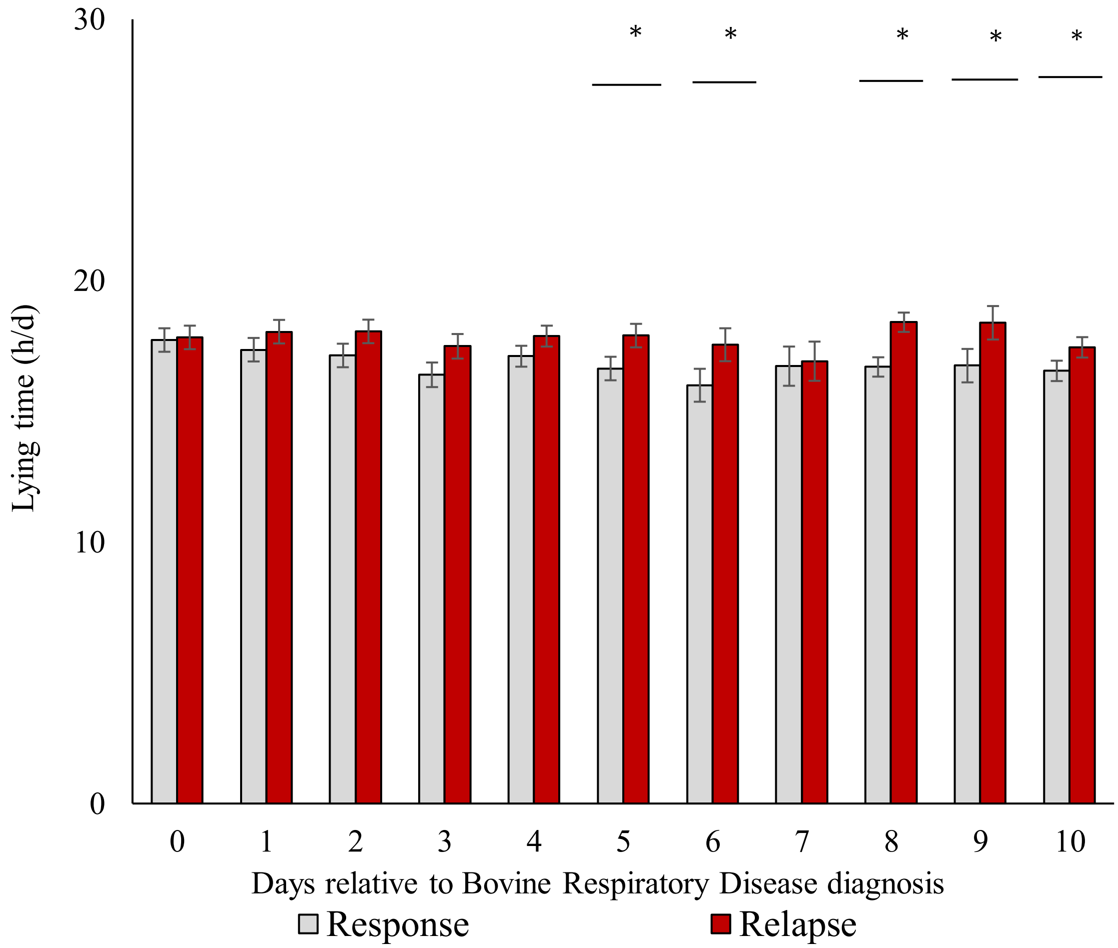
The association of recovery status[1] with daily lying time **(2a)**, lying bouts **(2b)**, step counts **(1c)**, and acceleration activity index[2] **(2d)** (LSM ± SEM) by day for preweaned, pair-matched calves (n=19 pairs) treated for Bovine Respiratory Disease and offered 10 L of milk replacer/d by an automated feeder.

1Bovine respiratory disease (BRD) status was defined as a clinical score of ≥ 5 and lobar lung consolidation ≥ 3 cm2 and all calves were treated for BRD on day 0. Recovery status was defined as calves who either resolved symptoms of BRD within 10 days post-antimicrobial treatment (recovered) or had clinical BRD status from days 10 to 14 (relapsed).

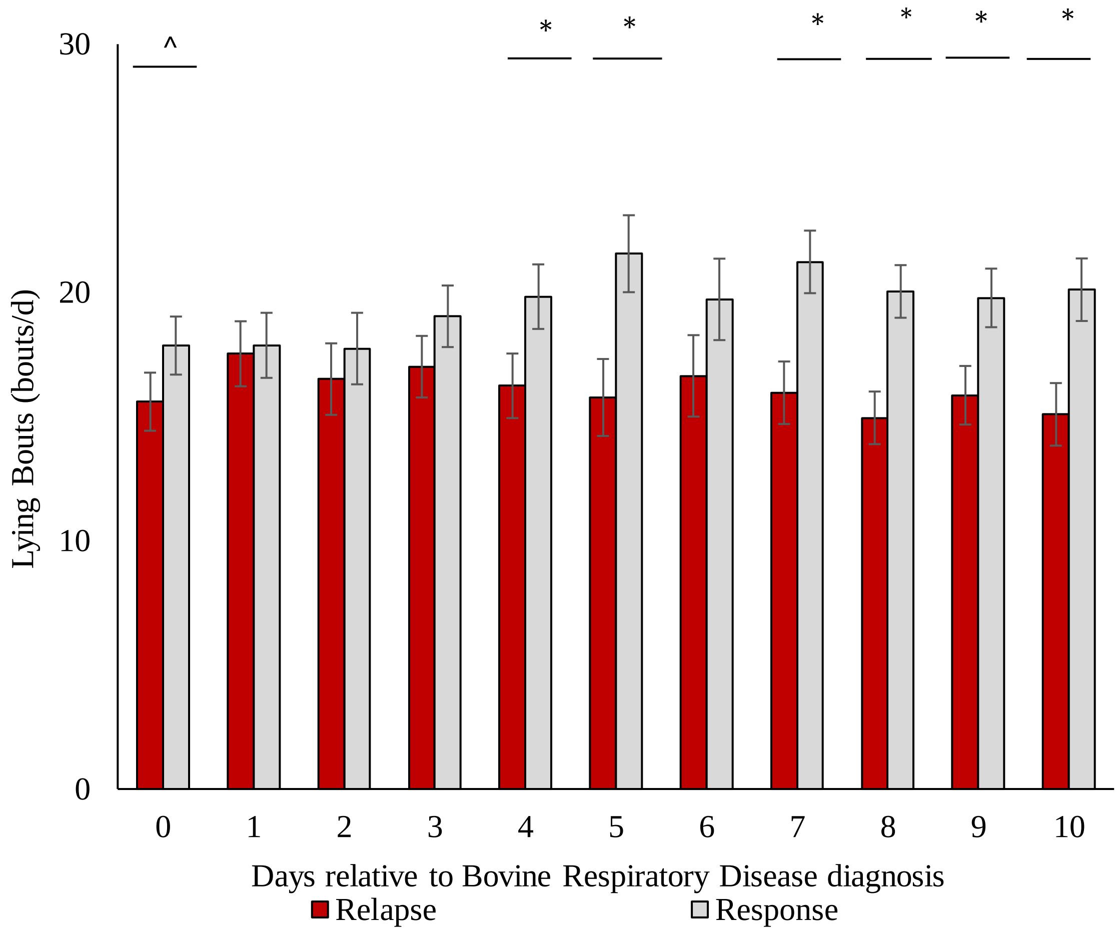
2The acceleration activity index was generated by a commercial algorithm (IceRobotics, Scotland) by recording the average daily rate of acceleration and step count using a commercial accelerometer attached to the calf's rear left leg

Significance*($P \leq 0.05$) and tendency^ ($P < 0.10$) indicate differences by BRD status by day

