OBJECTIVE MONITORING OF CATTLE

by

MILES E. THEURER

B.S., Kansas State University, 2012
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
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Abstract

There are multiple modalities available to evaluate health or stress status of animals. The objective of my research was to evaluate different modalities including behavior, rectal and nasal temperature, and blood samples to determine the relationship with these outcomes of interest in bovine respiratory disease (BRD) events, environmental conditions, transportation, and *Mannheimia haemolytica* challenge model. The objective for the final project was to determine whether diagnostic sensitivity or specificity resulted in greater economic value for the industry using simulation models for identification of BRD.

There was a positive association with rectal temperature and probability of not finishing the production cycle normally, but this relationship was not linear. Rectal temperature of feedlot calves at first treatment for BRD had limited value as a prognostic indicator of whether those calves would finish the production cycle normally. A positive association between rectal temperature and ambient temperature and temperature-humidity index was determined. Environmental conditions must be considered when rectal temperature is used as a diagnostic tool.

At 48 hours after initiation of transportation there were no differences in body weight, rectal temperature, and time spent at various locations in the pen detected between transported and non-transported control heifers. Transportation of heifers during periods of high ambient temperatures caused transient changes in physiologic and behavioral indices of heifers. Calves challenged with *Mannheimia haemolytica* had more changes in behavior, body weight, and blood biomarkers during high ambient temperatures compared to control calves. Results of this study may guide research in development of objective assessment tools for identification and management of cattle affected with BRD during extreme summer conditions.

For both low and high apparent prevalence cohorts, increasing diagnostic specificity resulted in more rapid, positive change in net returns compared to change in increasing sensitivity. Improvement of diagnostic specificity, perhaps through a confirmatory test or pen-level diagnostics, can increase diagnostic value. Mortality risk was the primary driver for net returns. Results from this study are important for determining future research priorities to analyze diagnostic techniques for BRD and provide a novel way for modeling diagnostic tests.

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Dedication

I dedicate this to my family.

Preface

The body of work in the following chapters integrates the use of different modalities in order to evaluate the health and well-being of animals in different stressed and diseased states. There are multiple methodologies available in order to remotely observe animals to determine pain or disease state. The veterinary literate uses multiple modalities in order to evaluate health or disease state in cattle. Those modalities involve clinical observations and remote technologies monitoring behavior and temperature (Chapter 1), or more invasive methods utilizing rectal temperature, body weight, or blood parameters (Chapter 2). We evaluated the relationship between rectal temperature at first treatment of bovine respiratory disease in calves and the probability of not finishing the production cycle (Chapter 3), environmental conditions on rectal and nasal temperatures (Chapter 4), transportation of beef heifers on physiologic and behavior indices during high ambient temperatures (Chapter 5), physiologic and behavior responses of calves challenged with *Mannheimia haemolytica* during high ambient temperatures (Chapter 6), and determined whether it was more valuable to improve diagnostic sensitivity or specificity for diagnosis of bovine respiratory disease (Chapter 7). The results from these chapters provide some quantification and baseline responses of some different modalities used to monitor health and well-being of cattle. These results will be important to prioritize future research diagnostic techniques for cattle.

Chapter 1 - Remote non-invasive assessment of pain and health status in cattle

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Synopsis

Cattle behavior is frequently monitored to determine health and wellness state of the animal. The objective of this review is to describe potential benefits and challenges of remotely monitoring cattle behavior with available methodologies including clinical illness scores, visual monitoring, accelerometers, pedometers, feed intake and behavioral monitoring, global position systems, real time location systems, thermography images, and rumen telemetry temperature bolus. The behavior of interest, labor required, and monitoring expenses all need to be taken into consideration before deciding which remote behavioral monitoring device is most appropriate. Monitoring the feeding behavior of an animal over a period of time allows establishment of a baseline against which deviations in subsequent behavioral patterns can be evaluated. Interpretation of multiple behavioral responses as an aggregate indicator of animal wellness status rather than as individual outcomes may be a more accurate measure of true state of animal pain or wellness status.

Key Points

- Cattle behavior is frequently monitored to determine health and wellness state.
- Available remote monitoring systems include:
    - clinical illness scores
    - visual monitoring
    - accelerometers
    - pedometers
    - feed intake and behavioral monitoring
    - global position systems
    - real time location systems
    - thermography images
    - rumen telemetry temperature bolus
- Selection of remote behavioral monitoring system is influenced by:
    - the behavior of interest (frequency and type)
    - labor required to monitor the animals
    - monitoring expenses
- Interpretation of multiple behavioral responses as an aggregate indicator of animal wellness status rather than as individual outcomes may be a more accurate measure of true state of animal pain or wellness status.

Keywords: behavior monitoring, remote sampling, animal welfare

Introduction

The ability to remotely identify cattle that require an intervention due to pain or disease is important for animal health providers and researchers. Behavior is frequently monitored to measure potential changes in animal well-being (Gonyou, 1994). Stress, pain, or disease may alter animal behavior relative to optimal wellness status, but monitoring these changes is challenging without a clear definition of the expected behavioral response to an adverse event (Levitis et al., 2009). Some behavioral definitions are vague, and they are not specifically tied to one pain or disease response. Improvement in behavioral monitoring techniques is needed for remote monitoring of activity to be useful as a diagnostic or research tools.

Multiple methods are available to monitor cattle behavior including subjective visual observation, objective measures of cattle activity, or determination of cattle location within the housing area. Subjective measurements of pain and cattle well-being include behavioral, depression, or illness scores based on observer impression of the animal's current wellness state. The challenges with utilizing subjective measures to determine cattle wellness state are related to potential differences both between observers and among observers over time.

An opportunity exists to more discretely identify potential behavioral changes via collection of data utilizing remote sensing technologies. Objective, continuous behavioral monitoring using accelerometers and pedometers has been used to assess cattle behavior in a variety of scenarios (Dockweiler et al., 2012; Hanzlicek et al., 2010; Pauly et al., 2012; Robert et al., 2009; Theurer et al., 2012a; Theurer et al., Accepted, In Press; Theurer et al., 2012b). Monitoring cattle location within a defined environment has also been used in an effort to identify and monitor potential behavioral changes (Theurer et al., 2012b; White et al., 2012).

The objective of this review is to describe potential benefits and challenges of remotely monitoring cattle behavior with available methodologies including clinical illness scores, visual monitoring, accelerometers, pedometers, feed intake and behavioral monitoring, global position systems, and real time location systems. Although all of these remote monitoring systems are not directly applicable in a clinical setting, the results from research based on these technologies provides valuable insights to practitioners on the associations between behavioral changes and pain and wellness states.

Observer monitoring clinical illness

One of the most common methods to determine wellness or painful state of an animal is having a trained observer monitor cattle for clinical signs of pain or disease. Multiple clinical signs and subjective assessments can be used to determine the animal's overall wellness status. Often a combination of findings can be categorized into a single value, or clinical illness score (CIS), which represents the current state of the animal. The potential benefit of determining a CIS is presumably that it correlates with the need for an intervention or the probability of a specific outcome (Hayes et al., 2010). Scoring systems that assign a value based on degrees of illness are relatively common (Perino and Apley, 1998) and are frequently used in disease research (Coetzee et al., 2012; Hanzlicek et al., 2010; White et al., 2012). Even when quantitative measurements, such as rectal temperature, are combined with subjective assessment,

the final disease classification remains subjective (Sanderson, 2006; Wenz et al., 2006). This subjectivity may impact how the results are interpreted if the CIS is used as one of the criteria in a treatment or preventative health program.

Research has shown very limited agreement among observers using the same CIS to identify calves with respiratory disease (Amrine et al., 2013). Potential sources of variation include differences among the experience and training of observers, cattle type, and environmental conditions. When a subjective scoring system is applied and interpreted by more than one individual, it should be repeatable among those individuals. Others have evaluated agreement among veterinarians assigning body condition scores to cows and determined even small amounts of training among the observers can increase the overall agreement (Kristensen et al., 2006). A clear case definition and educational programs can decrease the variation between observers and make the results more clinically applicable.

Although CIS are frequently utilized, true accuracy relative to disease state is difficult to determine. There is no gold standard to diagnose respiratory disease in cattle, but the presence or absence of pulmonary lesions at harvest has been compared with ante mortem diagnoses of clinical respiratory disease (Schneider et al., 2009; Thompson et al., 2006; Wittum et al., 1996). Results from these studies illustrate low correlations between lung scores and diagnosis of clinical illness. White and Renter (2009) estimated the sensitivity and specificity of using clinical signs of illness combined with rectal temperature to diagnose respiratory disease to be 61.8 % and 62.8 %, respectively. A test with imperfect sensitivity and specificity can underestimate or overestimate morbidity, thus leading to errors in the interpretation of preventative or therapeutic treatment efficacy (Amrine et al., 2013).

One way to improve CIS agreement among observers is the implementation of a refined scoring system with limited categories. The objective of assigning CIS to cattle is to accurately identify those animals which need an intervention (sensitivity) and those that would not (specificity); therefore, the system could be condensed to those two categories. If calves are deemed to require an intervention, the selection of the intervention would be based on clinician's judgment of the case. For example, a calf that was deemed to have clinical respiratory disease may require an intervention with an antimicrobial; while euthanasia may be a more appropriate intervention for an animal severely ill enough they become moribund and non-responsive to human approach. Dichotomizing the results would help agreement among observers and could

potentially increase accuracy of comparison of CIS among individual observers, as previous research has illustrated distinguishing illness severity based on CIS is challenging (Amrine et al., 2013; White et al., 2012). Much of the analysis of CIS data is based on the dichotomization of an animal into healthy or sick, therefore systems that have more than two main levels serve limited purpose.

Monitoring clinical illness by visual appraisal is a common procedure and the specific implementation of the scoring system influences final data interpretation. Although CIS are quantitative, they may not be repeatable between or among observers and do not provide an objective measure of the degree of clinical illness. Care should be taken to limit potential sources of variability among observers through training and selection of the appropriate scoring system for the situation.

Observer monitoring frequency of specific behavior

Comparing calf wellness status among treatment groups in a research environment or over time in a clinical application can also be performed by monitoring the frequency of specific behaviors associated with pain or disease. Researchers have noted increase in specific behaviors such as the number of head shakes, ear twitches, and foot stomps after a painful procedure such as castration (Gonzalez et al., 2009; Mellor, 1991; Robertson et al., 1994). Other researchers have documented a difference in head shakes and ear twitches following dehorning (Graf, 1999; McMeekan et al., 1999; Morisse et al., 1995; Stilwell et al., 2010; Vickers et al., 2005).

The frequency of all these behaviors has been associated with increased cortisol concentrations, and increased cortisol concentrations are often associated with stress and fearful events (Grandin, 1997; Mormede et al., 1982). However, neither cortisol nor counts of these behavior measurements have been determined as specific indicators of pain. Calves may increase counts of ear flicks, tail switches, and foot stomps following painful procedures, but these behaviors may also increase with high insect burden (Harris et al., 1987; Hillerton and Bramley, 1986). These behavioral counts are not specific for pain or wellness status, but they are cost effective and relatively easy to obtain through live observations or video analysis.

The most cost effective method to determine the frequency of these behaviors is having an observer document the activities as they occur in the field. A limitation of this method is that behavioral activities occurring at rapid rates (e.g. ear flicks) can be challenging to accurately record as they occur (Altmann, 1974). In a population environment, recording these behaviors on

more than one animal simultaneously can also be challenging. Cattle activity may also be difficult to interpret when the observer is in close enough proximity to document specific behaviors, as studies have shown the presence of a human observer to alter cattle behavior (Grignard, 2000; Ishiwata et al., 2006). Cattle behavioral patterns change throughout the day following a circadian rhythm (Robért et al., 2011), but it is difficult for an observer to continuously document cattle behavior for 24 hours a day. Due to these limitations, monitoring of these behaviors is commonly performed through video analysis.

The use of the video collection technology allows observers to analyze cattle behavior at their leisure and enables observation of tail flicks, ear twitches, stomping, postural behavior, and positional location just as collected during live observation. Video recording systems are relatively easy to set up and can be used in most scenarios. The output files can be viewed on a variety of common electronic devices including laptop computers and DVD players, allowing for minimal additional input costs to view the videos. The required quality of the video recording system is based on the specific behavior desired to document, the number of animals to observe, and environmental conditions.

Limitations of video analysis include the need to clearly identify and visualize individual animal activity, as well as the labor required to document the frequency of specific behaviors. Identification of individual animals is important to document while observing multiple animals in a pen level setting when the experimental unit is the individual, but identification on the video may be difficult using only a visual identification ear tag or coat coloration patterns. Animals may be uniquely marked with all-weather paint sticks, spray paint, and hair dye to ease the ability to identify animals on video, but all of these markings will wear away making it necessary to apply multiple times. Determining frequency of behavioral counts is difficult in low ambient light, but adding artificial lights has shown to increase the amount of time dairy cows spend lying down and reduced distance traveled (Phillips and Schofield, 1989). Depth perception is decreased while watching video footage compared to live observation that can make it challenging to determine if animals are actually eating or drinking or just spending time near the feeder or water. Another issue with video observation is the labor involved to view and document all the behavior activity desired.

Video viewing is a time consuming and tedious task. Software exists to make data recording of animal behavior easier for the viewer while watching the video (Hänninen, 2009;

Morrow-Tesch et al., 1998). Video sampling methods have been evaluated to reduce the amount of labor required to analyze the video. Continuous, scan, time, and focal animal sampling have all been used to minimize the labor required to classify segments of video, yet still accurately determine animal behavior.

Continuous sampling is observing animal activity for the entire period that data were captured at the same speed that video was recorded. Scan sampling is observing animal behavior for a brief period, then repeating the observation after a period of time (Mitlohner et al., 2001b). The portion of time passed between recording samples is the scan interval and is set at a pre-determined length. The frequency of behavioral activity monitored during the observation period is used to represent the percentage of behavior activity over the entire period of time (Colgan, 1978). Scan sampling has been shown to accurately evaluate frequency of cattle behaviors compared to continuous sampling, but when the scan interval was ≥ 30 minutes (a 30 minute gap between sampling periods), correlation to continuous sampling decreased (Mitlohner et al., 2001a).

Time sampling is identifying behavior for a period of 10 minutes at the beginning of each hour and then multiplying the frequency of behavioral activity by 6 to represent activity for the entire hour (Arnold-Meeks and McGlone, 1986). Time sampling has low correlation coefficients compared to continuous monitoring for describing standing, lying, feeding, drinking, and walking activity (Mitlohner et al., 2001a). This low correlation makes time sampling a less accurate method for classifying cattle behaviors based on recorded video.

Focal sampling is the monitoring of a portion of animals within the group for the entire period to determine behavioral activity for the group. Focal sampling of 1 animal out of 10 animals per pen was accurate for describing all 10 animals standing, lying, feeding and walking behaviors; however watering behavior required observing 4 out of 10 animals per pen to accurately describe drinking behavior (Mitlohner et al., 2001a). Individual animal variation in the behaviors of interest may influence accuracy of focal sampling, but this technique may be appropriate for some pen level studies. Observing video clips at the rate of 4 times faster than recorded speed has accurately depicted swine feeding and watering behavior in confined settings compared to real-time recording speed (Arnold-Meeks and McGlone, 1986).

Video recording and documenting counts of specific behaviors can be used to monitor potential changes related to pain or wellness status; however, the process is time and labor

intensive. The use of scan and focal sampling will reduce the amount of labor required to accurately determine animal behavior. Despite potential limitations, continuous video monitoring is considered as the "gold standard" by which other behavior monitoring devices are evaluated.

Monitoring activity with accelerometers

Accelerometers are devices that continuously measure gravitational force in multiple axes, and these values can be processed to determine activity and postural behaviors. Figures 1A and 1B show a three-dimensional accelerometer attached with the horizontal, vertical, and diagonal axes the accelerometer monitors gravitational force. Before remote continuous monitoring technology can be used to assess the physiological and behavioral patterns cattle display, the technology requires validation (Duff and Galyean, 2007; Weary, 2009). Accelerometers have been shown to accurately monitor calf behaviors of standing, lying, or walking with 97.7% agreement to video analysis (Robert et al., 2009). This high accuracy allows the user to effectively rely on the accelerometers to determine posture behavior rather than using a labor intensive process of analyzing video.

Assessing postural changes may be important in evaluating calf wellness or pain status, and several studies have illustrated differences in postural behavior following painful stimuli. Calves have been shown to increase the percentage of time standing in the hours immediately following castration based on accelerometer analysis (White et al., 2008). However, Pauly *et al.* determined calves spent more time lying down and less time walking in the five-day period following castration (Pauly et al., 2012). The difference between these two studies may be due to the length of the monitoring period and a potential time-dependent change in behaviors. Theurer *et al.* (2012) determined calves administered the non-steroidal anti-inflammatory drug, meloxicam, prior to cautery dehorning spent more time lying down for 5 days post-dehorning compared to control calves that did not receive analgesia as commonly performed in production practice (Coetzee et al., 2010; Theurer et al., 2012b). Lying behavior decreased in calves after being induced with experimental lameness using an amphotericin B synovitis-arthritis induction model (Schulz et al., 2011). Accelerometers are an effective tool for continuous monitoring of behavior changes in response to pain.

Accelerometers (GP1 SENSR, Reference LLC, Elkader, IA) have also been used to monitor disease and wellness state of cattle. Calves challenged with *Mannheimia haemolytica* spent more time lying down compared to unchallenged control calves (Theurer et al., 2012a).

This agrees with a common assumption that a primary clinical sign of respiratory disease is depression. In another respiratory disease trial, there was no difference in the amount of time morbid calves spent lying down or walking compared with baseline data collected prior to challenge (Hanzlicek et al., 2010). These findings suggest that the postural activity of cattle may be influenced by disease or pain state, but changes in standing and lying behavior may not be a specific response to changes in wellness status.

Daily environmental conditions, differences among individual calves, and circadian rhythms also affect the amount of time calves spend lying (Robért et al., 2011); therefore, it is important to make comparisons of behavioral activities to calves housed in the same environmental conditions. Monitoring control animals allows the observer to distinguish between the behavioral changes associated with administering a procedure from daily variation due to environmental conditions (Fuquay, 1981; Robért et al., 2011; Theurer et al., 2011). The placement of the accelerometer on the animal and accelerometer size and weight may transiently alter normal gait and behavior. A brief acclimation period may be needed for the cattle to adjust to having the accelerometer attached to their leg.

Limitations of using accelerometers to monitor behavior include cost, data processing, and technological constraints. Accelerometers are relatively expensive compared to other behavior monitoring techniques, such as video analysis. Transforming the accelerometer into useable behavioral measurements can be achieved with validated algorithms; however, generating the data processing technique is time consuming. The accelerometers must have sufficient battery life, on board memory storage (or the ability to wirelessly transmit data), and be small enough to be easily affixed to the animal in some method. The objective quantification of cattle postural behavior as determined by accelerometers provides valid data to compare potential changes in behavioral patterns associated with pain or wellness status.

Monitoring step count frequency with pedometers

Pedometers have been used to objectively quantify the number of steps traveled and total distance traveled. An on-board algorithm calculating the number of steps from the raw data is contained within the pedometer. Pedometers are relatively easy to attach and use, but the number of steps each calf travels varies considerably among days and environmental conditions.

The distance calves travel may be associated with painful and stressful procedures. The amount calves travel following a painful procedure such as castration may vary as some research

demonstrated calves traveled fewer steps for 4 days after castration (Devant et al., 2012); while other work was unable to detect a difference in the number of steps traveled in calves after castration (Currah et al., 2009). Stress may also influence the distance traveled as calves have been shown to take more steps for 3 days after weaning (Haley et al., 2005). Bulls travel more steps than steers per day indicating the need for accounting for gender in the analysis (Devant et al., 2012). In properly designed experiments, pedometers may be useful in determining changes in behavior following a painful procedure.

Pedometers have been used to detect early lameness in dairy cattle, but a 15% decrease in activity was needed before the pedometer could accurately identify 92% of lame cattle (Mazrier et al., 2006). The biological significance of a 15% decrease in activity has not been established, but there may be clinical implications in detecting cattle before a change this large is detected. O'Callaghan *et al.* demonstrated lame dairy cows traveled 22.5 fewer steps per hour compared to non-lame cows based on visual locomotion score throughout the majority of days into milk (O'Callaghan et al., 2003). As pedometers are directly measuring locomotion, they are a valuable tool in identifying and monitoring musculoskeletal pain. However, changes in step counts as measured by pedometers are not specific for only identifying pain as the use of the pedometer technology has been able to accurately detect the onset of estrus in cows due to increase activity levels (Redden et al., 1992; Roelofs et al., 2005).

Pedometers can be an effective monitoring device for evaluating pain response and health status of cattle. The relative lower cost of investment and labor intensity compared to other technologies makes pedometers an attractive tool to objectively monitor potential behavioral changes.

Feed intake and behavioral monitoring

Systems are available to measure individual cattle feeding behavior and intake in group housed situations. These systems have been used to identifying morbid cattle from healthy cattle based on differences in feeding behaviors (Sowell et al., 1999). Feed and water intake, duration, and frequency are specific behaviors that can be monitored with these systems. Systems that monitor feeding and watering behaviors that are commercially available include GrowSafe (GrowSafe Systems Ltd, Airdrie, AB Canada) and Insentec (Repelweg, Marknesse, Netherlands). GrowSafe utilizes radio frequency identification (RFID) ear tags to identify individual animals. Insentec on the other hand uses transponder collars to identify when animals

are at feeding or watering stations. Both systems have integrated software that allows for real-time monitoring and analysis of animal feeding or watering behavior.

Radio frequency identification technology has been used to document a reduction in the frequency of visits to feeders (Gonzalez et al., 2009). Researchers evaluating residual feed intake (RFI) found distinct differences in feeding behaviors among high and low RFI calves using both the GrowSafe and Insentec monitoring systems (Kelly et al., 2010; Nkrumah et al., 2004). Since feed inputs represent one of the largest costs in producing beef, monitoring behaviors that may identify calves with less than ideal feed efficiencies may be beneficial (Bingham et al., 2009). Monitoring the feeding behavior of an animal over a period of time allows establishment of a baseline against which deviations in subsequent behavioral patterns can be evaluated. Investigators have used algorithms with 7 day rolling average feeding times as baselines to identify behavioral changes correlated with painful locomotive conditions in dairy cows days before farm staff were able to diagnose lameness (Gonzalez et al., 2008).

Monitoring animal feeding behavior and intake can provide insight into potential changes in wellness or pain status. Setup, maintenance, training, and expense are all potential disadvantages that must be considered when evaluating remote feed intake and behavior systems. However, the feed intake and frequency data collection capabilities make these systems an attractive monitoring tool to use since feed costs are important to the producer.

Location determination: global positioning systems (GPS)

Global positioning systems (GPS) have been used to remotely monitor movement of wildlife and domestic animals (Davis et al., 2011; Moen et al., 2001). Advances in GPS technology have created lighter and more accurate receivers, but monitoring multiple animals in varied geographic regions is often cost prohibitive (Davis et al., 2011). Three of the largest challenges when monitoring cattle with GPS technology are the ability to have real time updates, decreased battery life, and spatial accuracy.

Current technology allows for the location of a GPS receiver to be updated every second, but this update rate exceeds the power sources available in most animal monitoring units (Tomkiewicz et al., 2010). Custom units with real time updates once every minute have been developed. However, battery life was only 3.7 days (Schleppe et al., 2010a). Others using non-real time receivers have successfully monitored cattle for longer durations (11 days) by only waking the system up from a deep sleep mode every 600 seconds; however, depending on the

environment these infrequent readings may not provide the level of data necessary to define specific behaviors (Trotter et al., 2010).

Positional accuracy of the systems are also an issue and some research shows a discrepancy between visual and tag position of an average ± standard deviation of 9 m ± 7 m (Schleppe et al., 2010b). Other work illustrates that 99.9% of positional fixes fell within 20 m and 97.3% within 10 m of a known point (Trotter et al., 2010). Based on these accuracies, the GPS can give approximate location of individuals, but readings are not discrete enough to delineate specific activities such as eating or drinking.

The tradeoffs of battery life and positional update frequency limit the potential uses of GPS systems in situations where the behavior needs to be continually monitored for longer periods of time. Accuracy of 10 m may be sufficient for questions of pasture usage and grazing activities, but is not sufficient for monitoring feeding and watering behaviors. These limitations make GPS difficult to use to monitor changes in pain or wellness status in cattle.

Monitoring movement in a defined system with real-time location systems (RTLS)

Real-time location systems (RTLS) are designed to locate the position of an item anywhere within a defined area. The architecture of an RTLS consists of receivers spaced around the desired monitoring space, active or passive tags which are placed on the objects one wants to monitor, computer hardware, and software to receive and translate positional data. Tags used with most RTLS systems are smaller and have considerably longer battery life than current GPS technology. Like GPS, most RTLS requires line of sight from tags to sensors for accurate readings. Figure 2 demonstrates a calf within the sensor area and shows how 3 of receivers locate the animal and triangulate its position. Amount of time is calculated by subtracting the time of arrival at that location from the previous time of arrival documented. While similar to RFID behavior and intake systems, RTLS has the distinct advantage of being able to monitor an animal's location anywhere within the pen thus not restricting evaluation to only feeding and drinking behaviors.

The system monitors location within the pen at pre-set time intervals and not the specific behavior the calf is engaged in while at that position. Therefore, for data to be useful, the positions must be matched with a known diagram of the facility structure with specific areas of interest (proximity to feed, water, shelter) identified on the same scale of axes as measured by

the RTLS system. Depending on the frequency of measurements, the RTLS can be used to document the percent of time animals spend in specific locations within the housing environment.

An advantage of RTLS is the ability to measure levels of activity such as distance traveled and time spent within a given proximity to other calves. By measuring location over discrete time intervals, the data can be compared to determine the distance an animal traveled over a given period with results similar to measurements taken using pedometers. Social interactions with other calves (or the lack thereof) can be monitored by comparisons the proximity of individual calves to other animals within the pen. Real-time location systems have been used to monitor potential changes in cattle behavior that may be associated with pain or alterations in wellness status.

Investigators have used RTLS technology (Ubisense, Denver, CO) to determine that certain behaviors, such as time spent at the feed bunk and distance traveled, were associated with clinical illness scores (White et al., 2012). The distance traveled by calves as monitored with RTLS was also associated with the level of lung consolidation, indicating that monitoring movement may be a reasonable tool for wellness status evaluation (White et al., 2012). Theurer *et al.* identified calves that were dehorned and given pain medication had different feeding behaviors as measured by RTLS technology compared to calves dehorned without pain medications (Theurer et al., 2012b). These associations with behavior changes indicate RTLS technology is a valid tool to generate quantitative measurements of cattle activities that can be used to monitor potential changes in wellness or pain status in response to an intervention.

Limitations of RTLS technology include expense and technological constraints. The RTLS systems are able to monitor animal behavior within a specific area, but those areas need to be equipped with multiple sensors to accurately monitor behavioral activity which may be cost prohibitive in many situations. Installation and calibration of a RTLS requires significant investment in time and resources and while the use of this technology for monitoring animals is relatively new, these systems have been used successfully for many years monitoring assets in large complex manufacturing environments.

Thermography images

Thermography imaging can be used to monitor and record surface temperatures in multiple species. This technology has been used to non-invasively monitor welfare in cattle

(Stewart et al., 2005) and monitor nasal mucosal temperatures (Willatt, 1993). Using thermography in cattle housed in high ambient temperatures has been shown to result in low sensitivity (70.7%) and adequate specificity (89.5%) of identifying animals above or below a rectal temperature cutoff value (Gomez et al., 2011).

Corneal surface temperatures have been monitored using thermography based on the hypothesis that changes in corneal temperature may be reflective of changes in core temperature resulting from pain or disease. In one study there was no difference in surface temperature 2-3 hours after dehorning procedure (Stewart et al., 2009). Maximum surface corneal temperature has been shown to decrease 0.27 °C from baseline 2-5 minutes in calves post disbudding without local anesthesia (Stewart, 2008). However cattle disbudded had higher surface temperature 5-15 minutes after disbudding compared to controls that were not disbudded (Stewart, 2008). Temporal relationships need to be taken into account when analyzing thermography images.

Cattle infected with foot-and-mouth disease virus have been monitored using infrared thermography imaging; however, this system resulted in a low sensitivity (61.1%) and adequate specificity (87.7%) of correctly identifying infected animals with foot-and-mouth disease (Rainwater Lovett, 2009). There was not a strong correlation between face surface temperature and rectal temperature, but there was a positive correlation between foot surface temperature and rectal temperature indicating peripheral extremities may be more illustrative of core body temperature. The use of infrared thermography imaging has also resulted in low sensitivity (67.6%) and adequate specificity (86.8%) of identifying calves with bovine respiratory disease (Schaefer et al., 2007).

Thermography images can be relatively easy to capture, but in order to capture the images correctly the distance from the camera and the animal needs to be relatively consistent. Environmental conditions impact the relative temperatures recorded on images and need to be standardized to collect images for comparison when designing a research trial. Interpretation of the thermography images needs to include the temporal relationship related to the procedure performed (establishment of a baseline reading in similar environmental conditions) in order to detect changes. Thermography imaging needs to have refinement of the cutoff values used to detect morbid or painful animals before becoming implemented into industry practice.

Rumen telemetry temperature bolus

Rumen telemetry temperature boluses have been used to non-invasively monitor the health parameters of cattle (Small, 2008). Rumen temperatures have been shown to increase in calves challenged with *Mannheimia haemolytica*, and rumen temperatures have also been shown to have a strong correlation ($R^2 = 0.80$) to rectal temperatures (Rose Dye, 2010). The use of reticulo-rumen boluses has a positive predictive value of 73% for identifying animals infected with BRD when compared to a physical exam (Timsit, 2011). The pyrogenic effect of lipopolysaccharide was shown to only transiently increase rectal temperatures when administered to dairy calves (Theurer et al., 2011), however rumen temperature increased 2 °C when administered lipopolysaccharide to beef heifers (Small, 2008).

Limitations of rumen bolus telemetry system include the expense and administration of the bolus into the animal. The continued automatic thermal documentation has potential advantages related to remote monitoring of temperature changes. Overall effectiveness of the rumen telemetry temperature bolus is impacted by specific facets of the system including:ability to use telemetry in the specific environment (interference, geographic distribution), data collection and management plan.

Tympanic bulla and intravaginal temperature monitors

Tympanic bulla temperature can be monitored using a portable data logger attached to a thermistor. Temperature readings obtained with tympanic bulla thermometers have been correlated to rectal temperature (Davis et al., 2003; Mader et al., 2002), and tympanic temperature has been shown to increase 0.78 and 0.65 °C by moving cattle around in the summer and winter respectively (Mader et al., 2005). The increase in body temperature due to processing needs to also be taken into consideration when evaluating the health status of an individual animal.

Intravaginal temperature can be monitored using a thermistor modified with finger-like projections in order to prevent expulsion from the vagina (Redden et al., 1992). The onset of estrus has been able to be determined using intravaginal temperature monitors (Redden et al., 1992; Rorie et al., 2002). However, there is little published literature describing the use of tympanic bulla and intravaginal temperature monitors to determine health or wellness states.

Summary

Determining animal wellness status is frequently based on visual appraisal or performance parameters. The use of multimodal, remote, quantitative monitoring techniques will become more critical in determining the physiological, behavioral, and performance responses cattle experience in different scenarios. Interpretation of multiple behavioral responses as an aggregate indicator of animal wellness status rather than as individual outcomes may be a more accurate measure of true state of well-being. Individual animals differ greatly in behavior and accurate interpretation of behavioral changes is dependent on the ability to establish normal baseline activity in calves in a specific housing environment.

Behavioral data should be interpreted carefully as none of the commonly monitored behaviors are truly specific for one type of illness or pain response. Statistical analyses should account for the hierarchy of repeated measures on individual calves, the effect of having multiple observers, housing effects, time of day, and seasonality. If these potential sources of variability are not included in statistical analysis, differences between treatment groups may be falsely detected or there may be differences that are undetected.

There are numerous remote monitoring methods available to assess the pain or well-being status of an animal; however determination of the specific behavior needed to monitor, labor, and expense all need to be taken into consideration before deciding which behavioral monitoring device to use. The selection of the appropriate system for the situation is dependent on the expected benefits compared to costs of operating the system. Utilizing remote monitoring system provides basic information on cattle behavioral changes that can be translated to other aspects of clinical practice and animal wellness evaluation.

Figure 1.1. Figure 1A and 1B. Position of the three-dimensional accelerometer (and illustration of measured X, Y, and Z axes) on the lateral aspect of the right rear limb in a standing (1A) and lying (1B) calf.
Borrowed from Robert B, White BJ, Renter DG, et al. Evaluation of three-dimensional accelerometers to monitor and classify behavior patterns in cattle. *Comput. Electron. Agr.* . 2009;67(1-2):80-84, with permission.

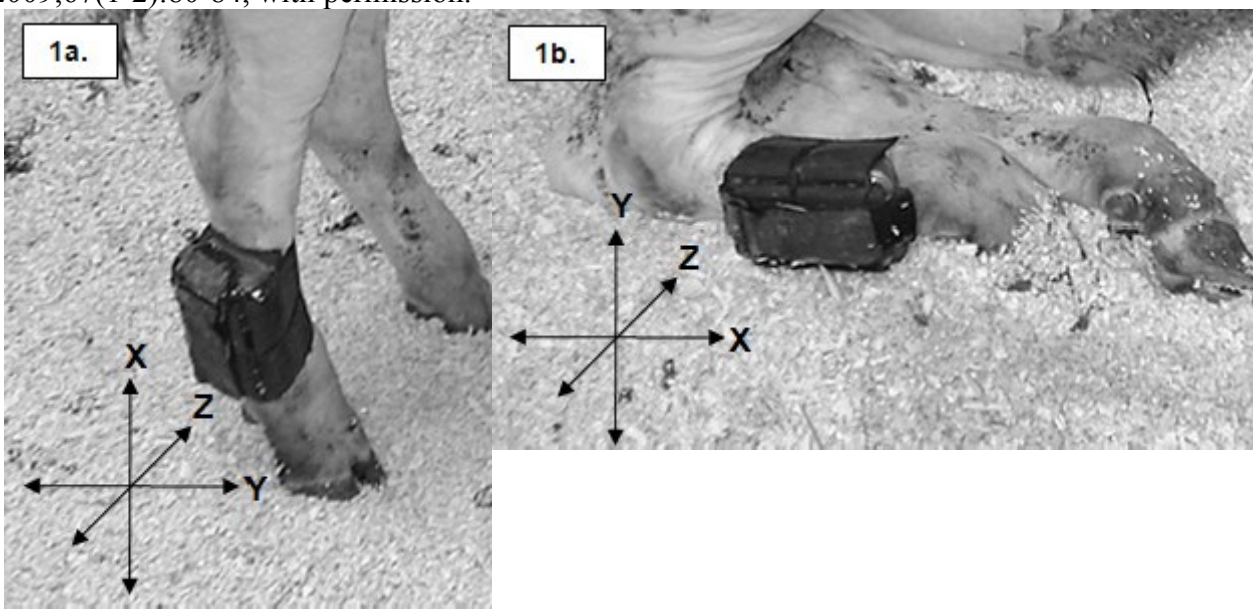
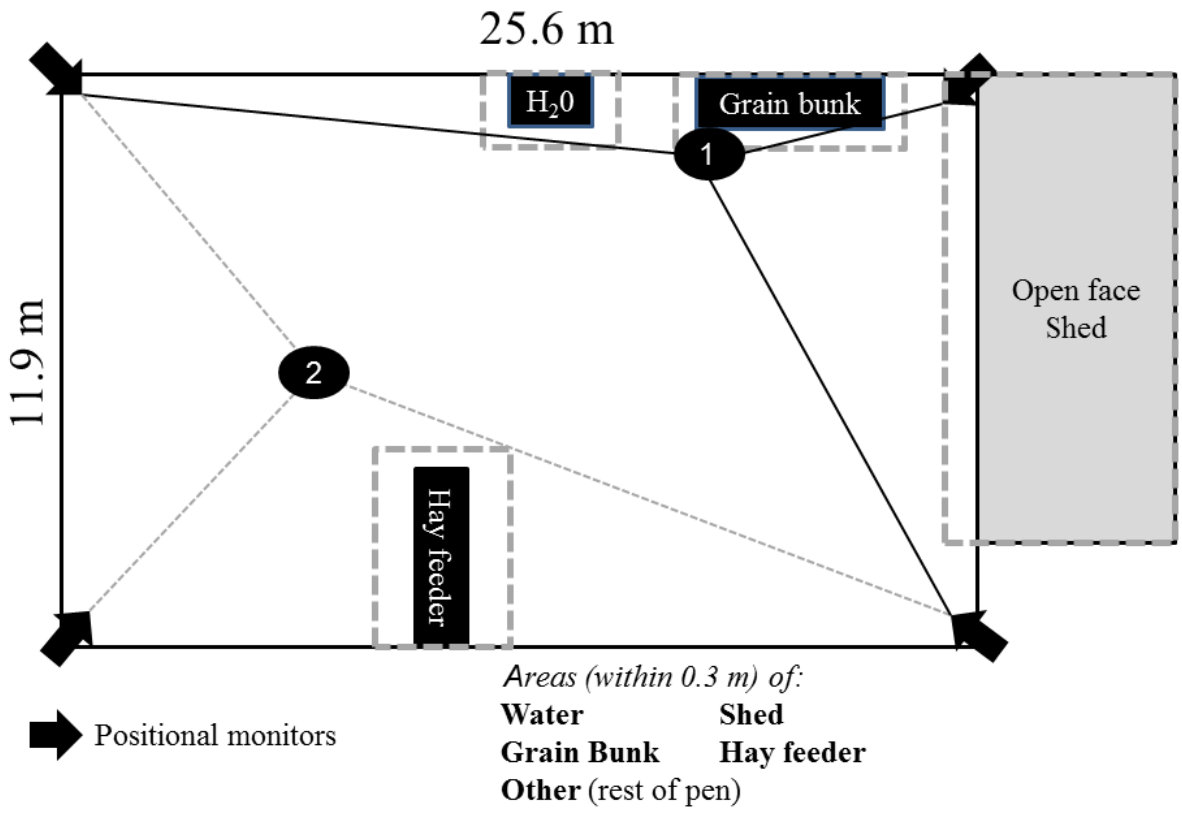


Figure 1.2. Stylized representation of a remote triangulation system with positional monitors (represented by arrows) able to triangulate animal position and compare to marked areas of interest including grain bunk, hay feeder, shed, and water.
Calf position is determined by the relative distance between the calf tag and at least three readers (represented by lines from the readers to the points within the pen). Amount of time at a location is determined by calculating the difference between time of arrival at that specific coordinates and previous triangulation time point. Circle 1 represents a calf that would be classified as being at the grain bunk, and Circle 2 represents a calf that would be classified as in the pen, but not next to a location of interest. *Adapted from* Theurer ME, White BJ, Coetzee JF, et al. Assessment of behavioral changes associated with oral meloxicam administration at time of dehorning in calves using a remote triangulation device and accelerometers. *BMC Vet. Res.*. 2012;8(1):48, with permission.



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Chapter 2 - Physiological modalities of health and wellness status in cattle

Abstract

Physiological indicators frequently are monitored to determine health or wellness state of cattle for research purposes. The objective of this article is to provide an overview of monitoring capabilities and technologies that have been used to evaluate animal health and wellness status, including rectal and nasal submucosal temperature, body weight, cortisol, substance P, haptoglobin, metalloproteinase, and tumor necrosis factor alpha. The potential collection method, use, and interpretation of results need to be taken into consideration prior to designing a research trial to detect differences between treatment groups. Diagnostic sensitivity, specificity, expense, and temporal delay from sampling to results all need to be taken into consideration before deciding which technologies to use in practice. Interpretation of these physiological indicators needs to be combined with other visual appraisal methods to determine true health or pain status of an animal. Use of these technologies in research settings can be applied to current production settings to determine ways to improve health and pain management protocols in field situations.

Keywords: physiological indicators, animal welfare, health monitoring, pain monitoring

Introduction

The ability to accurately identify cattle that are experiencing pain or morbidity is important for animal health monitors and research personnel. Animal behavior frequently is monitored to determine animal pain or wellness status, but with the wide variability in animal response to different states interpretation of individual behavior alone is hard to interpret (Gonyou, 1994; Theurer et al., 2013a). Treatment efficacy for different management programs, disease control, or reduction in painful stimuli are routinely evaluated using physiological and behavioral indices, which are compared among treatment groups, but several of these indices have not been validity for accurately distinguishing diseased or pain-affected animals from

healthy or controls animals. Confirmatory tests have been suggested to more accurately determine diseased from healthy cattle.

Several methods are available to determine physiological status of cattle including temperature monitoring, body weight measurement, and a variety of laboratory tests. While these tests all provide the user with a value to compare to an expected reference range, the challenge of utilizing these modalities include the time delay for performing the tests and determining what the appropriate reference range is. An opportunity exists determining which physiological parameter is more sensitive or specific in correctly identifying animal pain or health state. While there is a tradeoff of sensitivity and specificity values with the same diagnostic method, more research needs to be performed evaluating the efficacy and practical implications of utilizing some of these physiological indicators to determine animal pain or wellness state. Theurer *et al.* determined improving specificity of diagnostic test resulted in greater net returns for the producer compared to improvements in sensitivity for BRD diagnosis (Theurer et al., Submitted).

The objective of this article is to provide an overview of monitoring capabilities and technologies that have been used to evaluate animal health and wellness status, including rectal and nasal submucosal temperature, body weight, cortisol, substance P, haptoglobin, metalloproteinase, and tumor necrosis factor alpha. Although all of these physiological indicators are not directly applicable to clinical use, the results and interpretation from research trials utilizing these modalities provide insight to practitioners based on the associations between physiological changes and health and wellness states.

Monitoring pyrexia with rectal temperature

Rectal temperature commonly are used to evaluate health of individual animals and are relatively easy to capture. Temperatures can be collected using variety of modalities that range from simple analog rectal thermometers to digital, computerized thermometer systems that record the identity of individual calves and treatment history in order to evaluate disease progression. The pyrexia effect, which initiated by IL-1 and IL-6 cytokines to cause vasodilation may be quantified through temperature monitoring. Rectal temperatures have been positively correlated (0.59-0.63) to internal tail temperature, vulva temperature, auricular temperature, and body surface temperature in lactating Holstein cows indicating the use of rectal temperatures to evaluate thermal load animals are exposed (Martello, 2010).

Assessing rectal temperature may be important in evaluating calf wellness or pain status, and several studies have illustrated changes in rectal temperatures after disease challenge or stressful event. The timing of when the rectal temperature was collected in relation to the disease pathological progression needs to be taken into consideration, as calves challenged with *Mannheimia haemolytica* had increased rectal temperatures for 2 days after challenge compared to control calves (Burciaga-Robles et al., 2010; Confer, 2009; Corrigan et al., 2007; Rose Dye, 2010). Two days after challenge, however, there were no differences between calves challenged with *Mannheimia haemolytica* and un-challenged controls. These studies indicate there may be limited time early in the disease progression phase in which rectal temperatures may be accurately used to identify morbid animals.