(a)

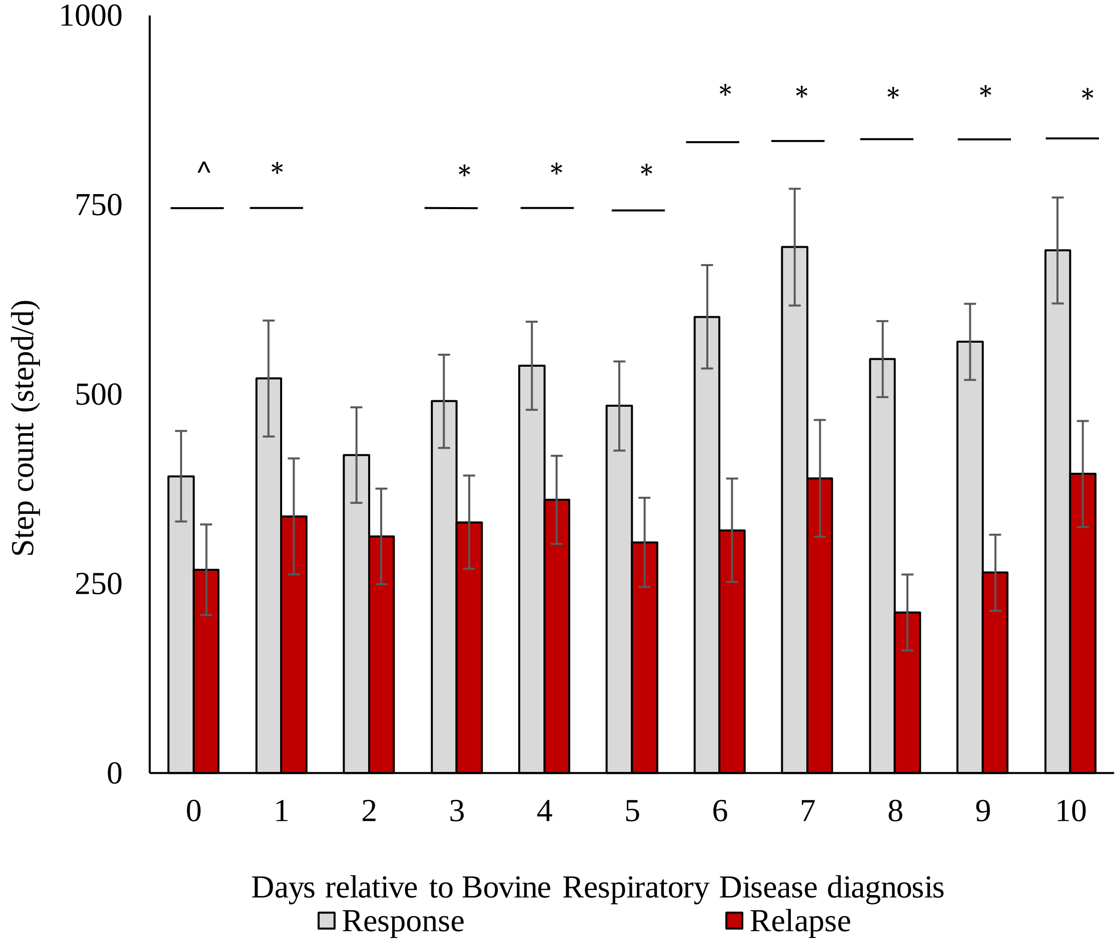

30
20
10
0
Lying time (h/d)
*
*
*
*
*
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(b)



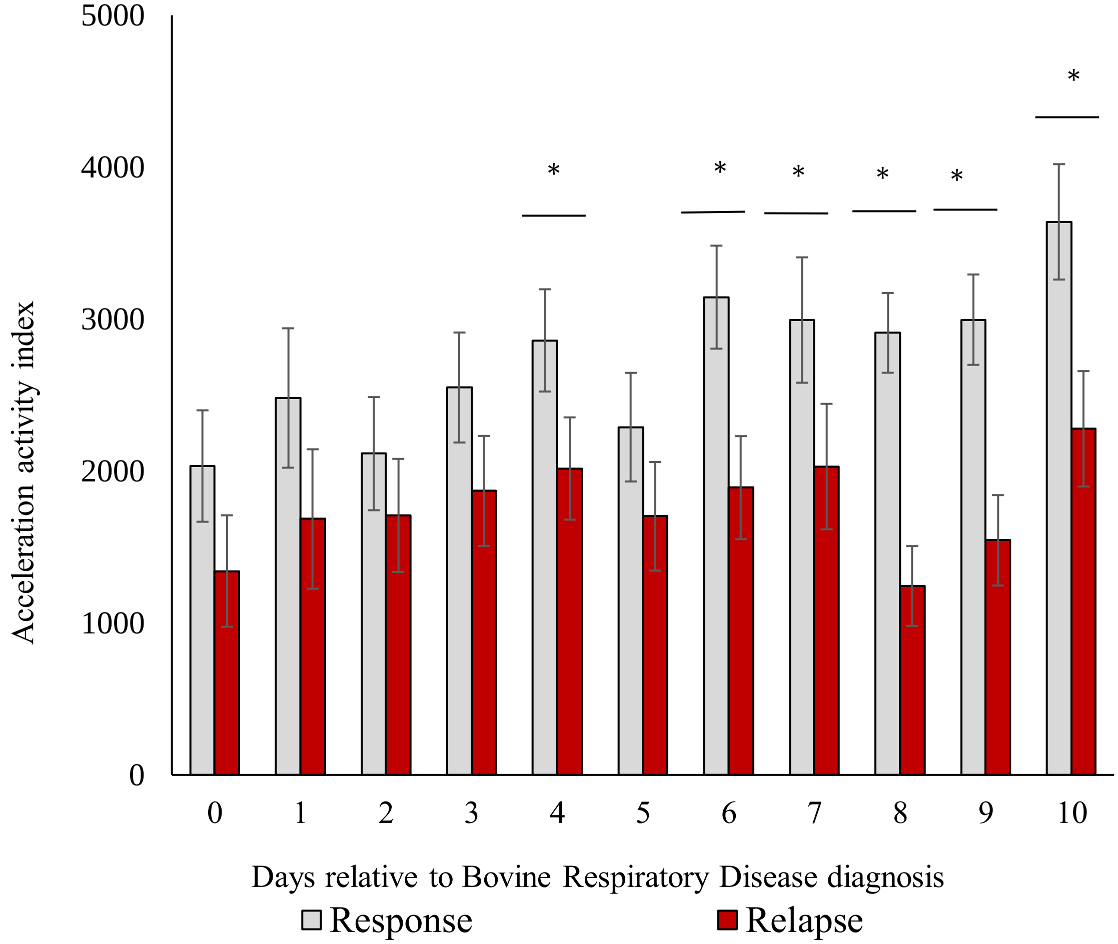
^
*
*
*
*
*
*
30
20
10
0
Lying Bouts (bouts/d)
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Relapse
Response

(c)



1000
750
500
250
0
Step count (stepd/d)
^
*
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(d)



5000
4000
3000
2000
1000
0
Acceleration activity index
*
*
*
*
*
*
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

Figure 4.3. a-e The association of recovery status with relative changes in feeding behavior in dairy calves within a 10-day period after antimicrobial intervention for Bovine Respiratory Disease

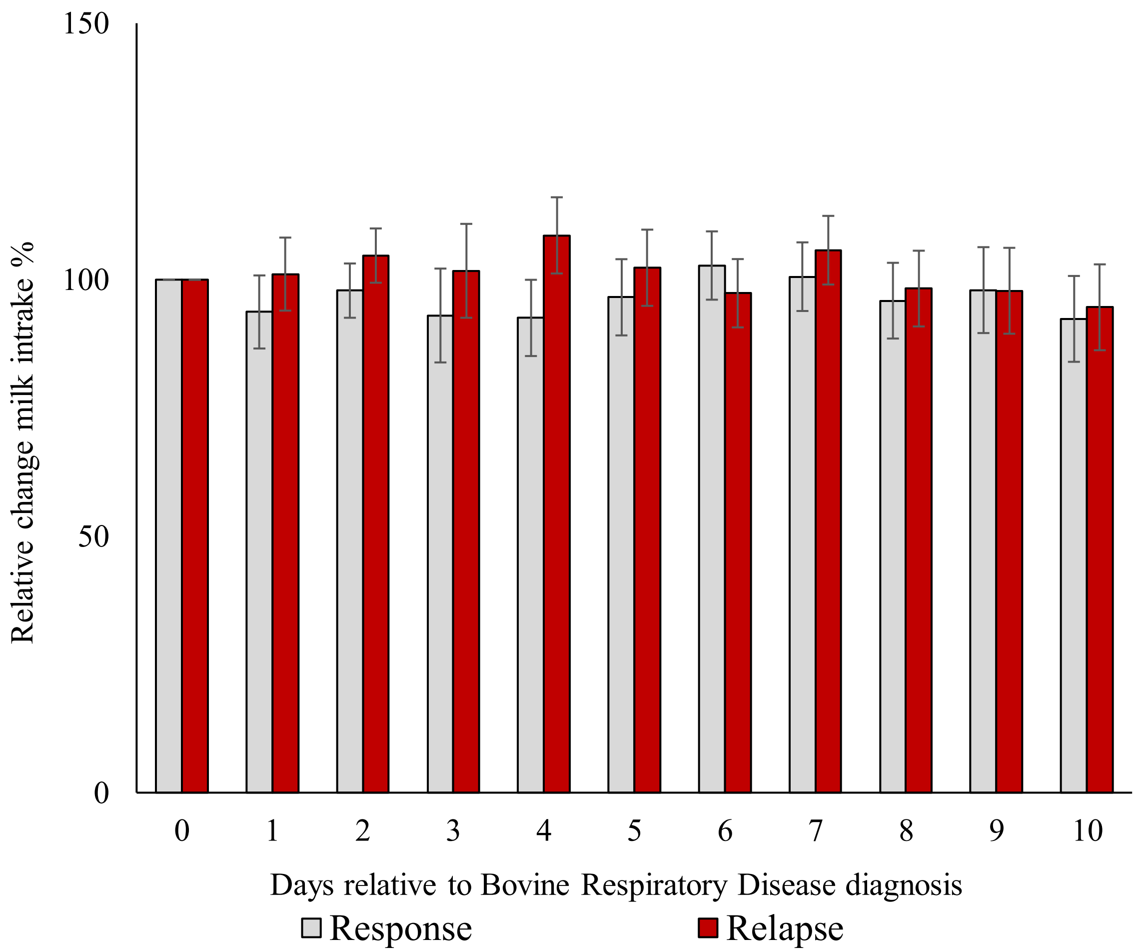
The association of recovery status[1] with relative changes[2] in milk intake % (**3a**), relative changes in drinking speed % (**3b**), relative changes in rewarded visits % (**3c)**, relative changes in starter intake (**3d)**, and relative changes in unrewarded visits (**3e)** (LSM ± SEM) by day for preweaned, pair-matched calves (n=19 pairs[3]) treated for Bovine Respiratory Disease and offered 10 L of milk replacer/d by an automated feeder.

1Bovine respiratory disease (BRD) status was defined as a clinical score of ≥ 5 lobar lung consolidations ≥ 3 cm2 and all calves were treated for BRD on day 0. Recovery status was defined as calves who either resolved symptoms of BRD within 10 days post-antimicrobial treatment (recovered) or had clinical BRD status from days 10 to 14 (relapsed)

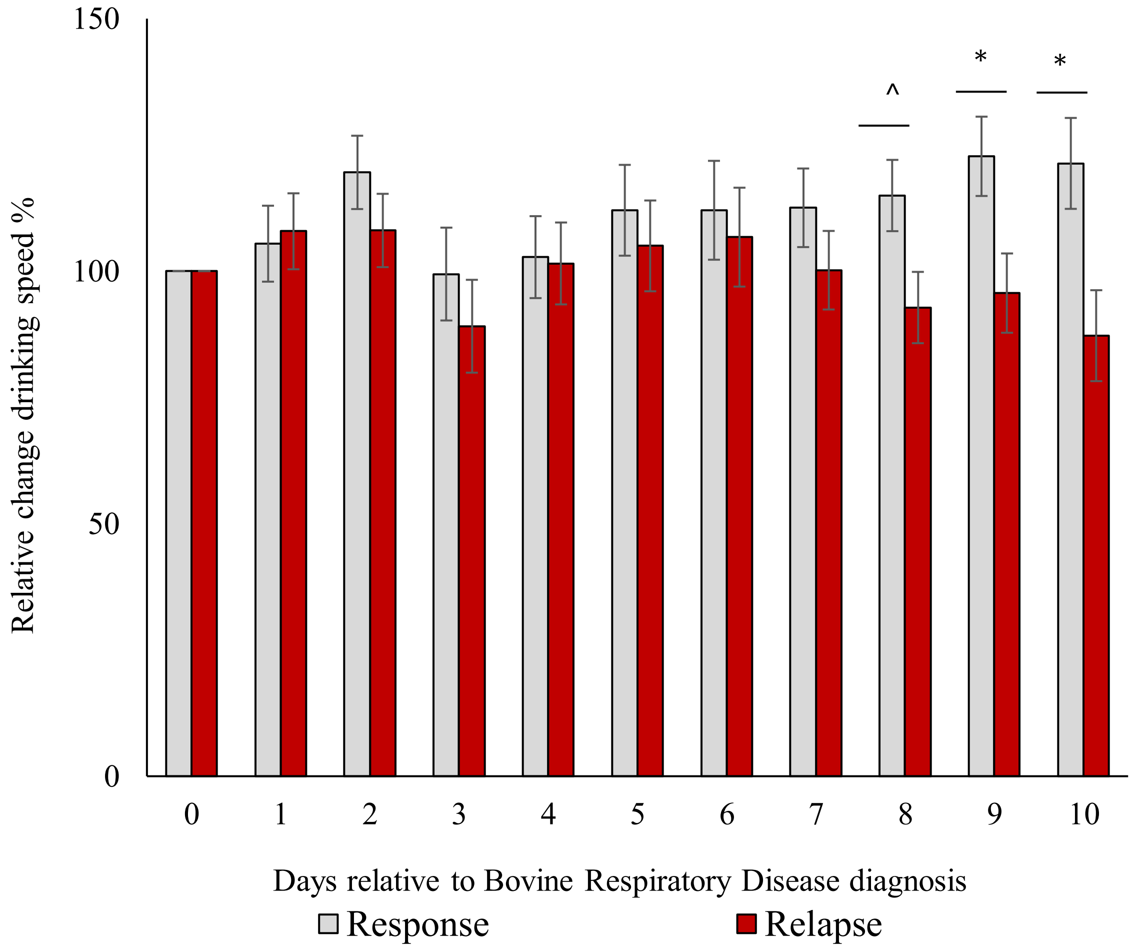
2Relative changes were calculated with day of antimicrobial treatment as a baseline (100%); relative changes were generated by dividing each day after BRD diagnosis (day 1 to day 10) by the baseline (day 0)

Significance*($P \leq 0.05$) and tendency^ ($P < 0.10$) indicate differences by BRD status by day