A field study evaluating treatment of BRD with gamithromycin or tulathromycin showed that for every 1°C increase in rectal temperature ≥ 40°C at first diagnosis for BRD, the odds that a calf would require retreatment was 1.8, regardless of treatment administered (Torres et al., 2013). A retrospective field data study with over 300,000 calves evaluating the relationship between rectal temperature at first treatment of BRD and probability of not finishing the production cycle normally (died or realized) showed the relationship of rectal temperature and not finishing the production cycle normally was not linearly associated (Theurer et al., Accepted, In Press). The relationship between rectal temperature and not finishing the production cycle normally was a relative flat relationship until 40.6°C and then increased with greater rectal temperatures at first identification of bovine respiratory disease. The mean and median rectal temperature in the database analyzed was 40°C, indicating that half of calves initially pulled and treated for BRD had a lower rectal temperature than has been used in some treatment protocols; however, accuracy of predicting the probability of not finishing the production cycle normally based on rectal temperature, days on feed, arrival weight, quarter arrived, and all significant interactions revealed poor accuracy, thus leading to the need for improved capabilities for diagnosis of BRD in field settings (Theurer et al., Accepted, In Press).

Rectal temperatures have been shown to increase in calves administered endotoxin due to the pyrexia effect of endotoxin (Borderas, 2008; Carroll et al., 2009). Transportation of calves has been shown to increase rectal temperature (Grigor et al., 2001; Stevens and Camp, 1979; Tennessen et al., 1984). Theurer *et. al* demonstrated that transportation caused a transient decrease in rectal temperature in calves that were transported compared to control non-

transported calves, and there were no differences detected 48 hours after transportation (Theurer et al., 2013c).

Rectal temperatures have been shown to have a diurnal pattern, which may impact interpretation of temperatures based on time of day (Hanzlicek et al., 2010). Sixty-three percent (5 out of 8) of control calves had rectal temperatures that exceeded 39.5°C, which has been established as upper reference limit in diagnosing morbid cattle (Radostits, 2001; Theurer et al., 2013b). Rectal temperature has been positively correlated with ambient temperature and temperature humidity index (Theurer et al., 2014). This may lead to establishing seasonal adjustment to protocols in regards to treating sick animals based on rectal temperature alone, as a change in 1°C may indicate a different diagnosis in animals or treatment protocol. Tympanic bulla temperature has been correlated to rectal temperature (Davis et al., 2003; Mader et al., 2002), and tympanic temperature has been shown to increase 0.78 and 0.65°C by moving cattle around in the summer and winter, respectively (Mader et al., 2005).

Limitations of using rectal temperature include the diurnal and environmental conditions that may affect interpretation of measurements. Also, timing of rectal temperature measurements in relation to the disease processes is a limiting factor, as we are not able to clinically identify duration of disease on animals when pulled for disease process. The increase in body temperature due to processing also needs to also be taken into consideration when evaluating the health status of an individual animal. While rectal temperature may provide the observer with more information, the additional information may lead to increases in false positive morbid animals. Refinement and adjustment of these threshold values needs to be established in order to accurately assess health status. Rectal temperature results must be interpreted in conjunction with other clinical signs displayed by animals.

Monitoring pyrexia with nasal submucosal temperature

Nasal submucosal temperatures can be determined by implanting biothermal sensors in the nasal mucosa caudal to the alar cartilage. Biothermal sensors can be placed submucosally in the nasal passage using the application device. The sensors have radiofrequency transponders that are activated using an electronic reading device with an accuracy of +/- 0.1°C. Biothermal sensors can be used in place of surface thermography images to determine peripheral temperature in calves.

Nasal mucosal temperature has been shown to decrease in calves after administration of endotoxin or *Mannheimia haemolytica* (Theurer et al., 2013b; Theurer et al., 2011). The decrease in nasal temperature has been attributed to effects of endotoxin from administration or apoptosis of gram negative bacterial colonies released into systemic circulation, causing vasoconstriction of the peripheral blood vessels to shunt more blood to vital organs (Theurer et al., 2013b). Severity of vasoconstriction has not been determined and more research needs to be performed in this area to examine the pathophysiological progression for decreased nasal temperatures. It may be important to consider environmental conditions when administering intranasal vaccines, as the nasal mucosal temperature may be too high, thus deactivating modified live vaccine, ultimately leading to an increase in vaccine failures. Modified live vaccines have been shown to become inactivated at temperatures exceeding 39°C (Mills et al., 1971; Pastoret, 1980).

Implantable biothermal sensors are able to accurately determine nasal submucosal temperature and are not subject to human error if properly implanted. The biothermal radiofrequency implants we used are commercially available, inexpensive, and easily applied. Limitations of the nasal mucosal temperatures include the practical application and interpretation of results. Consideration of implanting a sensor in the animals which may enter the food chain also needs to be taken into account. More research needs to be performed to determine if temperature-sensitive vaccines are inactivated when nasal mucosal temperatures exceed 39°C, and also evaluate relationships between nasal mucosal temperature, to rectal temperature, and other clinical signs displayed by animals.

Body weight change

Cattle body weight is monitored routinely to evaluate performance of animals. Weight can be monitored by individually weighing each animal in the group or weighing the entire group to determine lot average weight. Shrink is a common term that refers to the percentage of body weight lost by cattle during transportation. There are two types of shrink: fill shrink and tissue shrink. Fill shrink is where cattle lose body weight through urine and feces during the transportation period. When the cattle arrive at destination point, calves are able to eat and refill this body weight lost. Tissue shrink which occurs when cattle lose more bodily fluids through urine and feces from other sources than the rumen. Fluids are excreted from the cellular level to maintain homeostasis due to osmolality gradient. The amount of weight lost during

transportation has been positively associated with the risk of developing BRD and performance of cattle (Camp et al., 1981; Cernicchiaro et al., 2012).

The percent change in body weight in cattle exposed to different pain or health states varies considerably in published literature. This variation can be attributed to the pain or health procedure the animal is subjected to, type and age of cattle, environmental conditions in which cattle are housed, and time of the day when body weights are measured. All of these events can have varying impact on change in body weight of calves and these events all need to be accounted for when performing research trials.

The advantage of collecting and interpreting body weights is that it is relatively easy to perform and several producers and veterinarians routinely have some sort of scales available to measure weight in cattle; however, these scales may not be located in close proximity to where cattle are housed and there may be some body weight loss from cattle prior to the initial weight being collected. Limitations of using body weight include the daily variation in the body weight of animals, as weight can vary significantly during the day depending on time from when feeding period occurred. Growing calves are also gaining weight each day, making interpretation of change in body weight more difficult the further from the initial comparison weight. Use of metaphylaxis treatment of calves on arrival is routinely performed based on weight of calves (Nickell and White, 2010; Thomson and White, 2006).

Monitoring stress response using cortisol

In response to fear or stress, the body produces cortisol, through the hypothalamus, pituitary, and adrenal cortex axis. Cortisol is a modulator of inflammatory response produced by the body. Cortisol analysis has been used widely as a stress marker, as magnitude and duration of cortisol response coincide with predicted noxiousness of different procedures (Broom, 2001; Mellor et al., 2000). Stressful events have been interpreted by peak of cortisol concentration (Mellor et al., 2000).

Several studies have analyzed the cortisol response and duration of calves associated with castration procedures (Coetzee et al., 2008; Fisher et al., 2001; Molony and Kent, 1997; Stafford et al., 2002). Cortisol concentrations have been shown to increase post castration procedures when compared to sham castration periods or pre-castration periods. Process and handling animals has been shown to increase cortisol concentrations (Crookshank et al., 1979; Kent, 1983; Kent and Ewbank, 1986; Molony and Kent, 1997). Concentration of cortisol levels has been

shown to vary considerably between animals (Stafford et al., 2002). This variability between animals may be attributed to individual animals having a wide range of pain thresholds (Stafford and Mellor, 2005a). Others have shown cortisol concentrations did not increase in proportion to the severity of the treatment administered, where cortisol concentrations may plateau (Coetzee et al., 2007; Molony and Kent, 1997).

Cortisol has been used to quantify the amount of stress animals are exposed during transportation. Cortisol concentrations have been shown to increase almost 4 fold during transportation compared to pre-transportation concentrations (Crookshank et al., 1979; Kent, 1983; Theurer et al., 2013c). However, Theurer *et al.* monitored cortisol concentrations in calves transported during high ambient temperatures and determined cortisol concentrations increased during the midpoint of transportation (4 hours) but then returned to baseline concentrations measured (Theurer et al., 2013c). Grandin determined that individual animal factors, such as previous exposure to the event occurring, may influence the physiological response to stress (Grandin, 1997).

Changes in cortisol concentrations in response to respiratory disease vary in publish literature. In one study, they were not able to detect difference between calves challenged with *M. haemolytica* and control calves (Corrigan et al., 2007). In another study, Hewson *et al.* demonstrated highly variable concentrations of cortisol after challenge and could not determine a difference between control calves and calves challenged with *M. haemolytica* until 5 days after challenge when control calves had higher concentrations (Hewson et al., 2011). However Theurer *et al.* was able to detect a difference between control calves and calves challenged with *M. haemolytica* in the early pneumonia period (1 day after challenge) (Theurer et al., 2013b). This variability in literature may be associated with sample collection intervals or that cortisol may not be specific for evaluating health status of cattle.

Cortisol levels may not always reflect the extent of pain response in animals (Coetzee, 2011). Exposure to novelty events, such as process and handling, needs to be controlled for when using cortisol as a modality to quantify pain and health status. Control of these parameters may be accomplished by exposing the animal to these processing procedures multiple times through sham procedures to reduce the increased response of handling alone. Comparison to control animals exposed to the same handling procedures will also control for this variability. It has been suggested that small changes in cortisol levels to painful stimuli may be due to high pain

thresholds in animals, thus confounding the interpretation of cortisol results (Stafford and Mellor, 2005b). The diurnal pattern of cortisol concentrations needs to be considered when analyzing cortisol responses as well (Thun, 1981). The advantage of cortisol analysis is that methods to analyze cortisol concentrations are more readily available in some research facilities.

Neuroendocrine response evaluation using substance P

Substance P is an 11-amino acid neuropeptide that regulates the excitability of dorsal horn nociceptive neurons and can be detected in areas involved with pain and distress (DeVane, 2001). Substance P has been shown to be an effective biomarker of pain in castration trials in which there were higher concentrations in castrated calves compared to control animals, while there was no difference detected in cortisol concentrations between treatment groups (Coetzee et al., 2008). The neurophysiological processing of pain and stress may be different, but there may be cross-linking between cortisol and substance P responses (Coetzee, 2011).

Theurer *et al.* monitored substance P in calves transported during high ambient temperatures and determined substance P concentrations peaked at the midpoint of the transportation (4 hours) but then returned to baseline concentrations, which was similar to the cortisol response (Theurer et al., 2013c). Van Engen *et al.* (2014) also observed that transportation induced a 50% increase in substance P concentrations compared to baseline. Published literature analyzing the response of substance P in disease models is lacking. Theurer *et al.* demonstrated a wide range of substance P response in a bovine respiratory disease challenge model (Theurer et al., 2013b).

The advantages of using substance P to evaluate the health or wellness status of cattle include that substance P may be more specific for pain evaluation, and diurnal pattern has not been determined for substance P (Coetzee et al., 2008). Substance P is a volatile neuropeptide that may be influenced by a variety of conditions adding to the importance of proper sample collection methods and laboratory analysis (Mosher et al., 2014). The availability of the laboratory methods needed to accurately analyze and interpret substance P is limited, thus limiting the practical use of substance P. The response to disease status indicates that substance P may not be a specific indicator for disease; however, more research is needed to determine the pathophysiological response of substance P to determine health status.

Acute phase protein: evaluation of haptoglobin

Acute phase proteins have been monitored to determine health status in cattle. Haptoglobin is an acute phase protein that is predominantly produced by the liver and then released systemically. This acute phase protein binds to free hemoglobin dimers in the blood so that iron is not available to the organisms. Haptoglobin is part of the positive acute phase proteins complex that increase in plasma and serum due to an inflammatory process (Godson and Campos, 1996; Stockholm and Scott, 2012).

Haptoglobin concentrations have been shown to increase beginning 4 days post-infection of bovine respiratory syncytial virus (BRSV) challenge model (Grell et al., 2005). However by day 13 post-challenge, they were unable to detect a difference between calves challenged with BRSV and control calves in haptoglobin concentrations. Differentiating between acute and chronic an infection is important to determine the pathological progression the disease has already taken to further improve the treatment protocol. Haptoglobin has been shown to be the most specific and efficient biomarker available as haptoglobin was 76% specific and 73% accuracy overall in differentiating between acute and chronic inflammatory diseases (Horadagoda, 1999). In that trial, determination of acute and chronic infections was performed by lesions found at necropsy and clinical signs exhibited ante-mortem. Calves purchased through auction markets have been shown to have higher concentrations of haptoglobin compared to calves that originate from a single source (Step et al., 2008). This increase in haptoglobin concentrations may be correlated to morbidity incidence rates exhibited by various types of cattle.

Haptoglobin has been proposed as a diagnostic screening tool to identify calves that will become morbid with BRD at time of arrival. However, sensitivity and specificity have been relatively low (64% and 71%, respectively) in identifying calves with clinical respiratory tract disease (Svensson, 2007). In that study, they were using treatment as the definition of truly diseased state and clinical observations have been shown to result in poor sensitivity (62%) and poor specificity (63%) in identifying calves with lesions at harvest (White and Renter, 2009). The low sensitivity and specificity associated with haptoglobin as a diagnostic tool for identifying respiratory disease may be attributed to not properly defining the true disease state. Theurer *et al.* determined an increased concentration in haptoglobin levels in calves challenged with *Mannheimia haemolytica* for up to 7 days post-challenge (Theurer et al., 2013b). Angen *et*

al. compared clinically healthy calves to calves diagnosed with BRD and discovered in all 3 herds where pneumonia was present, haptoglobin concentrations were greater in the pneumonia calves compared to the clinically healthy calves (Angen, 2009). Haptoglobin concentrations have been shown to not be able to differentiate fatal BRD from non-fatal BRD (Aich et al., 2009).

A field study evaluated the effectiveness of predicting occurrence of BRD using haptoglobin as a potential biomarker and determined haptoglobin was not an accurate predictor for identifying BRD (Burciaga Robles et al., 2009). This may be attributed to varying disease state of BRD of calves coming in to the feedlot. Haptoglobin concentrations were higher in calves treated for BRD compared to clinically healthy calves (Burciaga Robles et al., 2009). Performance of animals has been shown to not be associated with haptoglobin concentrations, but cattle with detectable haptoglobin concentrations had high odds of being treated 3 times for BRD compared to cattle that did not have detectable haptoglobin concentrations on arrival at the feedlot (Holland et al., 2011). Haptoglobin has been analyzed to determine efficacy of treating case of BRD with antibiotic. Calves with BRD had a large and variable haptoglobin result, but calves treated with an antibiotic had lower haptoglobin levels when compared to calves clinically diagnosed with BRD but not administered an antibiotic treatment (Wittum et al., 1996). Haptoglobin concentrations have also been shown to increase in cattle with infected conditions when compared to cattle having non-infectious and chronic conditions with an haptoglobin concentration >0.4 g/L indicating a severe acute infection (Skinner et al., 1991).

Haptoglobin concentrations have been shown to increase in heifers that were tail-docked compared to heifers that were not tail-docked (Eicher et al., 2000). Transportation of cattle has been shown to cause lower concentrations of haptoglobin for 3 days compared to non-transported cattle (Arthington et al., 2003). There was no difference in haptoglobin concentrations in cattle that were administered meloxicam prior to transport and at arrival compared to cattle that were not transported 1,440 km and have similar performance for 21 days after transportation indicating the potential use of meloxicam in transport cattle (Filho et al., 2014).

Limitations of haptoglobin analysis include the delay in the time from when the blood sample was collected to when the sample is evaluated. While haptoglobin concentrations may not be able to predict morbidity rate for BRD, there is evidence to show haptoglobin concentrations may be useful in determining if an animal is truly diseased based on the high

specificity of the acute phase protein, as haptoglobin is nearly undetectable in healthy cattle. More research needs to be performed in development and refinement of chute side haptoglobin analysis kits to increase specificity values for diagnosing BRD in the field to use as a confirmatory test.

Inflammatory indicator evaluation with tumor necrosis factor-α

Tumor necrosis factor-alpha (TNF-α) has involvement of physiological functions including fever, appetite, energy metabolism, and endocrine activity (Klasing, 1988). Tumor necrosis factor-alpha is one of the earliest mediators produced by the animal, and TNF-α is primarily produced by macrophages, but can be produced by other cells in the body including natural killer cells, lymphocytes, and adipocytes. Along with other cytokines in the body, TNF-α mediates other inflammatory responses such as endotoxemic shock and mastitis (Elsasser et al., 1997; Wenz et al., 2010). During infection of cattle, TNF-α has been shown to be produced (Spurlock, 1997). The acute phase proteins, such as haptoglobin, are produced during the acute phase response of a disease, which is indicated by fever, leukocytosis, and alterations in plasma concentrations of trace minerals and hormones (Eckersall and Bell, 2010). Control of the acute phase response is believed to be mediated by the activated cytokine cascade, including TNF-α, IL-1, and IL-6 (Baumann and Gauldie, 1994).

Administration of TNF-α to calves has been shown to increase rectal temperature and haptoglobin concentrations 3 hours after administration of TNF-α, and has been shown to induce the acute phase response (Kushibiki et al., 2000; Van Der Poll et al., 1991). Pro-inflammatory cytokines, such as TNF-α, have been shown to increase the accumulation of leukocytes in the lung, causing more pathological changes. Changes in TNF-α concentrations in cattle exposed to stressful stimuli vary in publish literature. Transportation of cattle has been shown to have no effect on TNF-α concentrations (Theurer et al., 2013c); however, Van Engen *et al.* (2014) demonstrated a 1.5-fold increase in TNF-α concentration 24 hours after transportation compared to TNF-α concentrations prior to transportation. The difference between these two studies may be the amount of time cattle were transported, as Theurer *et al.* only transported calves only 8 hours, whereas as Van Engen *et al.* transported calves for 16 hours (Theurer et al., 2013c; Van Engen et al., 2014). Rats exposed to heat stress have been shown to suppress circulating TNF-α levels (Kluger et al., 1997).

Limitations of TNF-α include several diseases can increase TNF-α concentrations of an calf including BRD, mastitis, acidosis, viruses, parasites, and other cytokines, thus having low specificity values for using TNF-α to identify a certain disease as cause for the increase concentrations (DeForge et al., 1990; Kushibiki, 2011). The direct mechanism of action between the cytokine release of TNF-α is poorly understood in determining level of pain or disease state an animal is experiencing, let alone clinical importance of the use and interpretation of the cytokine. More research is needed to further evaluate effectiveness of utilizing TNF-α concentrations in clinical research.

Inflammation response utilizing haptoglobin matrix metalloproteinase-9

Haptoglobin matrix metalloproteinase-9 (Hp MMP-9) complexes are released from neutrophils during systemic neutrophil activation and degranulation (Bannikov et al., 2011; Bannikov et al., 2007). These complexes may be formed and released independently from haptoglobin, and may be important to identify clinically ill animals in the acute phase of a disease process. The Hp MMP-9 complex has been shown to be more in acute phase of disease compared to chronic cases.

During the acute inflammation phase of disease in cattle, Hp MMP-9 may be more sensitive for identifying diseased animals compared to haptoglobin concentrations (Bannikov et al., 2007). Hp MMP-9 has been shown to increase after transportation of calves (Van Engen et al., 2014). Limitations of using Hp MMP-9 include the current available laboratories that are able to perform the assay tests. While Hp MMP-9 may be more sensitive than haptoglobin alone, more research needs to be performed to evaluate specificity of the parameter. Temporal increases in Hp MMP-9 concentrations in clinically healthy calves has been determined, increasing reservation about its specificity (Theurer et al., 2013b). To the author's knowledge, there has been no research evaluating the change in concentrations of Hp MMP-9 concentrations with painful stimuli, thus we are not able to determine the practical application of utilizing Hp MMP-9 as a pain wellness evaluation.

Summary

Determining the animal health and wellness status is frequently based on combination of visual appraisal followed by some physical monitor of physiological status. The interpretation of the physiological measure aggregated with the visual appraisal rather than as any individual

outcome may be a more accurate measure of true state of health and wellness status. Individual animal physiological state varies considerably from animal to animal as well as through the day.

Blood samples are routinely collected to monitor physiological condition of an animal. Cortisol, substance P, haptoglobin, haptoglobin-metalloproteinase-9 complex and, tumor necrosis factor-α (TNF-α) all have all been used to quantify stress or health parameters in research trials. These results have augmented the physiological response to pain, stress, and health status of animals to determine the pathophysiological response to adverse events. The quantification of this animal response is necessary in order to evaluate and implement changes to production systems to improve animal welfare and performance.

There are multiple technologies available to assess physiological indicators to determine pain or health status of an animal; however, sensitivity, specificity, expense, and temporal delay from sampling to results all need to be taken into consideration before deciding which technologies to use in practice. Interpretation of these physiological indicators needs to be combined with other visual appraisal methods to determine true health or pain status of an animal. Use of these technologies in research settings can be applied to current production settings to determine methods for improving health and pain management protocols in field situations.

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Chapter 3 - Relationship between rectal temperature at first treatment for bovine respiratory disease complex and the probability of not finishing the production cycle normally in feedlot calves

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Abstract

Objective—To determine the relationship between rectal temperature at first treatment for bovine respiratory disease complex (BRDC) and the probability of not finishing the production cycle normally in feedlot calves.