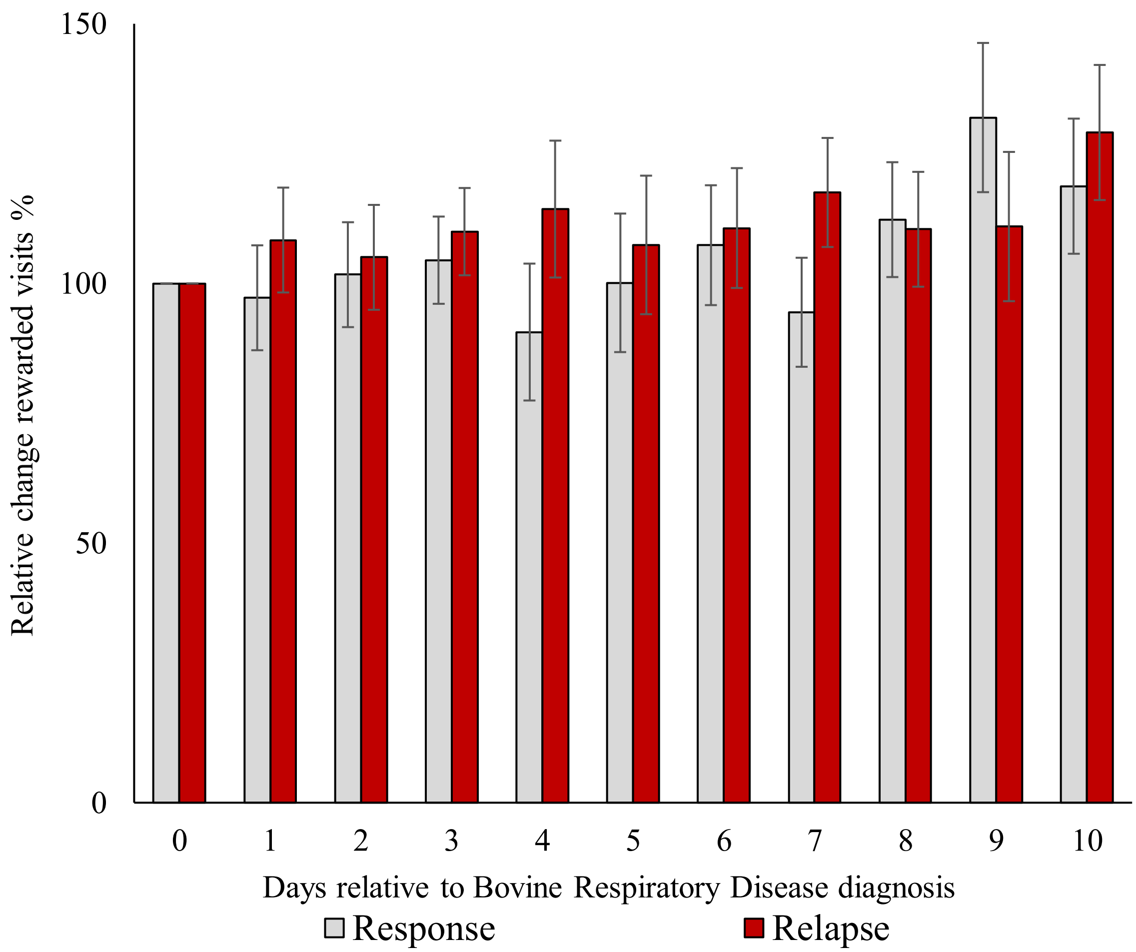
(a)


150
100
50
0
Relative change milk intrake %
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

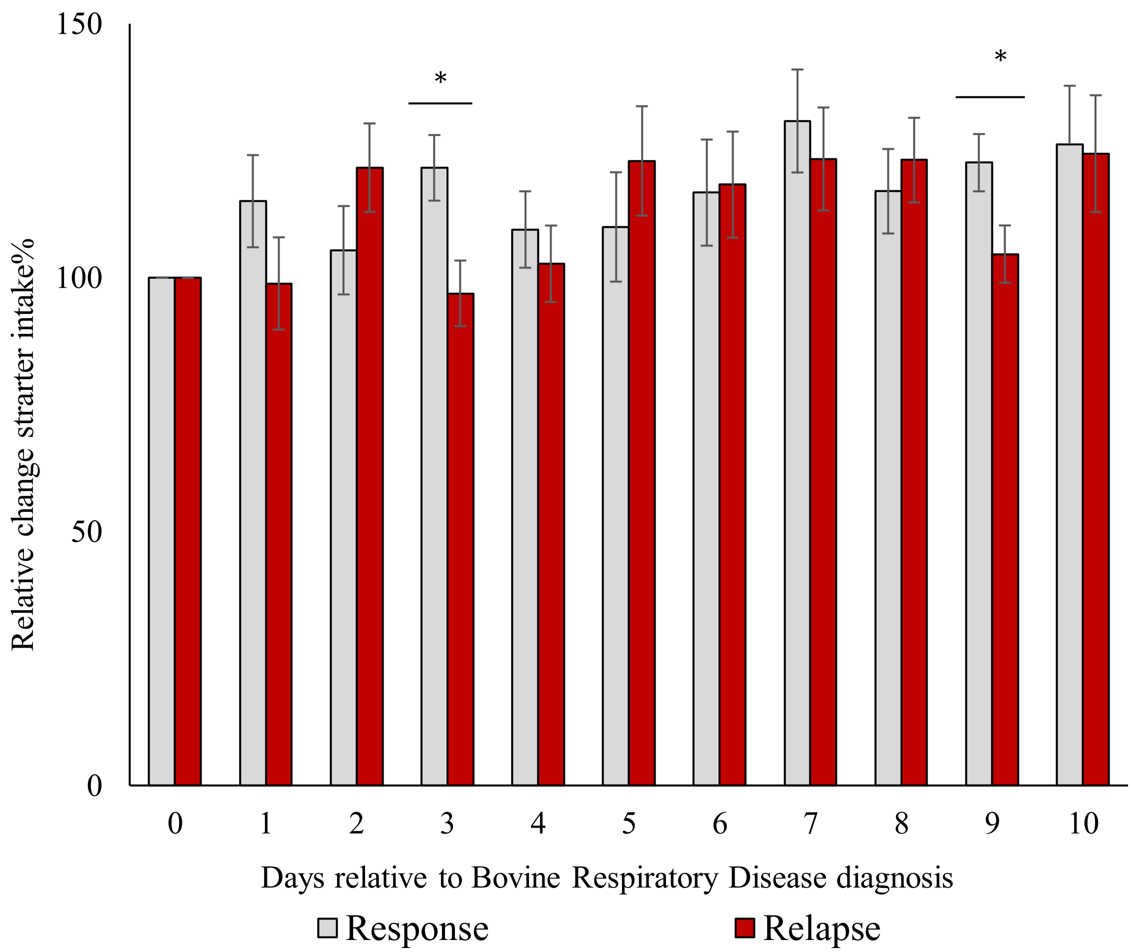
(b)


150
100
50
0
Relative change drinking speed %
^
*
*
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(c)


150
100
50
0
Relative change rewarded visits %
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(d)



150
100
50
0
Relative change strarter intake%
*
*
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(e)

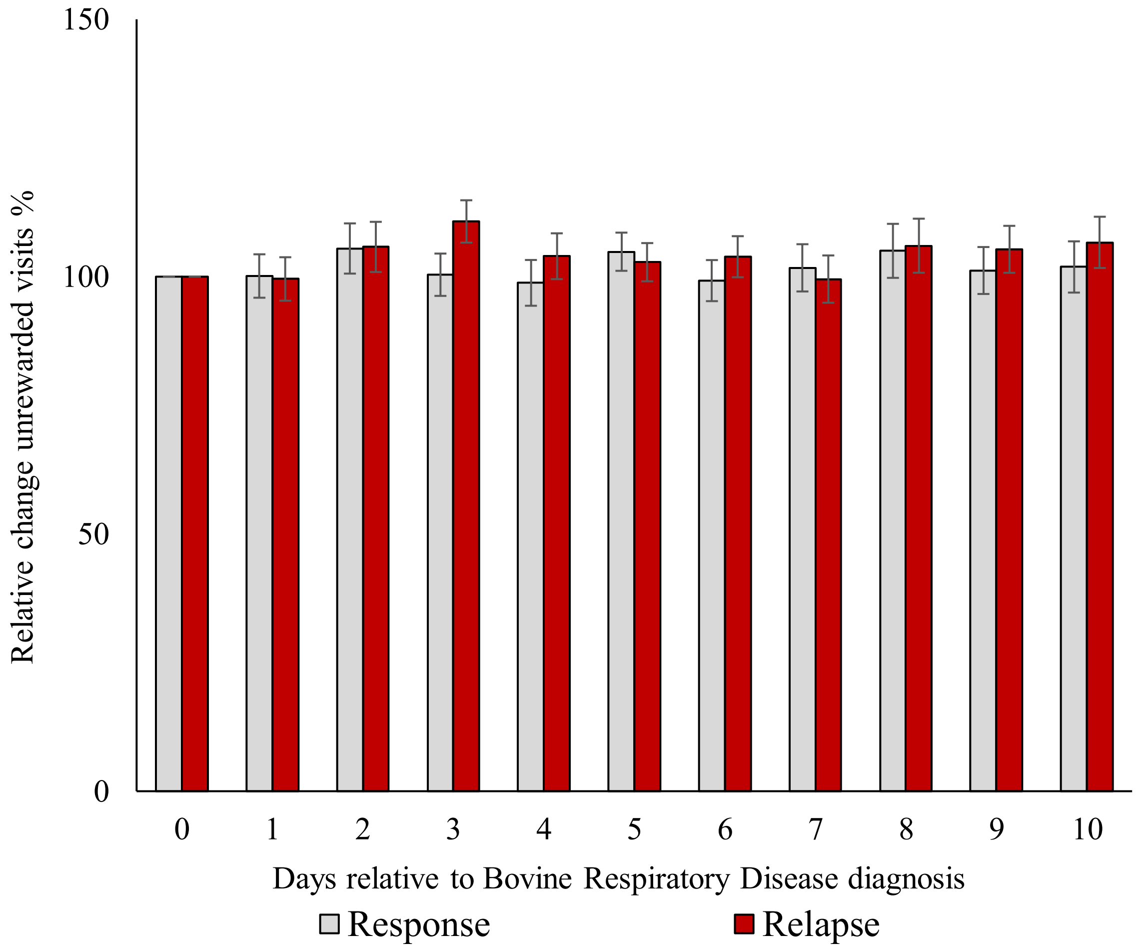

150
100
50
0
Relative change unrewarded visits %
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

Figure 4.4. The association of recovery status with relative changes in activity levels in dairy calves within a 10-day period after antimicrobial intervention for Bovine Respiratory Disease

The association of recovery status[1] with relative changes[2] in lying time (**4a**), relative changes in lying bouts (**4b)**, relative changes in step counts (**4c)**, and relative changes in the acceleration activity index[3] % (**4d)** (LSM ± SEM) by day for preweaned, pair matched calves (n=19 pairs) offered 10 L of milk replacer/d, and starter, *ad libitum* by an automated feeder.

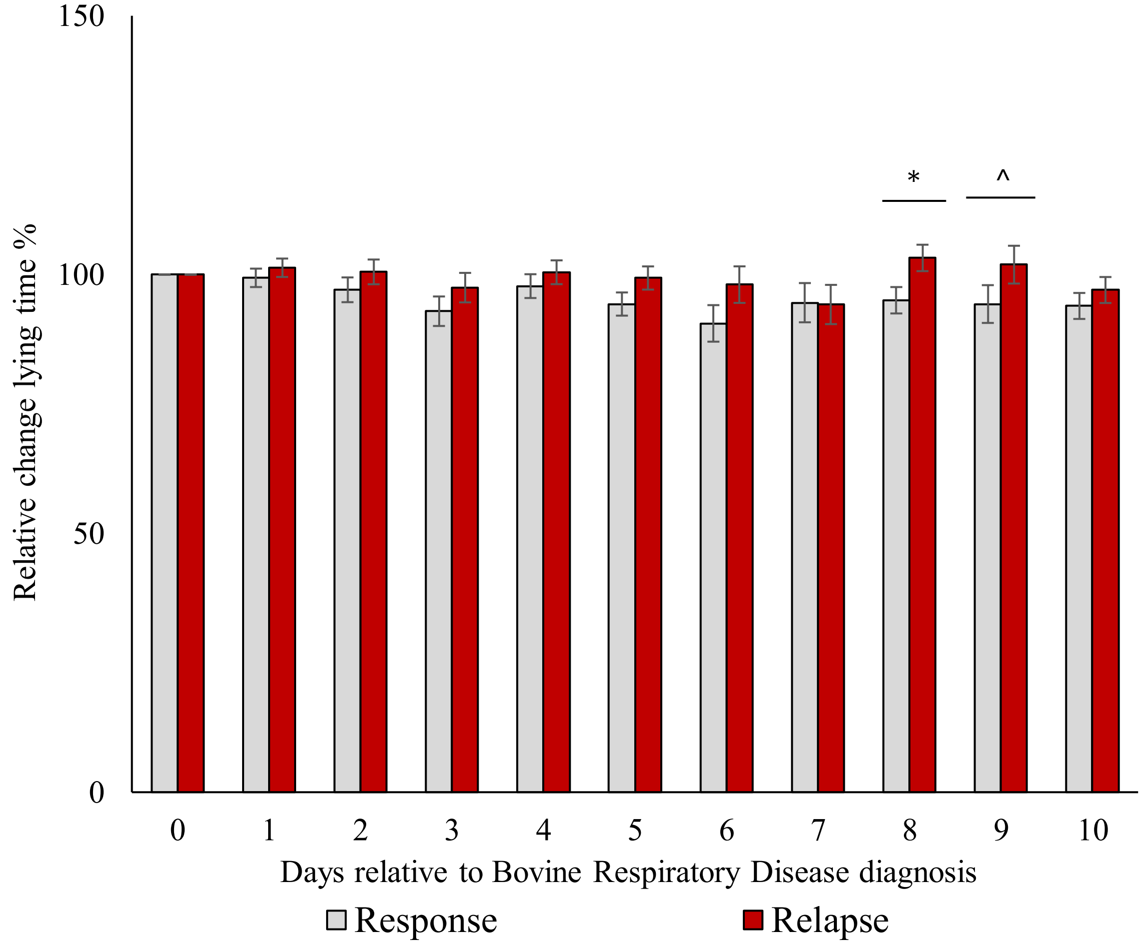
1Bovine respiratory disease (BRD) status was defined as a clinical score of ≥ 5 and lobar lung consolidation ≥ 3 cm^2 and all calves were treated for BRD on day 0. Recovery status was defined as calves who either resolved symptoms of BRD within 10 days post-antimicrobial treatment (recovered) or had clinical BRD status from days 10 to 14 (relapsed)

2Relative changes were calculated with day of antimicrobial treatment as a baseline (100%); relative changes were generated by dividing each day after BRD diagnosis (day 1 to day 10) by the baseline (day 0)