Design—Retrospective data analysis.

Animals—344,982 calves identified by feedlot personnel as having BRDC from 19 US feedlots from 2000 to 2009.

Procedures—For each calf, data for rectal temperature at the initial treatment for BRDC and various performance and outcome variables were analyzed. A binary variable was created to identify calves that did not finish (DNF) the production cycle normally (died or culled prior to cohort slaughter). A mixed general linear model and receiver operating characteristic curve were

created to evaluate the associations of rectal temperature and days on feed at BRDC diagnosis, weight and quarter of year at feedlot arrival, sex, and all 2-way interactions with rectal temperature with the probability that calves DNF.

Results—27,495 of 344,982 (7.97%) calves DNF. Mean rectal temperature at first treatment for BRDC was 40.0°C. As rectal temperature increased, so did the probability that a calf DNF; however, that relationship was not linear and was influenced by quarter of year at feedlot arrival, sex, and days on feed at BRDC diagnosis. The area under the receiver operating characteristic curve for correct identification of a calf that DNF was 0.646.

Conclusions and Clinical Relevance—Results indicated that, rectal temperature of feedlot calves at first treatment for BRDC had limited value as a prognostic indicator of whether those calves would finish the production cycle normally. (*J. Am. Vet. Med. Assoc.* 2014;245:1279-1285)

Abbreviations

BRDC	Bovine respiratory disease complex
DNF	Did not finish
DOF	Days on feed
ROC	Receiver operating characteristic

Introduction

Bovine respiratory disease complex is the most economically consequential disease affecting the beef feedlot industry (Galyean et al., 1999). Identification of calves with BRDC is routinely performed on the basis of visual observation of signs of depression, nasal discharge, lack of rumen fill, and anorexia (Smith et al., 2001). Those clinical observations have low sensitivity and specificity for identification of calves with BRDC (Amrine et al., 2013; White and Renter, 2009). Rectal temperatures are routinely obtained for calves with signs BRDC to improve diagnostic specificity, and the final decision to treat a calf may be made on the basis of a rectal temperature that exceeds a predetermined threshold. The rectal temperature of a calf with BRDC might also influence the selection of the antimicrobial used to treat that calf.

Despite the frequency with which rectal temperature is used as a determinant for the diagnosis of BRDC, published literature on how to most effectively use rectal temperature as a

metric for BRDC in feedlot calves is sparse. Results of 1 study (Torres et al., 2013) indicate that rectal temperature at initial diagnosis of BRDC is positively associated with retreatment and case-fatality risks. Most analyses have assumed a linear relationship between rectal temperature and case outcome. Because of the positive association between environmental temperature and rectal temperature of cattle, some investigators (Theurer et al., 2014a) have suggested seasonal changes to the rectal temperature threshold used to identify calves with BRDC to improve diagnostic accuracy. However, to our knowledge, studies to establish seasonal rectal temperature thresholds for diagnosis of BRDC in feedlot cattle have not been performed.

The primary objective of the study reported here was to determine the relationship between rectal temperature at first treatment for BRDC and the probability of feedlot calves not finishing the production cycle normally with their cohorts (ie, died or were prematurely culled or removed from the cohort prior to slaughter). In most production systems, early removal of individual animals from a cohort is associated with poor performance caused by chronic disease conditions. A secondary objective was to create an ROC curve to determine overall model predictive accuracy of rectal temperature at first treatment for BRDC as an indicator for a calf not finishing the production cycle normally. We hypothesized that rectal temperature would be positively associated with the risk of feedlot cattle not finishing the production cycle with their cohorts and that the overall predictive ability of the model with all input variables included would be high.

Materials and Methods

Feedlot data set

Individual cattle health data from 19 US feedlots for 2000 to 2009 were obtained. The study population consisted of calves that had clinical signs consistent with BRDC and were treated with an antimicrobial. The case definition for BRDC was determined by feedlot personnel, and treatments were administered in accordance with established feedlot protocols (ie, case definition for and treatment of BRDC were not standardized).

Data extracted for each calf included sex, body weight at feedlot arrival, quarter of year at feedlot arrival, rectal temperature at initial BRDC diagnosis, DOF at initial BRDC diagnosis, and total DOF. A binary outcome variable was created to distinguish between calves that did and did not finish the production cycle normally with their cohort. Calves classified as DNF included

those that died or were removed (culled) from their cohort prior to slaughter of that cohort (Amrine et al., 2014; Torres et al., 2013).

Data management

Extreme values were removed from the analysis to limit potential data entry errors and confine the external validity to the reference range or industry standard for each variable. Therefore, data for calves with rectal temperature ≤ 38.3°C (≤ 101°F; n = 1,508) and > 41.7°C (> 107°F; 2,748), and that were initially treated for BRDC > 126 DOF (9,499) were removed prior to analysis. Calf records from cohorts identified as Holstein (n = 10,108) and with arrival weights < 136 kg (300 lb; 12,144) and > 408 kg (900 lb; 4,504) were also removed prior to analysis as were those from cohorts identified as mixed sex (ie, only data from cohorts that were comprised exclusively of male or female calves were analyzed; 20,425).

Statistical analysis

Data for rectal temperature at first treatment for BRDC (rectal temperature) were categorized into 12 categories (≤ 38.6°C [101.5°F], > 38.6° to 38.9°C [101.5 to 102.0°F], > 38.9° to 39.2°C [102.0 to 102.5°F], > 39.2° to 39.4°C [102.5 to 103.0°F], > 39.4° to 39.7°C [103.0 to 103.5°F], > 39.7° to 40.0°C [103.5 to 104.0°F], > 40.0° to 40.3°C [104.0 to 104.5°F], > 40.3° to 40.6°C [104.5 to 105.0°F], > 40.6° to 40.8°C [105.0 to 105.5°F], > 40.8° to 41.1°C [105.5 to 106.0°F], > 41.1° to 41.4°C [106.0 to 106.5°F], and > 41.4°C). Data for body weight at feedlot arrival were categorized into 6 categories (≤ 180 kg [397 lb], > 180 to 226 kg [397 to 498 lb], > 226 to 272 kg [498 to 600 lb], > 272 to 318 kg [600 to 701 lb], > 318 to 363 kg [701 to 800 lb], and > 363 kg). The DOF at time of first treatment for BRDC were categorized into 5 categories (≤ 10 days, 11 to 20 days, 21 to 30 days, 31 to 40 days, and > 40 days). The month calves arrived at the feedlot was categorized into 4 categories (quarter 1 = January through March; quarter 2 = April through June; quarter 3 = July through September; and quarter 4 = October through December).

A mixed general linear model was used to determine the probability that calves DNF the production cycle normally. Fixed effects included in the model included the categorized data for rectal temperature, body weight at feedlot arrival, DOF at first treatment for BRDC, sex, quarter of feedlot arrival, and all 2-way interactions with rectal temperature. Random effects were included in the model for year, feedlot, and lot number. The model was constructed by including all potential effects and removing nonsignificant effects ($P > 0.05$) 1 at a time in a stepwise

manner; the final model included only variables with a value of $P \leq 0.05$ as determined by type 3 likelihood tests. All analyses were performed with commercial statistical software.[a]

ROC curve analysis

A parametric ROC curve was created for the final mixed general linear model to determine its overall accuracy for predicting that calves will be classified as DNF (Dohoo et al., 2009). The ROC curve allows for evaluation of sensitivity and specificity. Sensitivity was defined as the frequency with which the model correctly identified calves that DNF. Specificity was defined as the frequency with which the model correctly identified calves that finished the production cycle normally. Sensitivity and 1 – specificity were plotted against each other and the area under the curve determined the overall accuracy of the model.

Results

Following the removal of calf records with extreme values, the records of 344,982 calves treated for BRDC by feedlot personnel were included in the analysis, and 27,495 (7.97%) of those calves were classified as DNF. Rectal temperatures at the time of first treatment for BRDC were normally distributed, with a mean and median of 40.0°C **(Figure 1)**.

The probability that a calf would be classified as DNF (probability of DNF) was significantly associated with the interaction between rectal temperature and DOF at first treatment for BRDC ($P < 0.01$; **Figure 2**), the interaction between rectal temperature and sex ($P < 0.01$; **Figure 3**), the interaction between rectal temperature and the quarter of the year during which the calf arrived at the feedlot ($P < 0.01$; **Figure 4**), and body weight at feedlot arrival ($P < 0.01$; **Figure 5**). The interaction between rectal temperature and body weight at feedlot arrival was not significantly ($P = 0.55$) associated with the probability of DNF. The area under the ROC curve for the final multivariable model was 0.646 **(Figure 6)**.

Discussion

The present study was conducted to assess the relationship between rectal temperature of feedlot calves at the time of first treatment for BRDC and the probability that those calves would not finish the production cycle normally (ie, died or were prematurely culled or removed from the cohort prior to slaughter). Elucidation of this relationship was important to determine whether rectal temperature at first treatment for BRDC could be used as a prognostic indicator for feedlot calves. Results of this study indicated that rectal temperature of feedlot calves at first treatment for BRDC had limited valued as a prognostic indicator of whether those calves would

be classified as DNF. Case-fatality rate has commonly been used to evaluate the success of BRDC treatment protocols (Edwards, 2010). Net economic returns for feedlot cattle with chronic BRDC that do not die and fail to be marketed with their arrival cohorts are significantly lower than those for untreated healthy feedlot cattle (Brooks et al., 2011).

Rectal temperatures are a common component of health monitoring protocols for feedlot calves and often influence treatment decisions (Radostits, 2001). Rectal temperatures of cattle have a diurnal pattern and are positively associated with ambient temperature during periods of extreme heat (Hanzlicek et al., 2010; Theurer et al., 2014a). In the present study, the rectal temperature for calves at the time of initial treatment for BRDC was normally distributed with a mean and median of 40.0°C. Prior to the analysis, we assumed that most calves with BRDC would be pyrexic and the distribution of the rectal temperature data would be skewed to the left. The phase of BRDC during which affected calves were identified and treated might have had an effect on rectal temperature. Calves experimentally infected with *Mannheimia haemolytica* were pyrexic for only 1 to 3 days after the challenge inoculation before becoming normothermic (Ames et al., 1985; Hewson et al., 2011; Theurer et al., 2013; Vestweber et al., 1990). Thus, calves with BRDC identified during the latter stages of the disease may not always be pyrexic.

The association of the interaction between rectal temperature and DOF at the time of BRDC diagnosis with the probability that a calf would be classified as DNF (probability of DNF) had an interesting pattern. The probability of DNF remained fairly constant until rectal temperatures increased > 40.6°C, at which point the probability of DNF increased for all DOF categories. The probability of DNF was greatest for calves in which BRDC was diagnosed between 10 and 20 DOF and lowest for calves in which BRDC was diagnosed > 40 DOF. In general, the probability of DNF increased as the DOF at which calves were first treated for BRDC decreased. These results may help feedlot veterinarians and managers forecast the economically important outcome of cohort mortality risk on the basis of the proportion of calves treated for BRDC and the rectal temperatures of those calves at the first BRDC treatment (Irsik et al., 2006; Schroeder et al., 1993; Theurer et al., 2014b).

Although the interaction between rectal temperature and sex was significantly associated with the probability of DNF, the probability of DNF did not vary significantly between male and female calves within any rectal temperature category. Similar to DOF at BRDC diagnosis, the probability for DNF remained relatively constant until rectal temperature increased > 40.6°C, at

which point it increased for both male and female calves. Results in another study (Reinhardt et al., 2009) indicate that sex has no significant effect on the overall mortality rate of feedlot cattle; however, cattle that were culled prematurely (ie, realized) because of chronic disease were not included in that analysis.

The effect of the interaction between rectal temperature and quarter of year during which calves arrived at the feedlot on the probability of DNF was unexpected. We hypothesized that calves that entered the feedlot during the summer months (quarter 3) would have a low probability of DNF because high ambient temperatures could increase rectal temperatures of calves and lead to more healthy calves being treated for BRDC on the basis of pyrexia than during the other seasons. Seasonal adjustments to the rectal temperature cutoff used to diagnose BRDC in field settings have been suggested to account for the effect of high ambient temperature on rectal temperature (Theurer et al., 2014a; Theurer et al., 2013). The rectal temperatures of healthy calves can be increased from the reference range during periods of extreme heat, and calves with heat stress frequently have clinical signs that mimic those of BRDC (Theurer et al., 2014a; Theurer et al., 2013). Healthy calves with heat stress that are treated for BRDC solely on the basis of results of clinical observations should respond well to treatment and finish the production cycle normally because they were never diseased (Theurer et al., 2014a; Theurer et al., 2013). For most of the rectal temperature categories, the probability of DNF was greatest for calves that entered the feedlot during quarter 4 (October through December). This finding is most likely associated with standard management practices in which most beef calves are weaned and transported to feedlots (ie, stressors that put them at risk for BRDC) during quarter 4.

The negative relationship between body weight at feedlot arrival and probability of DNF in the present study was consistent with results of another study (Babcock et al., 2013). In the present study, calves with a body weight ≤ 226 kg at feedlot arrival had a 7.5% probability of being classified as DNF, which is greater than the probability of not finishing the observation period (1% to 5%) for similar calves in other studies (Bateman et al., 1990; Kelly and Janzen, 1986). However, in those studies (Bateman et al., 1990; Kelly and Janzen, 1986) calves were followed for only 63 days after feedlot arrival and only calves that died were included in the probability of not finishing the observation period, whereas in the present study, calves were followed throughout the entire feeding period and the probability of DNF included calves that

died as well as those that were euthanized or realized. The case-fatality rate for BRDC in feedlot calves typically ranges between 5% and 10% and may be influenced by type of calves being evaluated (Edwards, 2010). In general, compared with calves with a low body weight (≤ 226 kg) at feedlot arrival, calves with a higher body weight at feedlot arrival are less susceptible to BRDC and spend less time in the feedlot (ie, reach finished or slaughter weight quicker).

The accuracy of the final multivariable general linear model of the present study for predicting the probability that a calf would be classified as DNF was relatively low on the basis of the area under the ROC curve (0.646). Management of health outcomes is an important component to improve profitability and manage economic risk in feedlot settings.[24,25] On the basis of the findings of this study, a multivariable model that included sex, body weight at feedlot arrival, quarter of year at feedlot arrival, DOF feed at BRDC diagnosis, and rectal temperature at first BRDC treatment did not accurately predict whether an individual calf was going to be classified as DNF.

The economic tradeoff between the sensitivity and specificity for BRDC diagnosis of calves in feedlot settings may not be equal because a false-negative classification does not incur the same costs as a false-positive classification in terms of calf performance, frequency of misclassification, and true disease status (Theurer et al., 2014b). Management decisions for calves with BRDC might be altered on the basis of rectal temperature at initial BRDC treatment to maximize overall net returns. For example, a false-positive diagnosis of BRDC could result in healthy calves that would have finished the production cycle normally with their cohorts being unnecessarily treated and realized. Conversely, a false-negative diagnosis of BRDC could result in BRDC-affected calves not being appropriately identified and treated, which in the long term, could result in additional treatment and feed costs for those calves, when the most economically sound decision would have been to realize those calves early in the disease process. Although the economic tradeoff value for BRDC diagnostic sensitivity and specificity is unknown, the curvilinear line for sensitivity versus 1 – specificity (ROC curve; Figure 6) created on the basis of the results of the mixed general linear model was relatively flat; thus, improvement of the predictive ability of the model for identifying calves that will be classified as DNF is limited. This limitation in improvement might be a consequence of the case-definition of BRDC used in field settings, which has a low estimated sensitivity and specificity (White and Renter, 2009). Another reason for the relatively flat relationship between sensitivity and specificity might be a

result of the syndromic nature of BRDC and the fact that the multiple etiologic agents of BRDC cause varied pathophysiologic changes in affected calves in field settings. Additional research is necessary to evaluate how variables such as sex, body weight at feedlot arrival, and DOF and rectal temperature at BRDC diagnosis affect the predicted outcomes for BRDC-affected calves and to determine the most accurate method for identification of cattle with BRDC in a field setting.

In the present study, the probability of DNF was relatively stable until rectal temperature at initial BRDC treatment was > 40.6°C for most of the covariates assessed (sex, DOF at BRDC diagnosis, and quarter of year of feedlot arrival), at which point it began to increase as rectal temperature increased. An explanation for these results is that rectal temperatures > 40.6°C were indicative of severe BRDC, or that the diagnostic specificity for BRDC increased as rectal temperatures increased. Conversely, the case-fatality risk at high rectal temperatures (>40.6°C) might be a more typical response in truly ill calves, and the relative decrease in case-fatality risk at lower rectal temperatures is related to diagnostic inaccuracy or decreased specificity resulting in healthy calves being misclassified as affected with BRDC (ie, false positives).

The probability of DNF did not vary significantly among rectal temperature thresholds (39.4°C, 39.7°C, or 40.0°C) commonly used for BRDC diagnosis. The case definition and treatment protocols for BRDC can be influenced by rectal temperature; however, the present study did not identify a specific rectal temperature threshold that could be used to clearly delineate which cattle with BRDC will be classified as DNF. The data analyzed in this study were obtained from multiple feedlots across all seasons and many years, and care should be taken extrapolating these findings to a situation where the BRDC treatment protocol differs on the basis of a pre-existing rectal temperature threshold. The ROC curve developed for the model of this study indicated that, even when all variables were included, rectal temperature at first treatment for BRDC had a relatively low prognostic value for determining which calves would be classified as DNF.

The analysis of field collected data generally results in high external validity of the findings; however, a potential limitation of the data obtained for the present study was that it was collected retrospectively from multiple production systems. The data management process included steps to insure data validity, and potential recall bias was minimized owing to the electronic nature of the data management systems. Each operation used its own standard case

definition to identify calves with BRDC, and although differences in case definitions contributed to outcome variability, the collection of data from multiple feedlots that were representative of standard industry practices should provide good external validity of the study findings. Several significant interactions between cattle demographics and arrival characteristics were identified in this study, and extrapolation of these findings to specific cattle populations should be done with caution.

Results of the present study provided some knowledge about the probability that feedlot calves with BRDC will finish the production cycle normally with their unaffected cohorts; however, we were not able identify a rectal temperature that could be used as a cutoff threshold on which BRDC treatment decisions can be made. All calves included in this study were identified and treated for BRDC, and these findings indicated that rectal temperature of feedlot calves at initial BRDC treatment alone is not an accurate prognostic indicator for treatment outcome.

Footnotes

a. SAS, version 9.3, SAS Institute Inc, Cary, NC.

Figure 3.1. Histogram of rectal temperature at first treatment for BRDC for 344,982 feedlot calves identified with BRDC by feedlot personnel from 19 US feedlots from 2000 to 2009.

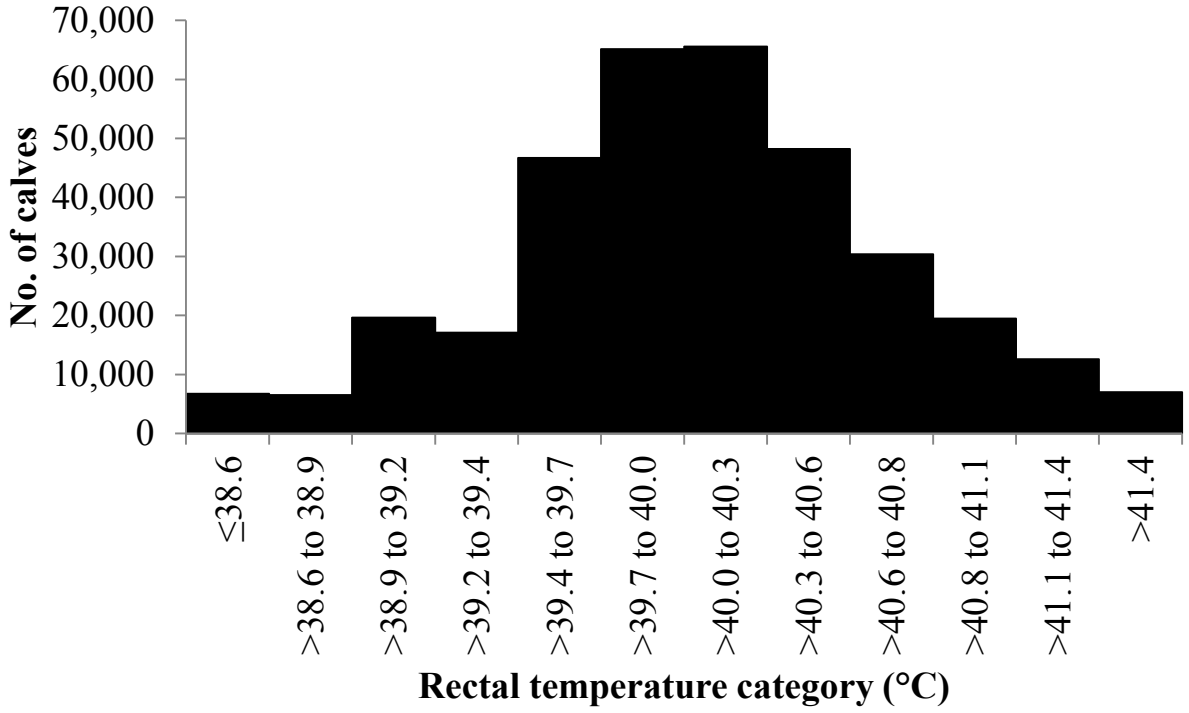


Figure 3.2. Model-adjusted least square mean ± SE probability that calves of Figure 3.1 would be classified as DNF (probability of DNF) by rectal temperature category and DOF (≤10 days [black line with black squares], 11 to 20 days [dotted line with white triangles], 21 to 30 days [dashed line with black diamonds], 31 to 40 days [gray line with white squares], and > 40 [gray line with black triangles]) at BRDC diagnosis.
Fixed effects included in the model included rectal temperature category, category of body weight at feedlot arrival, quarter of year at feedlot arrival, sex, and all significant ($P \leq 0.05$) 2-way interactions between rectal temperature and the other fixed effects. The interaction between rectal temperature category and DOF at BRDC diagnosis was significant ($P < 0.01$). See Figure 3.1 for remainder of key.