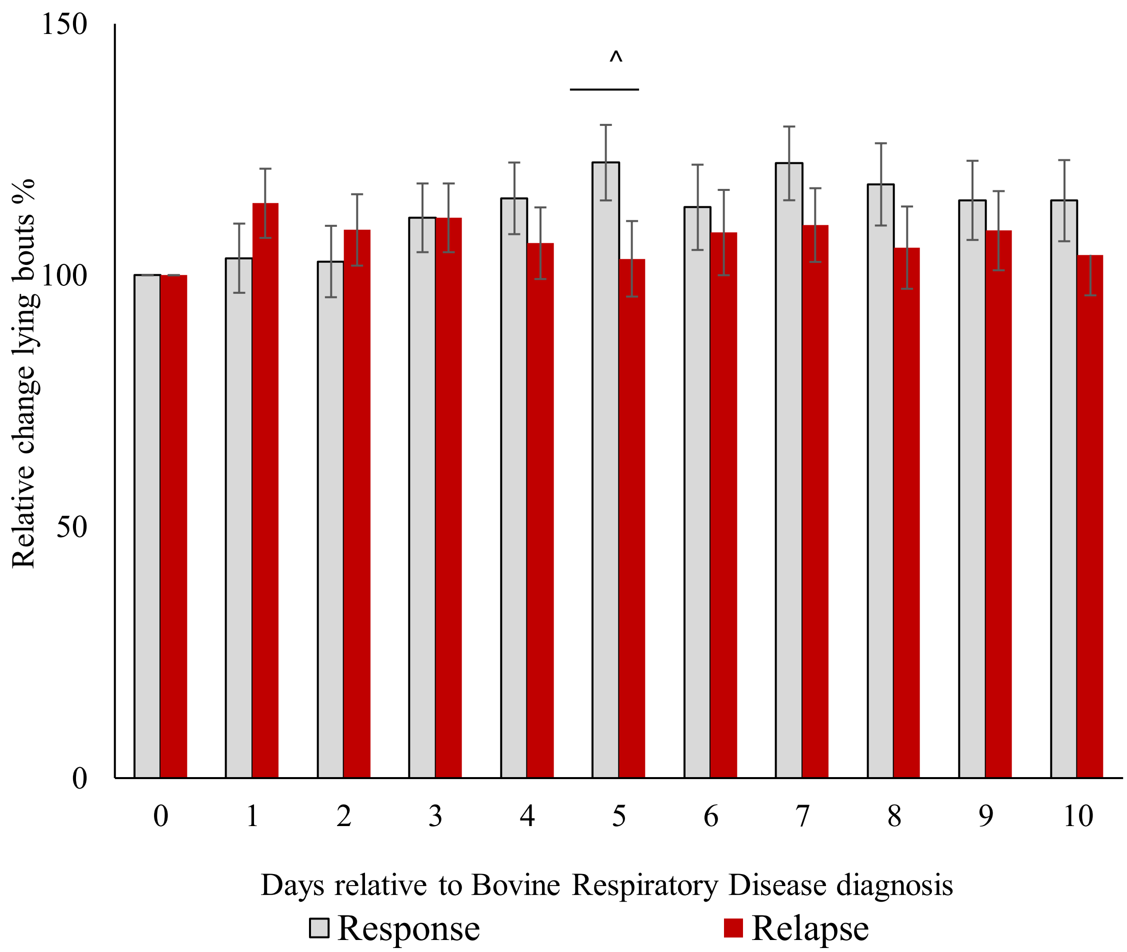
3The acceleration activity index was generated by a commercial algorithm (IceRobotics, Scotland) by recording the average daily rate of acceleration and step count using a commercial accelerometer attached to the calf's rear left leg

Significance*($P \leq 0.05$) and tendency^ ($P < 0.10$) indicate differences by BRD status by day

(a)

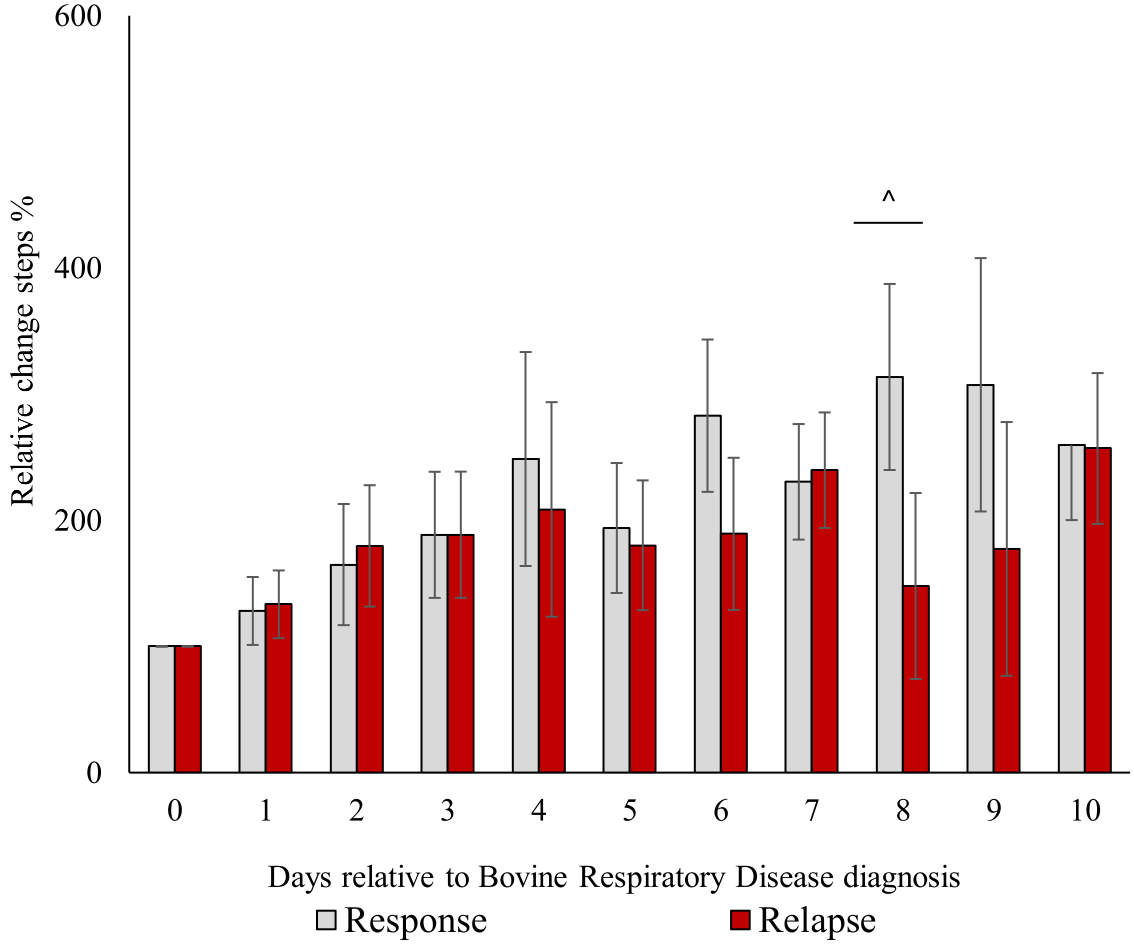

150
100
50
0
Relative change lying time %
*
^
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(b)