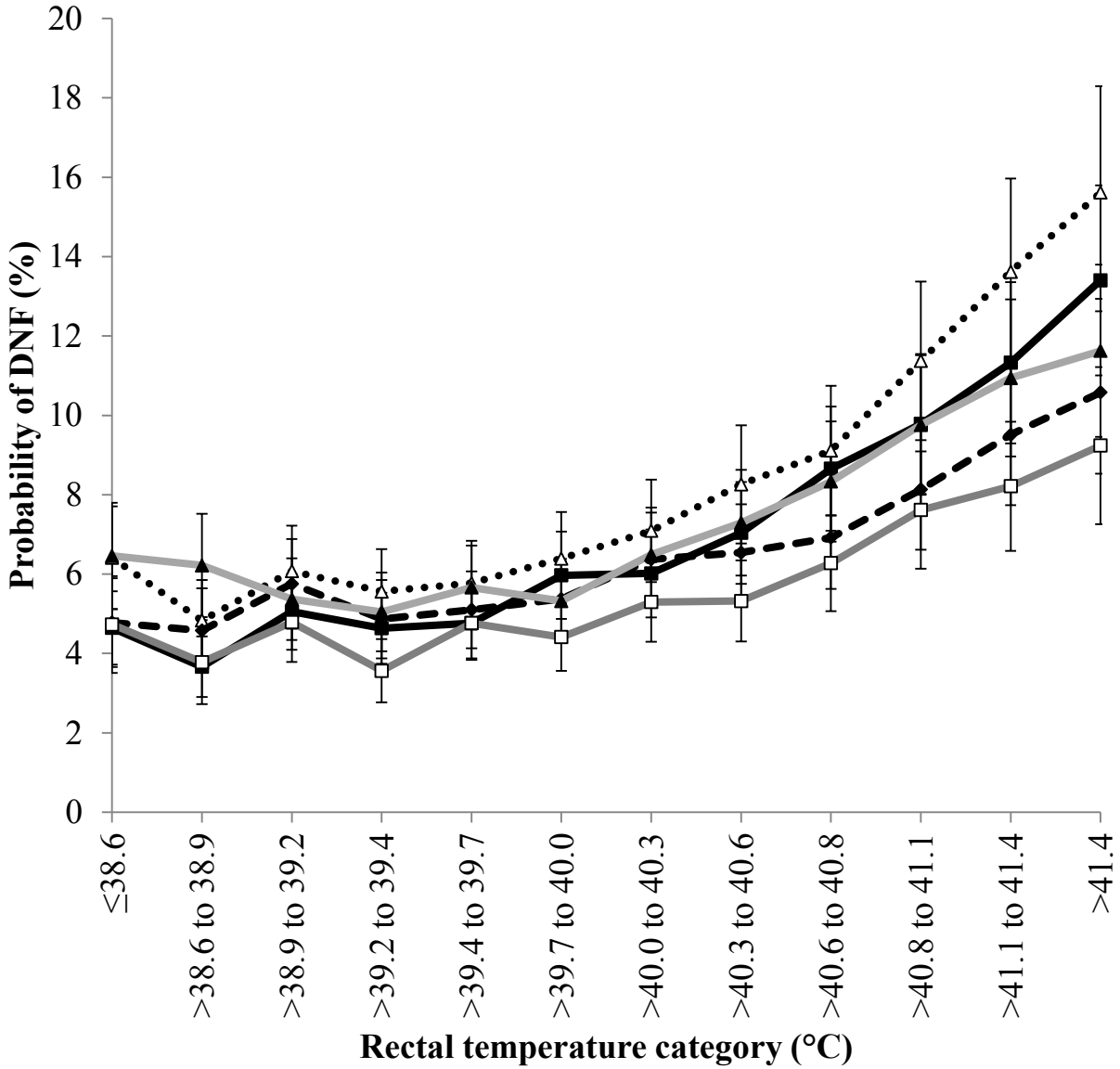


Figure 3.3. Model-adjusted least square mean ± SE probability of DNF for the calves of Figure 3.1 by rectal temperature category at first treatment for BRDC and sex (male [solid line] or female [dotted line]).
The interaction between rectal temperature category and sex was significant ($P < 0.01$). See Figures 3.1 and 3. 2 for remainder of key.

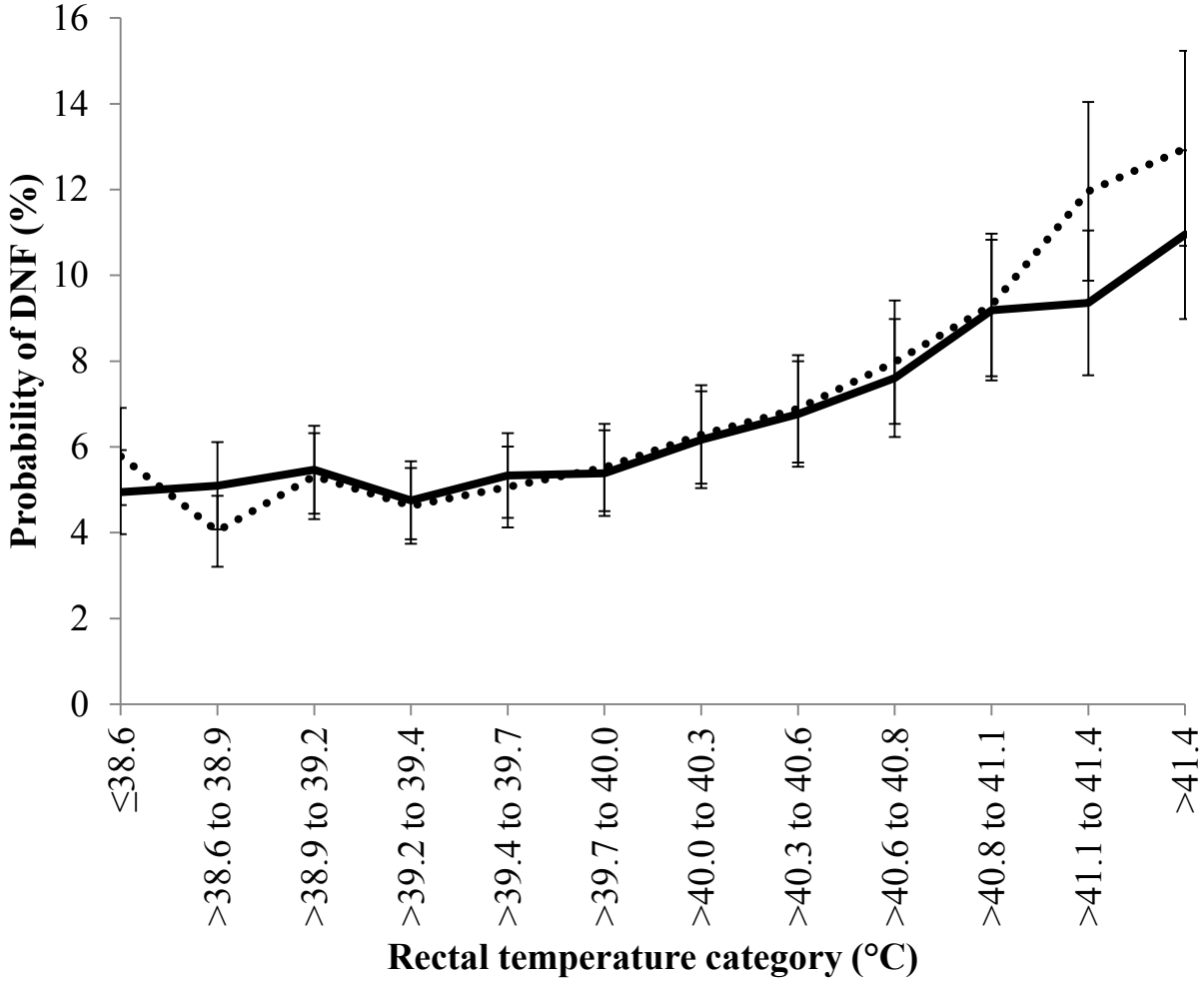


Figure 3.4. Model-adjusted least square mean ± SE probability of DNF for the calves of Figure 3.1 by rectal temperature category at first treatment for BRDC and quarter of year at feedlot arrival (quarter 1, January through March [black line with gray squares]; quarter 2, April through June [dotted line with gray triangles]; quarter 3, July through September [dashed line with white diamonds]; and quarter 4, October through December [gray line with black squares]).
The interaction between rectal temperature category and quarter of year at feedlot arrival was significant ($P < 0.01$). See Figures 3.1 and 3.2 for remainder of key.

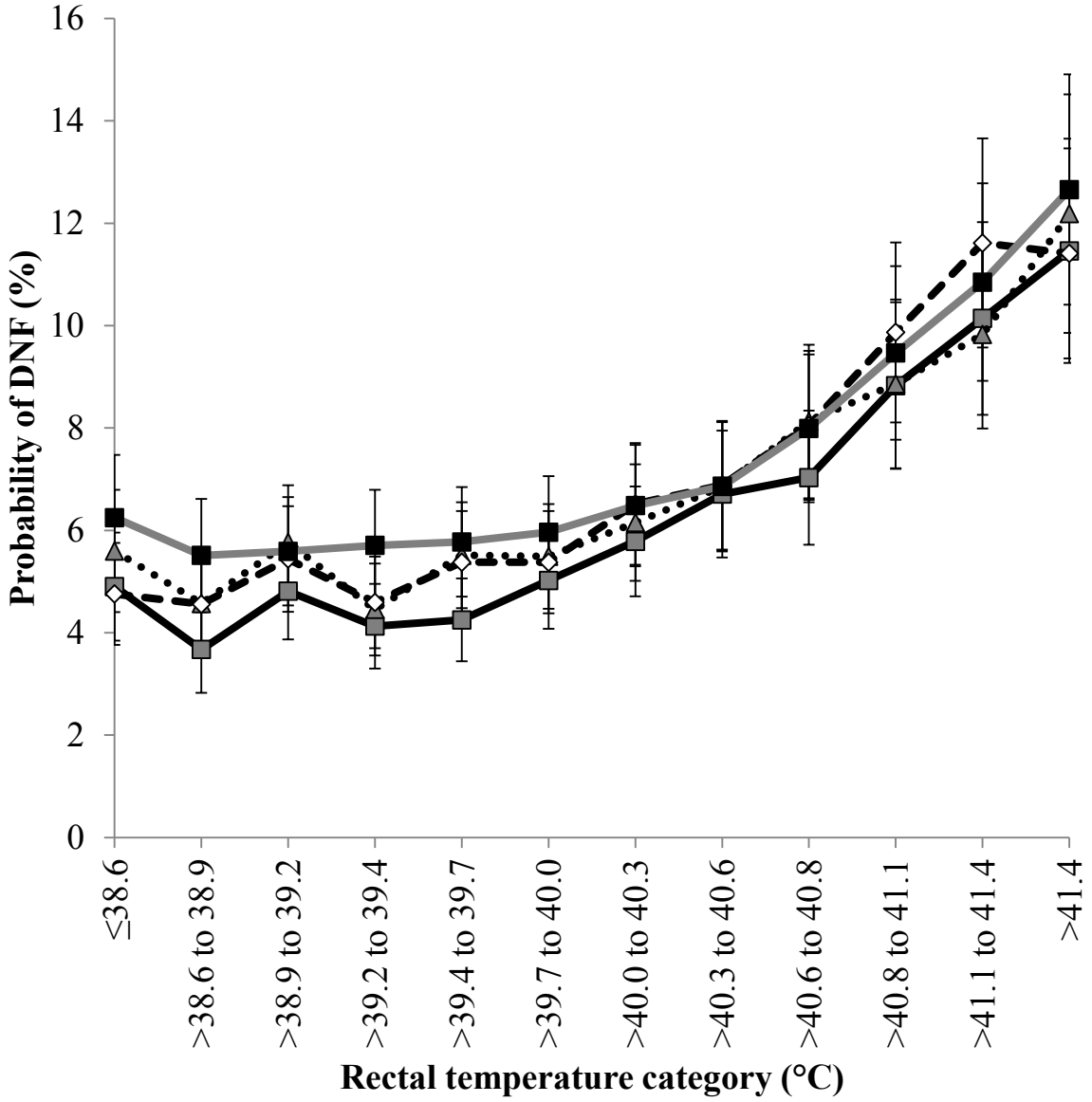


Figure 3.5. Model-adjusted least square mean ± SE probability of DNF for the calves of Figure 3.1 by category of body weight at feedlot arrival.
The interaction between rectal temperature category and category of body weight at feedlot arrival was not significant ($P = 0.55$). To convert kilograms to pounds, multiply by 2.2. *See* Figures 3.1 and 3.2 for remainder of key.

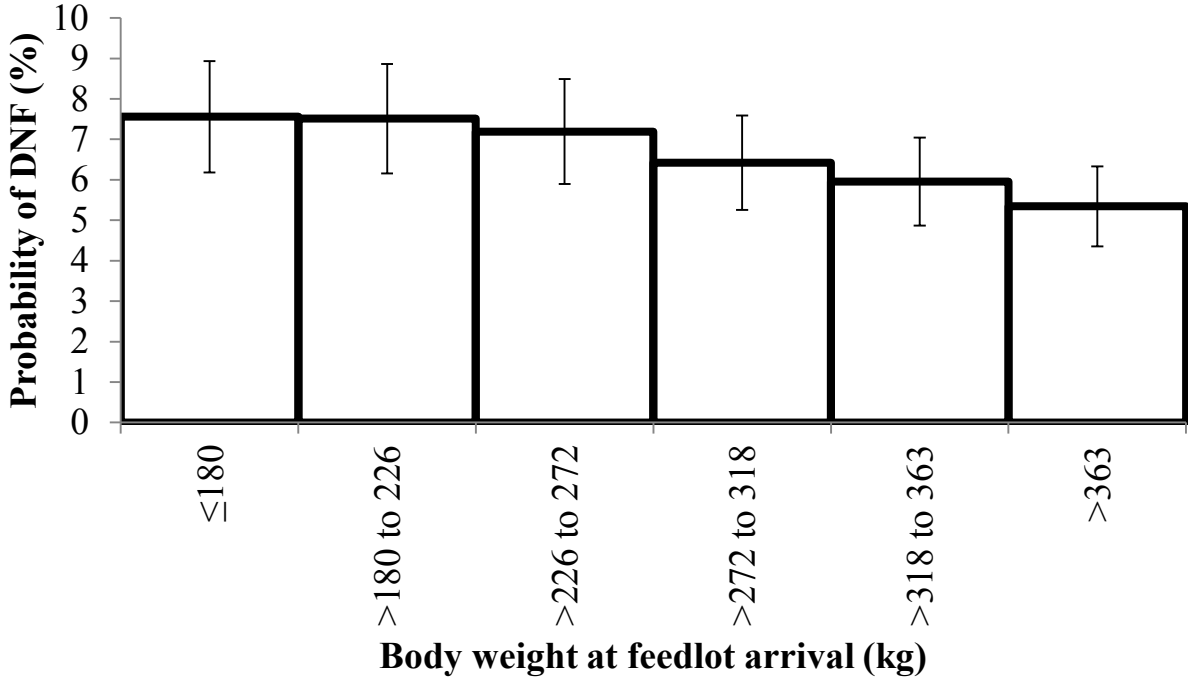
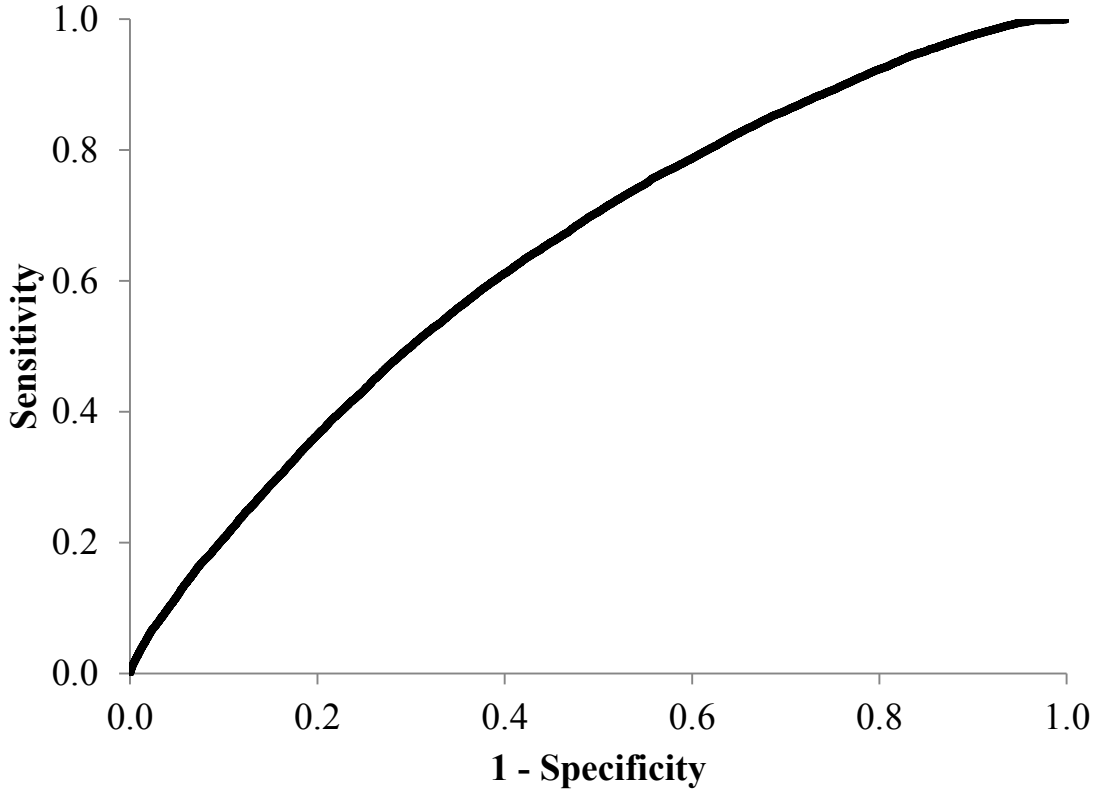


Figure 3.6. Parametric ROC curve for accuracy of the mixed general linear model described in Figure 3.2 to identify calves that were classified as DNF.
Random effects included in the model were year, feedlot, and lot number. The area under the ROC curve was 0.646. *See* Figure 3.2 for remainder of key.



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Chapter 4 - Effects of weather variables on thermoregulation of calves during periods of extreme heat

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Abstract

Objective—To determine effects of ambient temperature, relative humidity, wind speed, relative barometric pressure, and temperature-humidity index (THI) on nasal submucosal and rectal temperatures in cattle during extreme summer conditions.

Animals—20 black crossbred beef heifers (mean body weight, 217.8 kg).

Procedures—Nasal submucosal and rectal temperatures were monitored every 2 hours for 24 hours on 3 nonconsecutive days when ambient temperature was forecasted to exceed 32.2°C. Ambient temperature, relative humidity, wind speed, and relative barometric pressure were continuously monitored at a remote weather station located at the research facility. The THI was calculated and used in the livestock weather safety index (LWSI). Relationships between nasal submucosal or rectal temperature and weather variables were evaluated.

Results—Nasal submucosal and rectal temperatures were related to all weather variables monitored. A positive relationship was determined for ambient temperature and THI with both nasal submucosal and rectal temperatures. A negative relationship for nasal submucosal and

rectal temperature with relative humidity, wind speed, and relative barometric pressure was evident. Nasal submucosal and rectal temperatures increased with increasing severity of LWSI category.

Conclusions and Clinical Relevance—Effects of environmental conditions on thermoregulation in calves exposed to extreme heat were detected. The positive relationship between nasal submucosal temperature and ambient temperature and THI raised concerns about the efficacy of intranasal administration of temperature-sensitive modified live virus vaccines during periods of extreme heat. Environmental conditions must to be considered when rectal temperature is used as a diagnostic tool for identifying morbid cattle. (*Am. J. Vet. Res.* 2014;75:296-300)

Abbreviations

LWSI	Livestock weather safety index
THI	Temperature-humidity index

Introduction

Economic losses as a result of reduced productivity in cattle occur when animals are outside their thermal comfort zone. Heat stress has been estimated to cause annual losses of $282 million in beef finishing cattle because of reduced dry-matter intake, decreased growth rate, and increased risk of death (St-Pierre et al., 2003). In addition, cattle responses to high ambient temperature and relative humidity can mimic the clinical signs of respiratory disease. Heat stress has been associated with increased respiratory rate and effort, decreased feed intake, decreased activity, and increased body temperature (Fuquay, 1981; Mitlöhner et al., 2001). The similarity in behavior between calves affected by heat stress and cattle with respiratory disease can create challenges for people responsible for monitoring animal health.

The THI, which was established to estimate the severity of risk from heat stress in cattle, is based primarily on ambient temperature and relative humidity (Mader et al., 2000; Mader et al., 2006). The THI is used in the LWSI to categorize environmental conditions: normal (THI, ≤ 74), alert (75 to 78), danger (79 to 83), and emergency (≥ 84) (Hahn et al., 2009; United States. Agricultural Marketing Service and Marketing, 1999). The LWSI was established by the USDA Agriculture Marketing Service to provide recommendations for transportation of cattle and swine during extreme summer conditions (United States. Agricultural Marketing Service and

Marketing, 1999). Ambient temperature and relative humidity have been positively correlated with panting scores in cattle during hyperthermal conditions (Mader et al., 2006).

Although thermography measurement of nares temperature in cattle has been discussed (Reid et al., 2012; Theurer et al., 2013a; Theurer et al., 2013b), no study has been conducted to determine the effect of environmental temperature on nasal submucosal temperatures. Nares thermography records the temperature of the air exiting the nasal passages. These measurements are subject to errors associated with the conditions in which the thermography images are obtained. Also, thermography equipment of sufficient quality to accurately measure these temperatures is expensive.

Current use of temperature-sensitive vaccines administered via the intranasal route requires that nasal mucosal temperatures do not exceed 39°C (Mills et al., 1971). To the authors' knowledge, the effect of extreme summer environmental conditions on nasal submucosal temperature has not been reported. The objective of the study reported here was to determine the effect of ambient temperature, relative humidity, wind speed, relative barometric pressure, and THI on nasal submucosal and rectal temperatures in cattle during extreme summer heat conditions. This information would be useful for understanding the effects of environment on thermoregulation.

Materials and Methods

Animals

Twenty black crossbred beef heifers with a mean ± SD body weight of 217.8 ± 12.1 kg were selected for the study. All calves were approximately 6 months old. Calves were owned by Kansas State University, and all procedures were approved by an institutional animal care and use committee.

Calves were housed in a single pen (12.2 X 24.4 m) throughout the study. Calves were fed a starter ration that included 2.3 kg of corn/d with trace minerals and 0.9 kg of alfalfa/d. In addition, calves had ad libitum access to brome hay, a salt block, and water. A south-facing open-face tin shed was available as a source of shade throughout the study. Cattle were humanely handled during each portion of the study and observed twice daily throughout the study to monitor health status.

Measurements were obtained during 3 nonconsecutive intensive 24-hour monitoring periods (measurements obtained for a total of 72 hours). Monitoring periods were selected on the

basis of weather forecasts that the ambient temperature would exceed 32.2°C. The intensive monitoring period began at 8 AM, and nasal submucosal and rectal temperatures were recorded every 2 hours for 24 hours. Calves were moved through a chute, and rectal temperatures were measured with a rapid equilibration probe[a] to allow for minimal handling during the measurement period. Commercially available, inexpensive, and easily applied biothermal sensors[b] were implanted in the left and right nasal submucosa approximately 100 mm caudal to the alar cartilages. The biothermal sensors were radiofrequency transponders activated by an electronic recording device and had an accuracy of ± 0.1°C. A remote weather station[c] was placed at the research facility where the calves were housed to enable monitoring of local ambient temperature, relative humidity, wind speed, and relative barometric pressure throughout the study. The weather station was set to record variables for the same time periods during which calves were moving through the chute. Results for the aforementioned variables were used to calculate THI for each time point by use of the following equation (Hahn, 1999; Thom, 1959):

$$\text{THI} = (0.81 \times \text{ambient temperature}) + (\text{relative humidity} \times [\text{ambient temperature} - 14.4]) + 46.4$$

Statistical analysis

Data were imported into a commercial statistical software package[d] for analysis. The mean value for the left and right nasal submucosal temperature was calculated at each time point and used for statistical analysis. Values for weather variables were rounded to the nearest whole number prior to analysis. A multivariate model was created to evaluate the potential relationships for nasal submucosal or rectal temperature with all environmental weather variables (ambient temperature, relative humidity, wind speed, relative barometric pressure, and THI). Individual generalized mixed models were used to evaluate potential relationships between nasal submucosal and rectal temperatures on the basis of ambient temperature, relative humidity, wind speed, relative barometric pressure, and THI. All analyses included a random effect for each calf because of repeated measures on the calves and a random effect for each day (24-hour period of sample collection) temperatures were recorded. The time of day of observations (2-hour intervals) was included as a fixed effect in models whereby the interaction between time and the effect of interest was evaluated or as a random effect in models constructed to evaluate the overall estimates of the amount of time spent in thermal zones. For all comparisons, values of $P < 0.05$ were considered significant. Correlation analysis between rectal temperature and nasal

submucosal temperature was performed; this analysis included random effects for repeated measures on calves, study day, and time of day.

The THI was categorized into groups on the basis of the LWSI for recommendations of transportation of cattle during extreme summer conditions (United States. Agricultural Marketing Service and Marketing, 1999). Nasal submucosal and rectal temperatures were evaluated for each LWSI category with generalized mixed models that included a random effort for repeated measures on calves and a random effect for each day of the study. Student *t* tests were used to evaluate differences in nasal submucosal and rectal temperatures for each LWSI category. For all multiple comparisons, values of $P < 0.01$ were considered significant.

Results

All calves remained healthy throughout the study, and the ambient temperature exceed 32.2°C for each of the 24-hour intensive monitoring periods. Sunrise was at approximately 6 AM, and sunset was at approximately 7 PM. Overnight low temperature was 25.5°C, 21.4°C, and 20.7°C, respectively, for the 24-hour intensive monitoring periods. Mean environmental conditions by time of day were summarized **(Table 1)**. Ambient temperature was lowest in the early morning (6 AM; mean, 22.6°C) and highest in the late afternoon (4 PM; mean, 36.8°C). Relative humidity was highest in the early morning (6 AM; mean, 80.0%) and lowest in the late afternoon (4 PM; mean, 39.7%). Wind speed was lowest in the early morning (8 AM; mean, 1.4 m/s) and highest in the late afternoon (4 PM; mean, 3.8 m/s). Relative barometric pressure was highest in mid-morning (100 AM; mean, 739.06 mm Hg) and highest in the late afternoon (6 PM; mean, 736.85 mm Hg). The THI was lowest in the early morning (6 AM; mean, 70.96) and highest in the late afternoon (4 PM; mean, 84.69).

During the 72 hours of monitoring, the calves were exposed to environmental conditions in several LWSI categories. The percentage of time the calves spent in each LWSI category was as follows: normal, 27.8%; alert, 13.9%; danger, 25.0%; and emergency, 33.3%.

A single multivariate model for comparing nasal submucosal or rectal temperature with all of the weather variables did not converge; therefore, results were reported for models created to evaluate relationships between nasal submucosal or rectal temperatures and individual weather variables. Nasal submucosal and rectal temperatures were significantly ($P < 0.01$) associated with ambient temperature, relative humidity, wind speed, relative barometric pressure, and time of day **(Figure 1**; **Table 1)**. Nasal submucosal and rectal temperatures were significantly ($P <$

0.01) associated with THI **(Figure 2)**. Rectal and nasal submucosal temperatures increased with increasing severity of LWSI category. Analysis revealed that there was a correlation ($r^2 = 0.77$) between nasal submucosal and rectal temperatures. Nasal submucosal temperature exceeded 39°C in 5 calves during the monitoring period.

Discussion

In the study reported here, nasal submucosal and rectal temperatures in beef heifers were correlated with weather variables during periods of extreme heat. This may be of importance when evaluating the response of cattle to intranasal administration of temperature-sensitive vaccines. Temperature-sensitive vaccines are inactivated at temperatures > 39°C (Mills et al., 1971). In the present study, calves exposed to extremely high ambient temperatures occasionally had nasal submucosal temperatures above this threshold. Nasal submucosal and rectal temperatures were strongly correlated. Nasal submucosal and rectal temperature measurements are not subject to human error and may provide a more accurate reflection of temperatures to which the nasal mucosa is subjected, compared with the accuracy for temperatures measured with nasal thermography.

Rectal temperatures are a common component of health monitoring protocols (Radostits et al., 2001). However, information on the evaluation of the environmental effects on rectal temperatures during conditions of extreme heat or a high THI is lacking. Diurnal variation in rectal temperature of cattle has been described (Davis et al., 2003; Hanzlicek et al., 2010). In one of those studies (Hanzlicek et al., 2010), the lowest rectal temperatures were recorded during the morning hours, and the highest rectal temperatures were recorded during the late afternoon and early evening hours. The diurnal variation was attributed to changes in the environmental conditions those calves were exposed to throughout the day (Hanzlicek et al., 2010).

In the present study, associations were detected between nasal submucosal and rectal and all weather variables monitored; however, changes in nasal submucosal and rectal temperatures were more dramatic with increases in ambient temperature and THI. Nasal submucosal temperature increased more rapidly than did rectal temperature with increases in ambient temperature and THI, but there was marked variation. However, the overall pattern was a positive relationship (increases in nasal submucosal and rectal temperatures with increases in ambient temperature and THI). The rectal temperatures in the present study were recorded during summer environmental conditions, which limited interpretation of the data during these summer

conditions. These results may prove useful in future studies on extreme heat in the Midwest, which is the location for most beef feedlots (Feuz and Umberger, 2003; Mintert, 2003).

Surprisingly, a slight negative relationship was found for nasal submucosal and rectal temperatures with relative humidity. As relative humidity increases, respiration rates increase in an attempt to dissipate body heat (Mader et al., 2006; Seath and Miller, 1946). However, the calves in our study were exposed to weather conditions that resulted in an inverse relationship between ambient temperature and relative humidity because the highest relative humidity was detected during the night and the lowest relative humidity was detected during the day. We did not have enough data points to compare animal responses to different amounts of relative humidity at similar ambient temperatures to separate the effects of relative humidity and ambient temperature. The relationship between nasal submucosal temperature and relative humidity was unclear because of the variation in nasal submucosal temperature, which resulted in no pattern that was clearly evident.

Nasal submucosal and rectal temperatures decreased with increases in wind speed, as expected. Wind may help cattle dissipate heat and allow them to thermoregulate more efficiently through evaporation (Stokka et al., 1996).

It has been suggested that the THI is the best, simplest, and most practical method used to predict risk of heat stress in cattle. The positive relationship for nasal submucosal and rectal temperature with THI was expected. Variation in the nasal submucosal temperature with changes in THI was not as great as the variation in the nasal submucosal temperature with changes in ambient temperature. Both nasal submucosal and rectal temperatures plateaued at a THI $\geq$ 86. This plateau may be the tolerance limit for nasal submucosal and rectal temperatures in healthy cattle during periods of extreme heat. It could be speculated that cattle would succumb to heat stress at a THI above this threshold, but such studies might endanger well-being of the cattle and has not been performed to the authors' knowledge.

The calves remained healthy throughout the present study. The heifers were monitored continuously, and none of them developed clinical signs of illness throughout the study. The physiologic capability of morbid animals to thermoregulate during periods of extreme heat conditions remains unclear. Heat stress environmental indices have been established that incorporate both solar radiation and wind speed (Mader et al., 2010). Intensity of solar radiation

was not monitored during the present study, but all calves were housed in a single pen with full exposure to sunlight, although an open-faced tin shed did provide shade.

A diurnal pattern in nasal submucosal and rectal temperatures was detected, but the changes were less marked than in other studies (Davis et al., 2003; Hanzlicek et al., 2010). The minimal diurnal fluctuation in the present study may have been related to the environmental conditions to which the calves were exposed. It is important to consider the time of day when rectal temperature is measured and used as a diagnostic tool, given that a change in rectal temperature of 1°C may result in a different diagnosis or treatment.

Limitations of the study included that all monitoring periods were during extreme summer heat, and the weather variables monitored were not independent of each other, nor were they controlled. We attempted to develop a multivariate model with all of the weather variables included, but it was not possible to create a final model with these data because of convergence issues. Data were collected during three 24-hour periods; however, these periods did not provide sufficient variation among weather and outcome variables to enable us to evaluate them in a single model. A larger data set with increased variation in environmental variables may enable researchers to generate a multivariate model and provide more insights into the true relationships between weather conditions and homeostasis in cattle. Additional studies need to be performed to determine the clinical implications of extreme summer conditions on monitoring, health, productivity, and management strategies of beef calves.

In the present study, weather conditions impacted thermoregulation in cattle. Overall, calves were efficient at responding to various extremes of ambient temperature and relative humidity. The positive relationship between nasal submucosal temperature and ambient temperature and THI raises concerns about the efficacy of intranasal administration of temperature-sensitive modified live-virus vaccines to cattle during periods of extreme heat. Environmental conditions and the THI need to be considered when rectal temperature is used as a diagnostic tool to identify morbid animals because weather variable are associated with rectal temperature.

Footnotes

a. Pavia Rectal Temp, Pavia Sales Group Inc, Plymouth, Minn.

b. Biothermal LifeChip with Bio-thermo technology for llamas and alpacas, Destron Technologies, Round Rock, Tex.

c. WS-2812, La Crosse Technology, La Crosse, Wis.

d. JMP, version 9, SAS Institute Inc, Cary, NC.

Figure 4.1. Mean ± SE rectal temperature (white triangles) and nasal submucosal temperature (black squares) in 20 black crossbred beef heifers exposed to ambient temperature (A), relative humidity (B), wind speed (C), and relative barometric pressure (D) during extreme summer weather conditions.

The line of best fit and equation for that line was determined for nasal submucosa temperature and rectal temperature. Data were obtained during 3 nonconsecutive 24-hour periods when ambient temperature was forecasted to exceed 32.2°C. The model used for analysis included effects for repeated measures on individual calves, repeated measures on study day, and time of day. All weather variables monitored had significant ($P < 0.01$) effects on rectal temperature and nasal submucosal temperature.

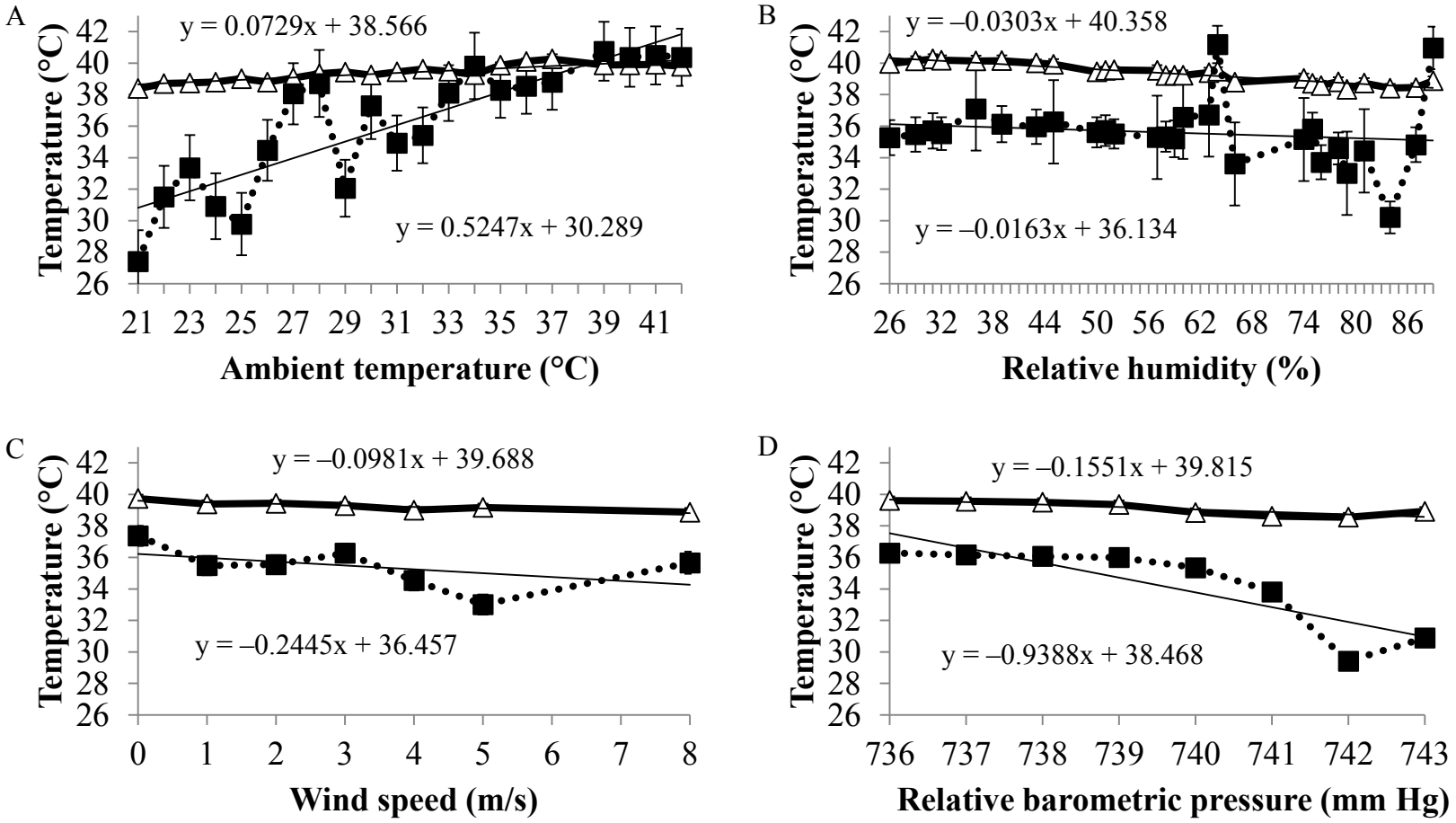

Figure 4.2. Mean ± SE rectal temperature (white triangles) and nasal submucosal temperature (black squares) in 20 black crossbred beef heifers by THI (A) and mean ± SE rectal temperature and nasal submucosal temperature by LWSI category (B).
In panel A, the THI had a significant ($P < 0.01$) effect on rectal temperature and nasal submucosal temperature. In panel B, the THI was used in the LWSI to categorize environmental conditions as follows: normal (THI, ≤ 74 [black bars]), alert (THI, 75 to 78 [gray bars]), danger (THI, 79 to 83 [white bars]), and emergency (THI, ≥ 84 [vertical-striped bars]).[6,7] [a–d] Within a temperature variable, LWSI categories with different letters differ significantly ($P < 0.01$). Notice that the scale on the y-axis differs between the panels.

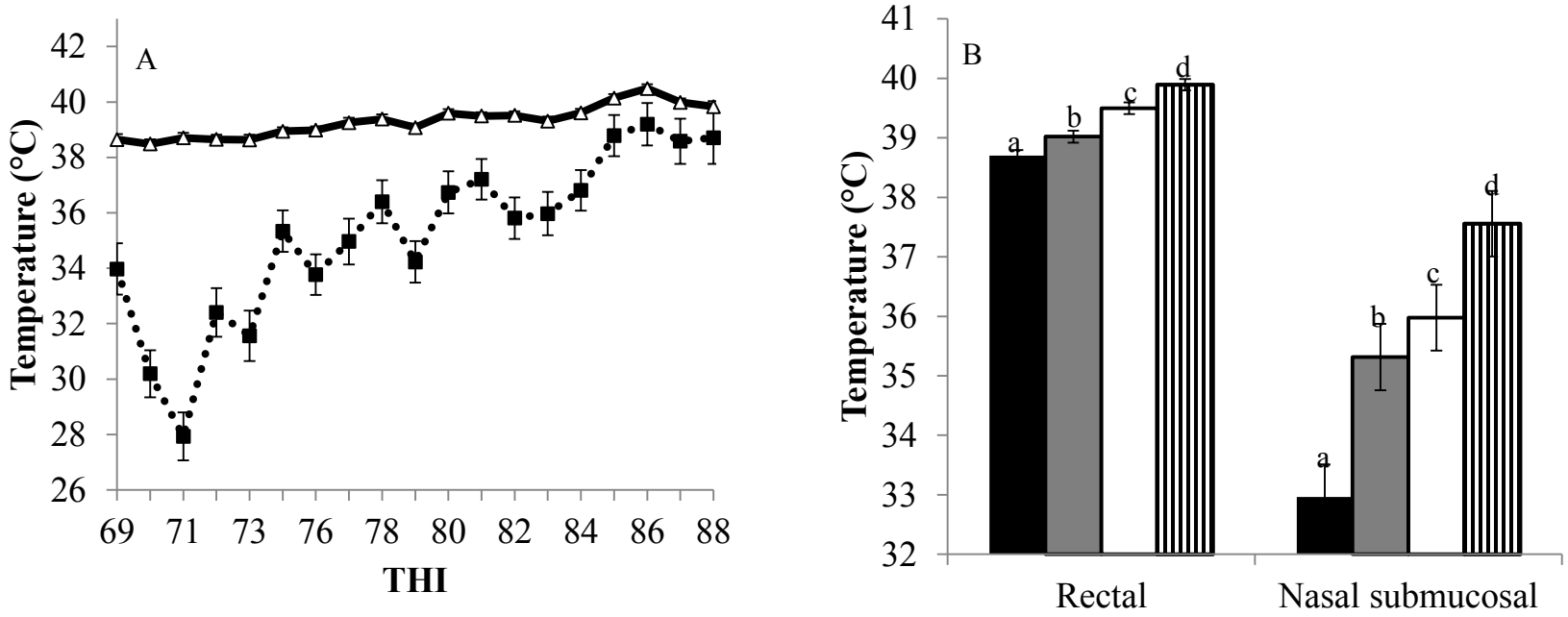

Table 4.1. Mean ± SD rectal temperature and nasal submucosal temperature by time of day in 20 black crossbred beef heifers exposed to extreme environmental conditions in summer.