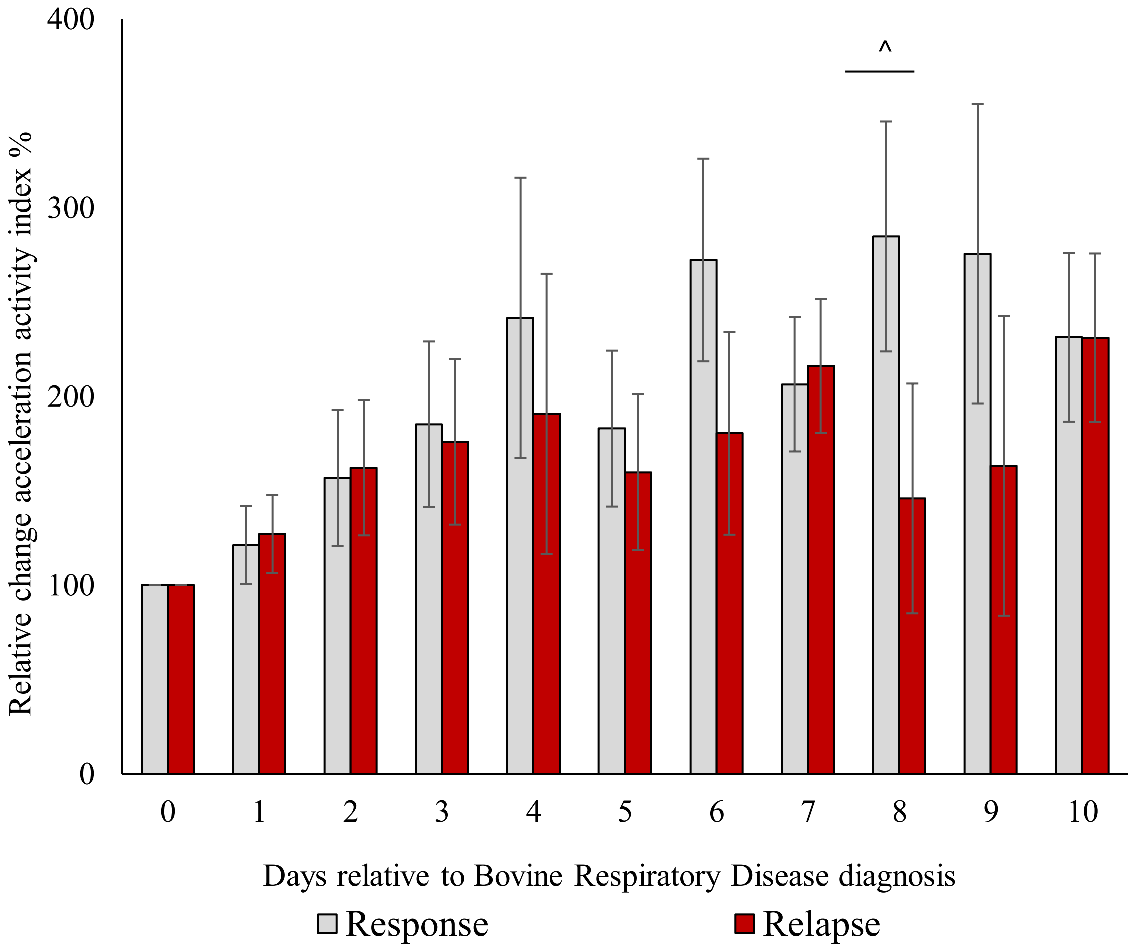
150
100
50
0
^
Relative change lying bouts %
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(c)



600
400
200
0
Relative change steps %
^
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

(d)



400
300
200
100
0
Relative change acceleration activity index %
^
0
1
2
3
4
5
6
7
8
9
10
Days relative to Bovine Respiratory Disease diagnosis
Response
Relapse

CHAPTER 5. DISSERTATION GENERAL DISCUSSION AND CONCLUSIONS

The objective of this dissertation was to determine if feeding behavior recorded by an automated feeder and activity levels recorded by an accelerometer were indicators of sickness behaviors in preweaned dairy calves for initial bovine respiratory disease (BRD_ detection, nutraceutical intervention, and to detect recovery status from BRD after antimicrobial intervention. Similarly, this dissertation aimed to describe true BRD development and BRD recovery in dairy calves. This research objective was chosen since a literature review revealed that multiple surveys suggested BRD, and diarrhea were the leading causes of morbidity and mortality of replacement heifers. Similarly, these diseases have welfare implications for the calf, and are sustainability issues due to being a contributor to antimicrobial usage on farm. Scoring systems commonly used to indicate BRD in calves also have limited operator sensitivity, suggesting a need to passively identify potential disease bouts in calves. Thus, it was warranted to not only describe sickness behavior in naturally diseased calves but determine if precision technology devices can detect disease development, serve as a tool for an intervention strategy, and detect disease recovery in dairy calves.

Precision dairy technologies were selected since precision livestock researchers in this area found that many dairy producers already have automated feeders and accelerometers on farm, and this industry was open to implementing these technologies. However, there was a gap in the literature detected; associations between feeding behavior and activity levels with disease bouts in calves were never cohort observational studies. These studies were either cross-sectional studies, providing one snapshot of prevalence in time, or studies which health scored calves infrequently (e.g., twice a week, or weekly). Similarly, much of the literature investigated health associations in relation to disease in calves as a secondary objective (i.e., disease challenge studies, or clinical trials). While those types of studies are warranted, the possibility of investigating disease development through time in calves was lacking. Similarly, there was no current knowledge on if calves modified their own individual behavioral patterns (relative change) prior to disease diagnosis and during disease recovery. Moreover, while preventative feeding of nutraceuticals such as colostrum had been lightly investigated, it was of interest to see if feeding behavior deviations could be used to identify and ameliorate disease severity of calves developing BRD.

To answer this dissertation's objective, a cohort of 120 calves were followed daily for health events to identify true disease development, and disease recovery status in calves. Calves were health scored for diarrhea, navel ill, and BRD for the first consecutive 90 days of life. Calves were fed 10 L/d milk replacer by automated feeder (milk intake, drinking speed, calf starter intake, rewarded and unrewarded visits), and calves wore an accelerometer at birth (lying time, lying bouts, step count, and an acceleration activity index). Twice weekly, weights were taken, and lungs were evaluated for true consolidation. Since stress was a variable associated with increasing the likelihood of BRD in the literature, calves were provided an enriched social environment that was not nutritionally restrictive.

The first study objective described the feeding behavior and activity levels of preweaned dairy calves for the five days before BRD diagnosis. Of the cohort of calves, 33/49 BRD bouts were enrolled, and pair matched to healthy calves. Then, a pair-match analysis was performed, considering day 5 before BRD diagnosis as a behavioral baseline. It was revealed in this study that calves destined to have BRD expressed sickness behavior, while healthy calves remained stable (e.g., relative changes in behaviors in healthy calves were 100% across multiple days). Specifically, BRD destined calves declined in their milk intake, starter intake, unrewarded visits, and were less active (less steps, and reduced indexes) compared to healthy calves within those 5 days before diagnosis. Moreover, compared to behavioral baseline day 5, soon to be sick calves also had negative relative changes in their starter intake, and their step counts, suggesting a motivational state of sickness behavior compared to healthy calves. This study suggests that relative changes in calf behavior for the few days leading up to disease may indicate a BRD bout. However, the study retrospectively evaluated these relationships, so future research for algorithm development is needed.

The second study was performed in parallel to the first study. It was of interest to use colostrum as a feeding intervention strategy to ameliorate a disease bout. Colostrum was selected as proof-of-concept, since the evidence suggested that preventative colostrum feeding lowered disease likelihood in multiple species. The health alarms were generated from the precision technology data, which were triggered by 84 calves who had a deviation from their 12-day rolling average milk intake or drinking speed, leading to a unique self-enrollment protocol. Calves received either a placebo by bottle of milk replacer, or one dose of colostrum divided across three days. There was no effect of colostrum replacer on ameliorating a diarrheal bout, nor did it improve calf performance. However, colostrum replacer lowered the likelihood of a BRD bout, and ameliorated the BRD bout severity in colostrum recipient calves. One limitation to this study is that we were not able to identify which constituent or even which combination of constituents in colostrum replacer improved disease outcomes for dairy calves developing a bout of disease. Future research is needed to determine which constituents in colostrum replacer help dairy calves recover from a BRD bout.