Time of day (h)	Ambient temperature (°C)	Relative humidity (%)	Wind speed (m/s)	Relative barometric pressure (mm Hg)	THI	Rectal temperature (°C)	Nasal submucosal temperature (°C)
8 am	28.9 ± 3.7	66.7 ± 11.9	1.4 ± 0.4	738.97 ± 0.94	79.1 ± 3.5	39.10 ± 0.47	36.3 ± 1.3
10 AM	34.2 ± 3.3	53.0 ± 12.2	1.8 ± 0.1	739.06 ± 0.94	84.2 ± 1.8	39.58 ± 0.58	37.9 ± 0.6
Noon	35.5 ± 3.9	47.3 ± 11.2	1.8 ± 0.8	738.72 ± 1.05	84.7 ± 2.7	39.64 ± 0.63	37.5 ± 0.6
2 pm	36.7 ± 4.1	41.7 ± 8.3	2.4 ± 0.6	738.12 ± 1.04	85.1 ± 3.4	39.70 ± 0.70	37.0 ± 0.6
4 pm	36.8 ± 4.1	39.7 ± 10.2	3.8 ± 3.0	737.53 ± 1.05	84.7 ± 2.8	40.05 ± 0.70	36.6 ± 0.8
6 pm	35.9 ± 3.8	41.3 ± 9.5	3.3 ± 1.5	736.85 ± 0.95	84.0 ± 2.8	39.90 ± 0.57	35.3 ± 0.8
8 pm	32.4 ± 2.9	52.0 ± 9.3	2.1 ± 1.0	737.28 ± 1.54	81.8 ± 2.3	39.71 ± 0.51	34.7 ± 0.9
10 PM	27.4 ± 3.6	61.7 ± 12.3	3.2 ± 1.3	738.12 ± 2.02	76.2 ± 3.3	39.25 ± 0.54	34.7 ± 1.6
Midnight	25.8 ± 3.3	68.7 ± 11.9	2.1 ± 1.0	738.04 ± 1.19	74.7 ± 3.4	38.99 ± 0.47	34.5 ± 1.3
2 am	24.8 ± 2.3	72.3 ± 11.0	1.6 ± 0.3	738.38 ± 2.00	73.8 ± 2.2	38.86 ± 0.49	34.4 ± 1.7
4 am	23.6 ± 2.7	76.3 ± 13.1	1.6 ± 0.7	738.89 ± 2.91	72.3 ± 2.8	38.73 ± 0.43	34.1 ± 2.7
6 am	22.6 ± 2.1	80.0 ± 11.4	2.7 ± 2.2	739.06 ± 2.24	71.0 ± 2.3	38.56 ± 0.38	33.9 ± 3.2

The model used for analysis included effects for repeated measures on individual calves for rectal temperature and nasal submucosal temperature and repeated measures on study day. Time of day had a significant ($P < 0.01$) effect on all variables evaluated.

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Chapter 5 - Effect of transportation during periods of high ambient temperature on physiologic and behavioral indices of beef heifers

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Abstract

Objective—To determine the effect of transportation during periods of high ambient temperature on physiologic and behavioral indices of beef heifers.

Animals—20 heifers (mean body weight, 217.8 kg).

Procedures—Heifers were transported 518 km when the maximum ambient temperature was ≥ 32.2 °C while the other 10 heifers served as untransported controls. Blood samples were collected from transported heifers at predetermined intervals during the transportation period. For all heifers, body weights, nasal and rectal temperatures, and behavioral indices were measured at predetermined intervals for 3 days after transportation. A week later, the entire process was repeated such that each group was transported twice and served as the control twice.

Results—Transported heifers spent more time near the hay feeder on the day of transportation, had lower nasal and rectal temperatures for 24 hours after transportation, and spent more time lying down for 2 days after transportation, compared with those indices for control heifers. Eight hours after transportation, the weight of transported heifers decreased 6%, whereas that of control heifers increased 0.6%. At 48 hours after initiation of transportation, weight, rectal temperature and time spent at various pen locations did not differ between transported and control heifers. Cortisol concentrations were higher 4 hours after initiation of transportation, compared with those determined just prior to transportation.

Conclusions and Clinical Relevance—Results indicated transportation during periods of high ambient temperatures caused transient changes in physiologic and behavioral indices of heifers.
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Abbreviations

BRD	Bovine respiratory disease
TNF	Tumor necrosis factor

Introduction

Throughout the United States, it is common for cow-calf producers to transport cattle substantial distances to feedlot facilities generally located in the central portion of the country (Feuz and Umberger, 2003; Mintert, 2003). In 2010, > 34 million cattle were slaughtered in the United States (USDA, 2011), and most of those cattle would have been transported at least once prior to slaughter. Handling prior to, during, and after transportation is stressful for cattle (Fike and Spire, 2006; Grandin, 1997; Swanson and Morrow-Tesch, 2001), and transportation regulations for cattle are being scrutinized (Transportation, 2011).

Bovine respiratory disease is one of the most economically important diseases affecting beef feedlot cattle. For immature beef cattle, the incidence of BRD commonly increases during the stress of weaning and transportation to a feedlot (Edwards, 1996; Fike and Spire, 2006). Bovine respiratory disease affects 14.4% of all cattle entering beef feedlots (USDA, 2000), and the immune responses of recently transported cattle are often suppressed (Mackenzie et al., 1997) because of increased cortisol concentrations (McEwen et al., 1997).

Factors associated with stress to cattle during transportation include management changes, novelty, social regrouping, ambient temperature, humidity, and transit time (Swanson and Morrow-Tesch, 2001). Knowles recommends not transporting cattle when the ambient temperature is > 30°C (Knowles, 1999). Because of the constant flow of cattle into feedlots, restriction of the transportation of cattle to when temperatures are < 30°C may cause logistic complications for the beef industry during summer months and some periods of the spring and autumn when high ambient temperatures persist for several consecutive days (APHIS, 2000). These complications may compound the stress endured by feedlot cattle, especially when movement to the feedlot is delayed; cattle exposed to severe or sustained stress may have increased susceptibility to disease (Moberg and Mench, 2000). Research to examine the physiologic responses of cattle to transportation during periods of high ambient temperatures is warranted to determine whether restrictions on cattle transportation are necessary.

The objectives of the study reported here were to determine the effects of transportation during periods of high ambient temperature (≥ 32.2°C) on physiologic and behavioral indices of beef heifers. Our hypotheses were that transported heifers would have increased body temperatures, increased concentrations of stress biomarkers in blood, and decreased activity after transportation. Evaluation of these variables may help identify important risk factors for morbidity after transportation of cattle.

Materials and Methods

Animals

Twenty black crossbred beef heifers with a mean ± SD body weight of 217.8 ± 12.1 kg were selected for the study reported here. All heifers were owned by Kansas State University and all study procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Throughout the study, the heifers were housed as a group in a single pen (12.2 X 24.4 m) and fed a ration calculated to provide each heifer 2.3 kg of ground corn/d with trace mineral and 0.9 kg of alfalfa/d in addition to *ad libitum* access to brome hay, a supplemental salt block, and water. The heifers were humanely handled during each portion of the study. The health status of the heifers was monitored via the same individual who recorded each heifer's heart rate and respiration rate and assigned a clinical health score to each heifer twice daily throughout the study.

Study design

The study had a double-crossover design. At study initiation, each heifer was matched to another heifer on the basis of weight to form a block of 2 heifers. Then each heifer within a block was randomly allocated to 1 of 2 groups such that each group contained 10 heifers. On each of 4 days, one group of heifers was transported 518 km in a livestock trailer (2.1 X 6.1 m; stocking density, 170 kg/m^2) while the other group served as untransported controls. The study protocol was repeated such that each group was transported twice and served as controls twice. The days during which cattle were transported were selected by a predetermined criterion that the maximum ambient temperature was forecasted to be ≥ 32.2°C.

A wireless remote weather station was installed at the research facility to allow continuous monitoring of the environmental conditions where the heifers were housed. On the morning of each day selected for transportation, none of the heifers were fed grain until after the transportation process was initiated and the heifers being transported did not have access to feed or water during the 8 hour period of transit and processing. The heifers being transported were loaded into the livestock trailer at 8:00 AM (hour, 0). Those heifers were then transported for 4 hours (noon; hour, 4) and approximately 259 km to a remote livestock-working facility where they were unloaded and worked through a chute system so that body weights and nasal and rectal temperatures could be measured and venous blood samples could be obtained. Then, the heifers were reloaded into the livestock trailer and transported 4 hours back to the research facility, arriving at approximately 4:00 PM (hour, 8). Three days after the first group of heifers was transported, the study groups were crossed over and the 10 heifers that served as controls during the first transportation day were transported. The entire process was repeated once such that each group of heifers were transported twice and served as controls twice.

Measurement of rectal, nasal, and surface temperatures

For each heifer, rectal temperature was measured with a rapid equilibration thermal probe.[a] Radiofrequency thermal sensors[b] were implanted at a depth of approximately 2 mm in the submucosa of the nasal mucosae on the dorsal and medial aspects of the left and right nares approximately 100 mm caudal to the alar cartilage. Each sensor contained a radiofrequency transponder that, when initiated by an electronic signal from a reading device,[c] sent the temperature (± 0.1°C) back to the reading device where it was recorded into an electronic database. A high-definition thermal sensor camera[d] was used to record surface temperatures of

the right and left nares, nasal planum, and cornea at hours 0 and 8. The mean temperature for the left and right nares was calculated and this value was used for subsequent statistical analyses. Rectal and nasal temperatures were recorded from all heifers immediately prior to (hour, 0) and at 4 (transit midpoint), 8 (transit end), 10, 12, 14, 16, 18, 20, 22, 24, 36, 48, and 56 hours after initiation of each transportation period.

Behavioral data acquisition

Prior to initiation of the study, a remote location monitoring tag[e] was applied to the left ear of each study heifer to record heifer behavior and activity as described (White et al., 2012). Briefly, the tag transmitted information about the heifer's location (ie, X [length] and Y [width] coordinates) within the pen to fixed wireless sensors at the periphery of the pen, which relayed the information to a computer database where it was stored for analysis.[f] The heifer's coordinates were recorded at 1-second intervals throughout the study and each set of coordinates was identified with a time stamp. A data mining software program[g] was used to compare each set of coordinates for a heifer with the known X and Y coordinates of specific locations (grain feeder, hay feeder, waterer, and shelter) within the pen, and each set of heifer coordinates were dichotomously (yes or no) classified as being within a 0.3-m radius of each location. The time stamps on the coordinates were evaluated to determine the amount of time a heifer spent at a particular location within the pen during a given period. Data obtained from heifers during transportation were not included in the statistical analyses.

Each heifer also had a commercial accelerometer[h] and pedometer[i] applied to the lateral aspect of the right hind limb just proximal to metatarsophalangeal (fetlock) joint. The accelerometer and pedometer were placed within a neoprene sleeve and affixed to the limb with a strap. The accelerometer recorded triaxial (X, Y, and Z axes) directional forces with an axis range of ± 10 *g* and recorded 100 measurements/s (Reference LLC, 2007). The accelerometers were programmed with validated settings (Robert et al., 2009) such that X, Y, and Z acceleration and mean and maximum vector magnitude were recorded at 5-second intervals. Values for the mean force of gravity and vector magnitude were calculated by summing the values for force of gravity and acceleration (values for X, Y, and Z axes combined), respectively, and dividing each by the number of measurements (ie, 5-second intervals) recorded during a specified time period. The maximum vector magnitude was the highest combined value for acceleration during the 5-second interval. Every 7 days throughout the study, the accelerometers were briefly removed

from the heifers so that data could be downloaded into a computer database and then were reapplied. Data obtained via the accelerometers was analyzed with a data mining software program[g] to determine the amount of time each heifer spent standing, lying down, or walking during each 5-second interval, which was then aggregated by day. Data obtained from accelerometers during transportation were analyzed separately from data obtained from accelerometers during the remainder of the observation period.

For each heifer, the number of steps/d was determined via the pedometer, which contained a 2-D accelerometer that monitored the up and down movement of the limb. Along with the accelerometers, the pedometers were briefly removed from the heifers every 7 days so that data could be downloaded into a computer database and then were reapplied. Although behavioral data was obtained throughout the study, behavioral activity was analyzed for a period of only 3 days after initiation of each transportation period.

Body weight

For each transportation period, all heifers were individually weighed immediately prior to (hour, 0) and at 4, 8, and 48 hours after initiation of transportation. For each heifer at 4, 8, and 48 hours, respectively, the percentage change in body weight was calculated as follows:([body weight at 0 hours – body weight at the time of interest]/body weight at 0 hours) X 100%.

Blood sample collection and analyses

During each transportation period, blood samples (12 mL) were collected via jugular venipuncture from the heifers being transported immediately prior to (hour, 0) and at 4, 8, 24, 36, 48, and 56 hours after initiation of transportation. The blood samples were immediately transferred to a 6-mL serum separator tube and 6-mL tube containing potassium EDTA. All blood samples were centrifuged at 1,500 X *g* for 10 minutes. A 2-mL aliquot of serum or plasma was separated from each sample, placed in a cryovial, and frozen at –80°C until analyzed for serum cortisol and TNF-α concentrations or plasma substance P concentration.

Serum cortisol concentration was determined via a solid-phase competitive chemiluminescent enzyme immunoassay[j] as described (Coetzee et al., 2007). The immunoassay's lower limit of detection was 5.5 nmol/L; therefore, the results for serum samples with cortisol concentrations < 5.5 nmol/L were recorded as 5.5 nmol/L.

Serum concentration of TNF-α was determined via a commercial ELISA[k] modified for use with bovine serum. The primary and secondary antibodies used for the assay were goat anti-

bovine TNF-α and biotinylated goat anti-bovine TNF-α, respectively. Horseradish peroxidase-labeled streptavidin[j] and a tetramethylbenzidine-hydrogen peroxide solution[l] were used for antibody detection and color development. The TNF-α concentration was calculated by subtraction of the absorbance value at 540 nm from the absorbance value at 450 nm and comparing that value to the curve for the TNF-α standard that was run on the same plate as the serum sample.

Substance P concentrations in plasma samples obtained at 0, 4, 8, 24, and 48 hours (substance P concentrations were not determined for plasma samples obtained at 36 and 56 hours) were determined via a commercial immunoassay kit[m] that had been validated for use with bovine plasma (Coetzee et al., 2008). Plasma samples were extracted by means of C18 cartridges[n] prior to immunoassay analysis and the assay used a polyclonal anti-substance P antibody. The concentration of substance P in plasma samples was inversely proportional to the intensity of the color detected at a wavelength of 405 nm. Any plasma sample with a substance P concentration outside of the standard curve was not included in statistical analyses.

Statistical analysis

Data were imported into 1 of 2 commercial statistical software packages for analyses.[o,p] Distributions of each variable were visually evaluated for normality, and when data appeared to be non-normally distributed, that variable was logarithmically transformed ($\log_{10}$). Mixed regression models were used to evaluate potential relationships between continuous outcome variables (ie, nasal temperature, rectal temperature, surface temperatures, heart rate, and respiratory rate) and independent variables of interest, which included transport status, trial hour, and the interaction between transport status and trial hour. All analyses included random effects for each heifer and transportation day to account for a lack of independence caused by repeated measures. For transported heifers only, the effect of trial hour on cortisol, substance P, and TNF-α concentrations, respectively, was analyzed by the use of a mixed regression models.

Logistic regression models were used to determine the probability of proximity to a specific location (grain feeder, hay feeder, waterer, or shelter) within the pen or engagement in a specific activity (standing, lying down, or walking). Independent variables included transport status, trial day, and the interaction between transport status and trial day. A first-order autoregressive correlation structure was defined to account for the repeated measures on heifers over time in all analyses (Agresti, 1996). Multivariable regression models were constructed by

the use of backward selection in a stepwise procedure, and the final multivariable model for each outcome included only variables with a type 3 likelihood ratio test $P < 0.05$. Within each trial day, differences between transported and control heifers were evaluated via *t*-tests, and the level of significance was set at $P < 0.01$ a priori to account for multiple comparisons.

Results

Animals

All heifers remained healthy throughout the study. The maximum ambient temperature exceeded 32.2°C on each transportation day **(Table 1)**. Mean respiratory rate did not differ significantly between transported and control heifers and did not vary significantly during the observation period. Transported heifers had a significantly higher mean heart rate, compared with the mean heart rate for control heifers at 8 hours after initiation of transportation **(Figure 1)**. Evaluation of nasal ($P < 0.01$) and rectal ($P < 0.01$) temperature data revealed a significant association between trial hour and transport status **(Figure 2)**. Transported heifers had lower mean rectal ($P < 0.01$) temperatures at 12, 14, 20, and 24 hours after initiation of transportation, compared with those of the control heifers. Similarly, transported heifers had lower mean nasal ($P < 0.01$) temperatures at 4, 8, 20, and 24 hours after initiation of transportation, compared with those of the control heifers. The mean surface temperatures of the nares, nasal planum, and cornea did not vary significantly between transported and control heifers at any time measured. For both transported and control heifers, the mean surface temperatures of the nares ($P < 0.01$), nasal planum ($P < 0.01$), and cornea ($P < 0.01$) obtained at 8 hours after initiation of transportation were significantly higher, compared with those obtained immediately before transportation (hour, 0).

A significant interaction was identified between transport status and trial day for the percentage of time heifers spent within 0.3 m of the grain feeder, hay feeder, waterer, and shelter, respectively **(Figure 3)**. Transported heifers spent significantly ($P < 0.01$) more time near the hay feeder on the day of transportation (day, 0) than did the control heifers. Otherwise, the percentage of time spent at a particular pen location did not differ significantly between transported and control heifers during the 3-day observation period after initiation of transportation.

During the 8-hour transportation period, the transported heifers spent a significantly greater percentage of time walking ($P < 0.01$); mean ± SE, 2.7% ± 0.3%), compared with the percentage of time spent walking (2.0% ± 0.2%) by the control heifers, whereas the control heifers spent a significantly greater percentage of time lying down ($P < 0.01$; 22.0% ± 2.0%), compared with the percentage of time spent lying down (4.0% ± 0.4%) by the transported heifers. A significant interaction was identified between transport status and trial day for the percentage of time heifers spent lying down ($P < 0.01$) and standing ($P < 0.01$). Transported heifers spent a significantly greater percentage of time walking ($P < 0.01$) on the day of transportation (day, 0) and days 1 and 2 after transportation and lying down on days 1 and 2 after transportation, compared with the percentage of time spent walking and lying down by the control heifers during the same period **(Figure 4)**. A significant interaction was also identified between transport status and trial day for the number of steps traveled by heifers. The mean number of steps traveled by the transported heifers was significantly greater, compared with the mean number of steps traveled by the control heifers on the day of transportation **(Figure 5)**.

Compared with the mean body weight immediately prior to transportation (hour, 0), the mean body weight of the transported heifers was decreased at 4 and 8 hours after initiation of transportation, whereas the mean body weight of the control heifers was increased at 4 and 8 hours after initiation of transportation **(Figure 6)**. Also, the percentage change in body weight from hour 0 differed significantly between transported and control heifers at 4 and 8 hours after initiation of transportation. However, at 48 hours after initiation of transportation, the mean body weight did not differ between transported and control heifers.

Data for cortisol, substance P, and TNF-α concentrations were not normally distributed; therefore, logarithmic transformations ($\log_{10}$) were applied to the data so that regression analyses could be performed. Heifers had an increased mean serum cortisol concentration at 4 and 8 hours after initiation of transportation, compared with that immediately prior to transportation **(Figure 7)**. Conversely, heifers had a decreased mean serum substance P concentration at 24 and 48 hours after initiation of transportation, compared with that immediately prior to transportation. The mean plasma TNF-α concentration did not vary significantly during the observation period.

Discussion

Results of the present study indicate that beef heifers transported during periods of high ambient temperatures (≥ 32.2°C) had a transient decrease in rectal and nasal temperatures and

body weight. Transported heifers also spent more time at the hay feeder on the day of transportation, walking during the 3 days after initiation of transportation, and lying down on days 1 and 2 after initiation of transportation, compared with time spent at the hay feeder, walking, and lying down by control heifers that were not transported during the same period. Transported heifers also had increased serum cortisol and substance P concentrations at 4 hours after initiation of transportation, compared with those immediately before transportation. To our knowledge, the present study was the first to monitor both physiologic and behavioral variables in cattle immediately before, during, and after transportation.

The transient decrease in the mean rectal and nasal temperatures of transported heifers for 24 hours after transportation was unexpected and differs from results of other studies (Grigor et al., 2001; Tennessen et al., 1984).[q] Given that the environmental conditions were similar for both transported and control heifers, we attributed thermoregulatory differences between the treatment groups to the continuous air flow within the trailer, which allowed the transported heifers to dispel body heat via convection. Infrared thermography is an effective tool for monitoring body temperature in cattle (Stewart et al., 2005). In the present study, we were unable to detect significant variation in the surface temperatures of the nares, nasal planum, and cornea in the transported heifers, although our ability to detect such variations may have been confounded by the environmental conditions.[r]

Behavioral changes were expected in the heifers during and after transportation. On the day of transportation (day, 0), all behavior data was affected because of the frequency with which body weight and temperature measurements were obtained. The amount of time that heifers spent standing during transit in the present study was similar to that for heifers of a similar age in another study (Kent, 1983). Some of the heifers of the present study laid down during transit, which suggested that cattle will lie down while being transported if they have sufficient room to do so. The stocking density of the livestock trailer used in the present study was well below that recommended by the Farm Animal Welfare Council and USDA (Council, 1993; United States. Agricultural Marketing Service and Marketing, 1999). The fact that the percentage of time that the transported heifers spent walking during the 8-hour transportation period was greater, compared with that of the control heifers was probably associated with the movement of heifers during loading and unloading of the trailer as well as movement of the heifers within the trailer during transit. The significantly greater number of steps traveled by the

transported heifers, compared with the number of steps traveled by the control heifers on day 0 was also likely a reflection of heifer movement during transit. The pedometers used in the present study recorded the number of steps each heifer traveled during a 24-hour period, and unfortunately, we were unable to separate the data obtained during the 8-hour transportation period from the rest of the data obtained on day 0.