The final study investigated if recovery status from the initial antimicrobial intervention for a BRD bout was associated with feeding behavior and activity levels in calves. Since timely disease detection improves a calf's outcome, and since this disease is painful for calves, this study aimed to seek an additional utility for precision technology devices on farm. Of the cohort of calves, 19 calves failed to respond and were re-classified as relapsed calves 14 days after the initial antimicrobial intervention. Then, a pair-match analysis to BRD calves who recovered was performed, considering the day of initial diagnosis as a baseline. Recovered calves show improved behavioral responses to treatment such as nearly double relative changes in their activity levels compared to relapsed calves, and greater starter intakes and unrewarded feeder visits across the 10 days after the initial diagnosis. Moreover, relapsed calves had fewer relative changes in their activity levels and starter intake, suggesting that sickness behavior further depressed feeding and encouraged lethargy within the 10 days compared to recovered calves. One limitation to this study was that it was retrospectively analyzed, thus further research is needed to develop algorithms. However, this evidence suggests that precision technology devices can also monitor for recovery status in recently treated calves. This is a new frontier for this technology, and sickness behavior may aid in detecting relapsed calves sooner.

In summary, this dissertation found that feeding behavior and activity levels are sickness behaviors which decrease for up to four days prior to clinical diagnosis for BRD calves. Similarly, when dairy calves had decreased feeding behavior and colostrum replacer was fed, there was less likelihood of BRD, and less severe disease. However, colostrum did not improve diarrhea or performance, so more research is needed to determine what nutrient or compound in colostrum replacer affected BRD in these calves. Moreover, calves who received antimicrobial treatments and recovered showed an increase of exploratory activity (i.e., greater relative changes in activity levels and feeding behavior) compared to relapsed calves. Furthermore, relapsed calves had negative relative changes in their feeding behavior and activity, suggesting that feeding depression and lethargy indicate sickness behavior in relapsed BRD calves. This dissertation answered that BRD development in calves may be associated with the motivational sickness response, and nutraceutical intervention may ameliorate it. Future research should investigate the potential of machine learning techniques to determine which combination of these behaviors indicates BRD; similarly, this research may be used to determine the optimal sampling frequency for disease detection in calves, but more research is required.

APPENDIX 1

The Mean ± (SD) chemical composition of calf starter (Baghdad Feeds, KY) and hay (sourced from the UK research station) offered to a cohort of 120 preweaned Holstein calves assessed daily for Bovine Respiratory Disease and fed 10 L/d milk replacer, and calf starter by automated feeder.

Variables	Calf Starter[1]	Hay[2]
% DM Basis		
DM %	89.47 ± 0.77	93.74 ± 2.03
CP %	22.94 ± 1.10	13.70 ± 2.72
Fat %	4.17 ± 0.34	1.57 ± 0.30
NDF %	13.48 ± 1.15	62.45 ± 7.40
ADF %	5.81 ± 0.58	43.57 ± 2.11
Starch %	35.64 ± 1.63	ND[3]
Ash %	5.81 ± 0.58	8.15 ± 0.72
Calcium %	1.13 ± 0.17	0.68 ± 0.21
Phosphorus %	0.57 ± 0.04	0.39 ± 0.05
Magnesium %	0.21 ± 0.02	0.22 ± 0.06
Potassium %	1.29 ± 0.07	2.23 ± 0.35
Sulfur %	0.27 ± 0.02	15.07 ± 3.02
ME (Mcal/kg)[4]	2.84 ± 0.01	2.09 ± 0.06

[1] Special Calf Starter and Grower, Baghdad Feeds, Baghdad, KY
[2] Late cut alfalfa farm hay (*Medicago sativa)* was provided to calves in a trough.
[3] ND = not determined
[4] ME = TDN × 0.04409 × 0.82; calculated according to (NRC, 2001) calculations

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VITA

1. Education

    a. Chaparral High School, Temecula California, 2003-2006

    b. Mt. San Jacinto College, Menifee, CA, 2007-2009

       Degree awarded: NA.

    c. University of Kentucky, Lexington, KY, 2009-2011

       Degree awarded: Bachelor of Science, Animal Science

    d. University of Wisconsin-Madison, Madison, WI, 2013-2015

       Degree awarded: Master of Science, Dairy Science

2. Professional positions

   Shift Supervisor, Starbucks, 2009-2015

   Dairy Herd Improvement Technician, Agsource Dairy, 2015-2017

3. Scholastic and professional honors

   Three-minute thesis, University of Kentucky

   Third Place, and People's Choice

   Intercollegiate Animal Welfare Judging Assessment

   Multiple placings 2014, 2018, 2019

   Invited Speaker at the Dairy Cattle Welfare Council, 2019

4. Professional publications

a. **Cantor, M.C**., D.L. Renaud, and J.H.C. Costa. Nutraceutical intervention with colostrum replacer: can we reduce disease hazard, ameliorate disease severity, and improve performance in preweaned dairy calves? J. Dairy Sci.; 104(6):7168-7176.

b. Reis, M.E., A. Toledo, A.P. da Silva, M. Poczynek, E. Fioruci, **M.C. Cantor**, L. Greco, and C.M.M. Bittar. Supplementation of Lysolecithin in milk replacer for Holstein dairy calves: effects on growth performance, health, and metabolites. J. Dairy Sci. 104(5): 5457-5466.

c. Woodrum Setser, M. M., **M. C. Cantor,** and J. H. C. Costa. 2020. A comprehensive evaluation of microchips to measure temperature in dairy calves. J Dairy Sci. 103(10):9290-9300.

d. Costa, J. H. C., **M. C. Cantor,** and H. W. Neave. 2020. Symposium review: Precision technologies for dairy calves and management applications. J. Dairy Sci. 104(1): 1203-1210.

e. **Cantor, M. C**., C. H. Pertuisel. and J.H.C. Costa. 2020. TECHNICAL NOTE: Validation of a partial weight scale attached to an automated feeder for estimating daily weight in dairy calves. J. Dairy Sci. 103(2):1914-1919.

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g. **Cantor, M. C**., J.H.C. Costa, and H.W. Neave. 2019. Current perspectives on the short- and long-term effects of conventional dairy calf raising systems: a comparison with the natural environment. Transl. Anim. Sci. 3(1): 549-563.

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j. Falk, M.L., **M.C. Cantor**, M. Hayes, J. Jackson, and J.H.C. Costa. 2018. Validation of radio frequency identification with a current transducer to quantify the use of an automatic grooming brush in pre-weaned dairy calves. In: 10th International Livestock Symposium (ILES X). (p. 1). Am. Soci. Ag. Biol. Eng.

k. Costa, J. H. C., **M. C. Cantor**, and H. W. Neave. 2018a. Bovine Diet. Encyclopedia of Animal Cognition and Behavior. J. Vonk and T. Shackelford, ed. Springer International Publishing, Cham.1-6.

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Extension Articles

a. Rice, E.M., **M.C. Cantor**, M.M. Woodrum Setser and J.H.C. Costa. From birth to weaning: a calf care guide. A poster published on behalf of: Kentucky Dairy Development Council. March 2021.

b. Costa, J.H.C., **M.C. Cantor,** H.W. Neave and M.E. Reis. Duas cabeças pensam melhor que uma! Por que adotar o sistema de alojamento de bezerras em pares. Leite Integral. October 2020.

c. **Cantor, M.C.,** H.W. Neave and J.H.C. Costa. Effectively raising pair housed calves: common questions from transitioning farmers. Progressive Dairy. September, 2020.

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f. Costa, J.H.C., H.W. Neave and M.C. Cantor. What you need to know before, during and after transitioning to group housing of dairy calves: key considerations. Western Canadian Dairy Seminar. University of Alberta, 2018.

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