Regarding the time heifers spent at specific locations within the pen, the only change in behavior detected after transportation was an increased percentage of time transported heifers spent near the hay feeder on the day of transportation, compared with that for control heifers. We anticipated that the transportation of heifers during periods of high ambient temperature would result in stress and less time spent by the heifers at the feeders in a manner similar to that described in other studies (Sowell et al., 1998; Sowell et al., 1999) involving feedlot heifers, in which morbid heifers spent significantly less time at a grain feeder, compared with healthy control heifers. In the present study, the transported heifers may have spent more time at the hay feeder on the day of transportation in an attempt to recover from the period during which they did not have access to feed. However, because of the limitations of the behavior monitoring system used, we cannot confirm that the heifers were actually eating during the time they spent at the hay feeder.

Interestingly, transported heifers spent approximately 3% more time lying down and 0.1% more time walking during the 3 days immediately after transportation than did the control heifers. This finding suggested that after transportation, heifers were walking when they were not lying down. The biological importance of this is unknown and warrants further investigation. Regardless, the results of the present study indicated that transportation of healthy feedlot heifers during periods of high ambient temperature did not have a detrimental affect on their behavior during the immediate 3-day period after transit and that the use of accelerometers was a sensitive and effective method for monitoring behavior in cattle.

The amount of weight cattle lose during transportation varies (Warriss, 1990). The distance cattle are transported has been associated with the percentage of weight loss, and the greatest proportion of weight is lost during the first hours of transit (Cernicchiaro et al., 2012b; Coffey et al., 2001; Cole et al., 1988). In the present study, the percentage of weight lost by heifers during transportation was similar to that lost by feeder steers in another study (Coffey, 1997). The percentage of body weight lost by heifers during transportation in the present study

was most likely the result of the withholding of feed and water in addition to excretion of feces and urine and moisture lost via respiration and sweating.

For cattle, it is important to monitor weight loss during transportation because the amount of weight lost is positively associated with the risk of developing BRD (Camp et al., 1981). The percentage of weight lost by cattle during transportation has been used as a determinant for the metaphylactic treatment of cattle entering feedlots (Nickell and White, 2010; Sanderson et al., 2008). The distance cattle were transported has also been positively associated with BRD morbidity and negatively associated with average daily gain (Cernicchiaro et al., 2012a). In another study (Phillips, 1991), the percentage of weight lost was greater when cattle were transported during periods of high ambient temperature (18° to 34°C), compared with that when cattle were transported during periods of low ambient temperature (–6° to 16°C). Results of yet another study (Gonzalez et al., 2012) indicate a positive association between the total amount of weight lost during transportation and body weight immediately prior to transport and ambient temperature, respectively. In the present study, heifers were transported during periods of high ambient temperature (≥ 32.2°C) and weight loss was transient; by 48 hours after initiation of transportation, the mean body weight for the transported heifers did not differ significantly, compared with that immediately before transportation.

In animals, the physiologic response to fear or stress is the release of cortisol from the adrenal cortex via stimulation of the hypothalamus and pituitary gland (Molony and Kent, 1997). In the present study, we expected serum cortisol concentration to increase in the heifers during transportation because of the novelty of the situation and the increased handling that was required for sample collection. The increase in mean cortisol concentration at the midway (hour, 4) point of transit, compared with that immediately prior to transit, was similar to results of other studies (Crookshank et al., 1979; Sowell et al., 1999). However the decrease in mean cortisol concentration between the midway point and the end (hour, 8) of transportation was unexpected and may have been caused by the lack of novelty for the heifers after being reloaded onto the trailer and transported for the second time within a short period of time. Results of research by Grandin (1997) indicate that individual animal factors such as previous experience influence the physiologic and behavioral responses to stressful events. The implementation of animal handling procedures that decrease stress should result in decreased cortisol release and improve animal welfare (Speer et al., 2001). Also, the increase in mean serum cortisol concentration in heifers

midway through transit may have been associated with the decrease in nasal and rectal temperatures as an effect of weight loss and peripheral vasoconstriction.

In the present study, we also evaluated plasma substance P concentration as a potential biomarker for transportation stress in cattle. Substance P is a neuropeptide that modulates the dorsal root nociceptive neurons and can be detected in areas involved with pain and stress (DeVane, 2001). Results of another study (Coetzee et al., 2008) indicate that substance P concentration is an effective biomarker for pain in calves. Concurrent evaluation of cortisol and substance P concentrations may be beneficial because of the different mechanisms by which each biomarker is released, which may allow for the quantification of the magnitude or severity of stress in cattle. Although the neurophysiologic processing of pain and stress may differ, there is a cross-link between cortisol and substance P concentrations. Cortisol release follows a circadian rhythm with increased secretion occurring with the onset of daylight (Thun et al., 1981). Conversely, release of substance P does not follow a circadian rhythm (Coetzee et al., 2008); thus, it may be more sensitive biomarker for stress when sampling frequency is limited. In the present study, only the short-term effects of transportation on substance P concentration in cattle were evaluated. Also, blood samples were not obtained from control heifers because of logistic limitations; therefore, cortisol and substance P concentrations could not be compared between transported and control heifers. Further research is necessary to elucidate the mechanism of cortisol and substance P release in cattle exposed to pain and stress in various situations.

For the heifers of the present study, the mean serum TNF-α concentration did not vary significantly at any sample collection time, compared with that immediately prior to transportation. In other studies (Arthington, 2003; do Amaral et al., 2010; Kluger et al., 1997; Suganuma et al., 2002), TNF-α concentration either increased or decreased after exposure of animals to heat stress or transportation. The TNF-α concentration results of the present study suggested that there was a lack of a proinflammatory response in heifers that were transported during periods of high ambient temperatures, and this was unexpected.

Extrapolation of the results of the present study to the general feedlot cattle population should be done with caution because the stocking density (170 kg/m^2) of the trailer during transportation was < half the recommended maximum stocking density (360 kg/m^2) for livestock trailers (Council, 1993) and did not reflect conditions under which cattle are commonly

transported in the United States. The location of heifers within a semitruck trailer during transportation has been associated with the subsequent morbidity rate of those heifers (White et al., 2009).

Results of the present study indicated that beef heifers that are transported during periods of high ambient temperature (≥ 32.2°C) have transient changes in body temperature and weight, serum cortisol and plasma substance P concentrations, and behavior. However, none of the study heifers developed detrimental health effects during the observation period after transportation. To our knowledge, the present study was the first to evaluate nasal mucosal temperature and plasma substance P concentration in beef heifers during and after transportation.

Footnotes

a. Pavia Rectal Temp thermometer, Pavia Sales Group Inc, Plymouth, Minn.

b. Bio-Thermo LifeChip, Destron Fearing, South St. Paul, Minn.

c. Pocket Reader, Destron Fearing, South St. Paul, Minn..

d. ThermaCAM S65, FLIR Systems, Wilsonville, Ore.

e. Ubisense Series 7000 compact tag, Ubisense, Denver, Colo.

f. Steggles P, Gschwind S. The Ubisense Space Platform. Advances in pervasive-computing (abstr), in Proceedings. 3rd Int Conf Pervasive Comput 2005;191.

g. Insightful Miner, Insightful Corp, Seattle, Wash.

h. GP1 SENSR, Reference LLC, Elkader, Iowa.

i. NL-800, New-Lifestyles Inc, Lees Summit, Mo.

j. Immulite, Siemens Medical Solutions, Los Angeles, Calif.

k. Bovine TNF-α, Pierce, Rockford, Ill.

l. Substrate Solution, R & D Systems, Minneapolis, Minn.

m. SP Correlate-EIA, ELISA kits, Assay Designs Inc, Ann Arbor, Mich.

n. Sep-Pak Vac 3cc C18 SPE, Waters Corp, Milford, Mass.

o. JMP, version 9, SAS Institute Inc, Cary, NC.

p. SAS, version 9.2, SAS Institute, Cary, NC.

q. Stevens DG, Camp TH. Vibration in a livestock vehicle (abstr), in Proceedings. Am Soc Agric Biol Eng 1979;10.

r. Gomez A, Vergara C, Cook NB, et al. Is thermography a possible new method to evaluate body temperature in fresh cows? (abstr), in Proceedings. Annu Meet Am Assoc Bovine Pract 2011;191.

Figure 5.1. Mean ± SE heart rate immediately prior to (hour, 0) and at 12, 24, 36, 48 and 56 hours after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperatures (≥ 32.2°C).
Prior to study initiation, each of 10 heifers was matched to another heifer on the basis of weight to form a block of 2 heifers. Then each heifer within a block was randomly allocated to 1 of 2 groups; therefore, each group contained 10 heifers. The study had a double-crossover design such that each group of heifers was transported twice and served as controls twice; thus, each data point represents the mean of 40 observations. There were 3 days between transportation days 1 and 2 and transportation days 3 and 4 and 1 week between transportation days 1 and 3. Interaction between trial hour and transport status was significant ($P < 0.01$). *Within an hour, values for heifers during transportation and control periods differ significantly ($P < 0.01$).

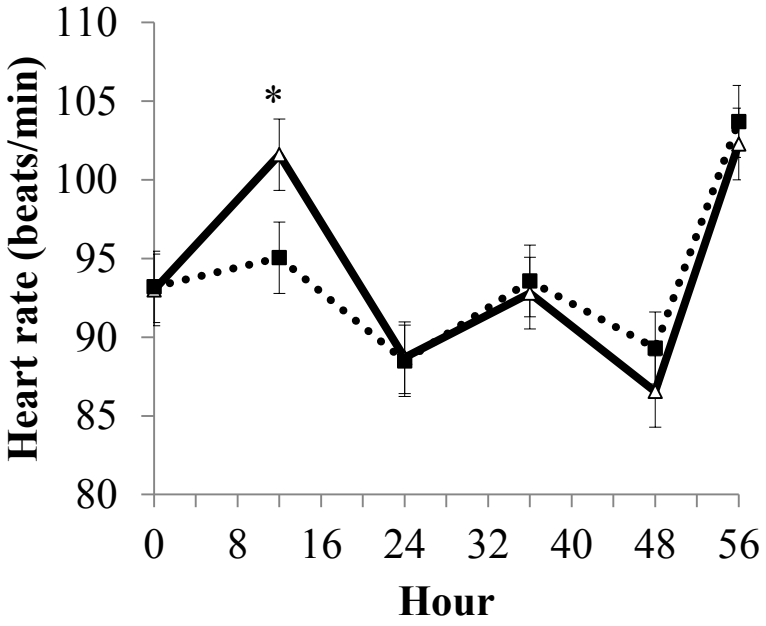


Figure 5.2. Mean ± SEM rectal (A) and nasal (B) temperatures immediately prior to (hour, 0) and at 4 (transit midpoint), 8 (transit end), 10, 12, 14, 16, 18, 20, 22, 24, 36, 48, and 56 hours after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C).

Rectal temperatures were measured via a rapid equilibration thermal probe. Nasal temperatures were determined by means of radiofrequency thermal sensors that were implanted at a depth of approximately 2 mm in the submucosa of the nasal mucosae on the dorsal and medial aspects of the left and right nares approximately 100 mm caudal to the alar cartilage. For each heifer, the nasal temperature recorded at each observation represented the mean of the temperature readings from the sensors in both the left and right nares. Notice that the scale of the y-axis differs between panels. Interaction between trial hour and transit status was significant ($P < 0.01$). *Within an hour, values for heifers during transportation and control periods differ significantly ($P < 0.01$). Notice that the scale of the y-axis differs between panels. *See* Figure 5.1 for remainder of key.

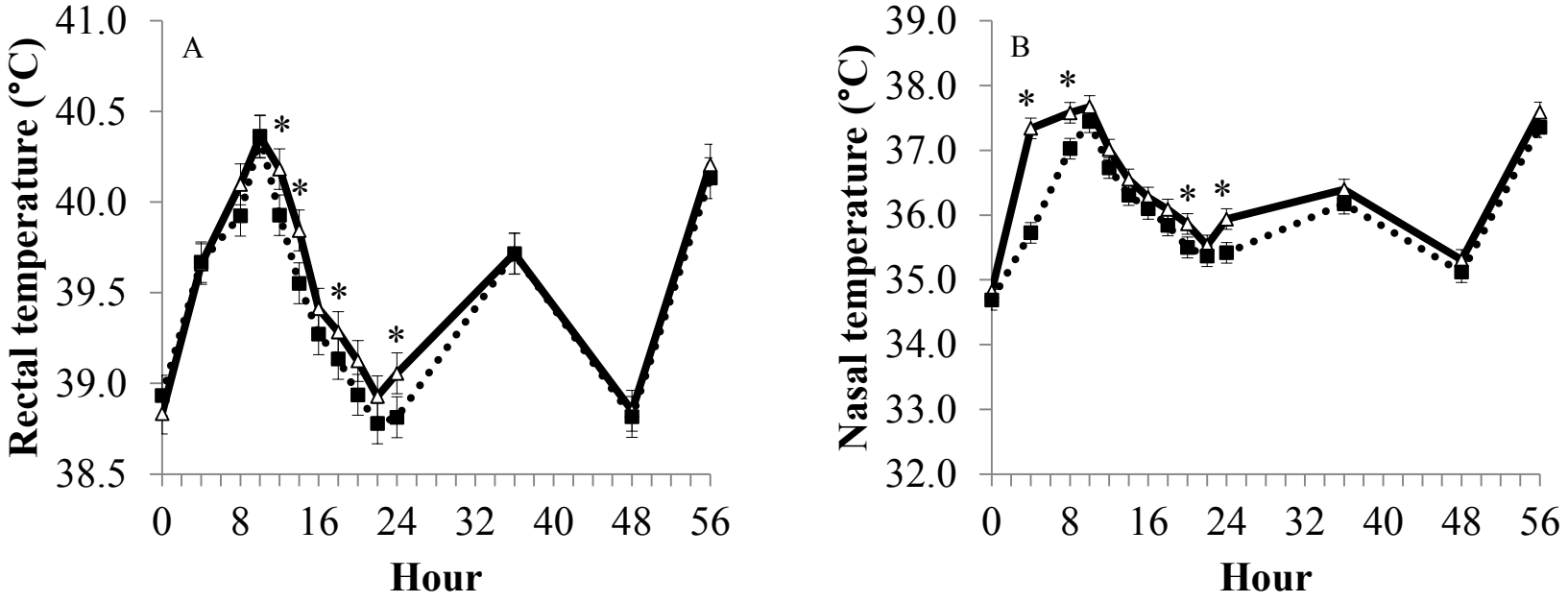
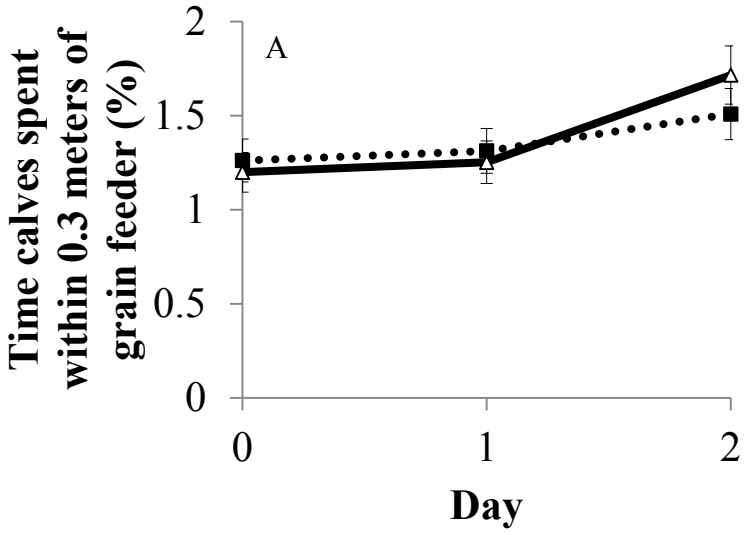
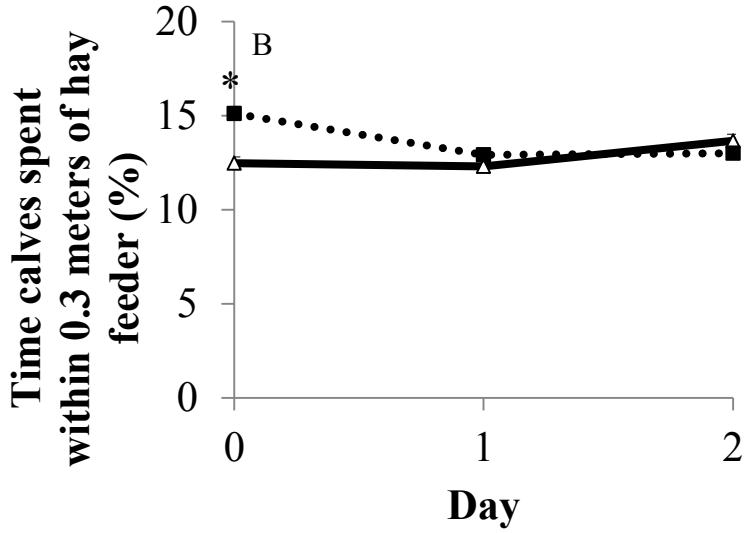
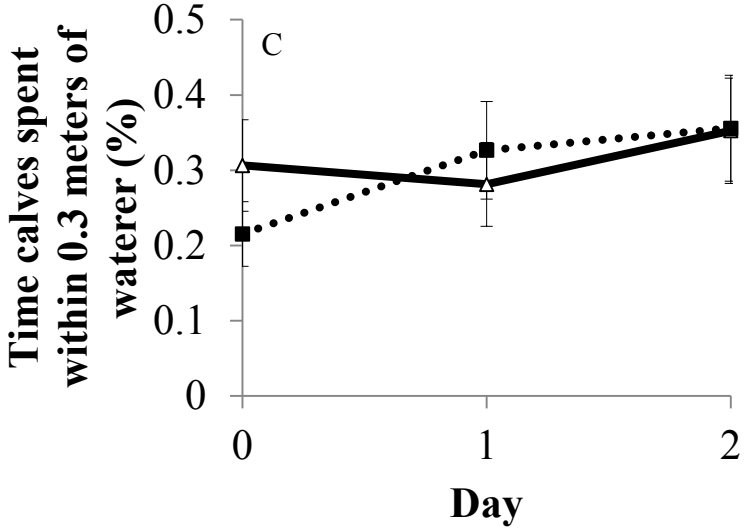


Figure 5.3. Mean ± SEM percentage of time spent within 0.3 meters of the grain feeder (A), hay feeder (B), waterer (C), and shelter (D) the day of (day, 0) and for the 2 days after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C).
The time spent at specific locations within the pen was determined by measurements obtained from a remote location monitoring tag that was applied to the left ear of each heifer prior to study initiation. The tag continuously transmitted coordinate data to wireless sensors located at the pen's periphery, which then transmitted the coordinates to a computer database for analysis. Notice that the scale of the y-axis varies among the panels. Interaction between trial day and transit status was significant ($P < 0.01$). *Within an observation day, values for heifers during transportation and control periods differ significantly ($P < 0.01$). *See* Figure 5.1 for remainder of key.


A
Time calves spent within 0.3 meters of grain feeder (%)
2
1.5
1
0.5
0
0
1
2
Day


B
*
Time calves spent within 0.3 meters of hay feeder (%)
20
15
10
5
0
0
1
2
Day


C
Time calves spent within 0.3 meters of waterer (%)
0.5
0.4
0.3
0.2
0.1
0
0
1
2
Day

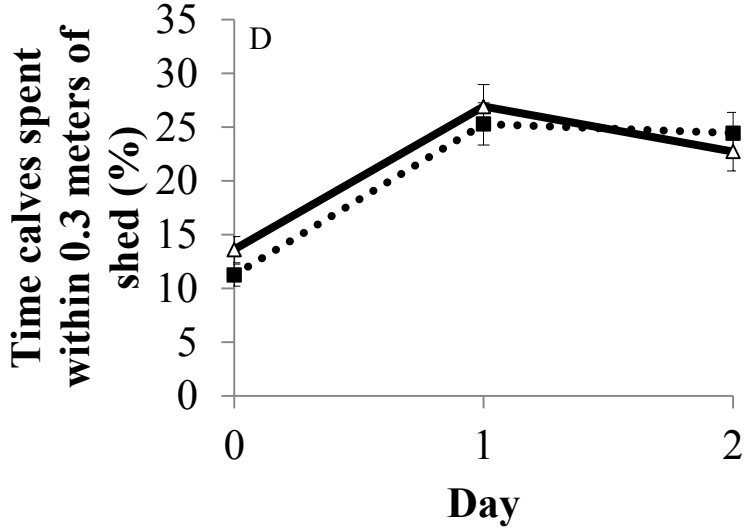

D
Time calves spent within 0.3 meters of shed (%)
35
30
25
20
15
10
5
0
0
1
2
Day

Figure 5.4. Mean ± SE percentage of time spent lying down (A) and walking (B) the day of (day, 0) and for the 2 days after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C).
Data were obtained via accelerometers that were applied to all heifers on the lateral aspect of the right hind limb just proximal to the metatarsophalangeal (fetlock) joint. The accelerometers were programmed such that acceleration along X, Y, and Z axes and mean and maximum vector magnitude were recorded at 5-second intervals, and the data obtained were aggregated by day. Notice that the scale of the y-axis differs between panels. Interaction between trial day and transit status was significant ($P < 0.01$). *Within an observation day, values for heifers during transportation and control periods differ significantly ($P < 0.01$). *See* Figure 5.1 for remainder of key.

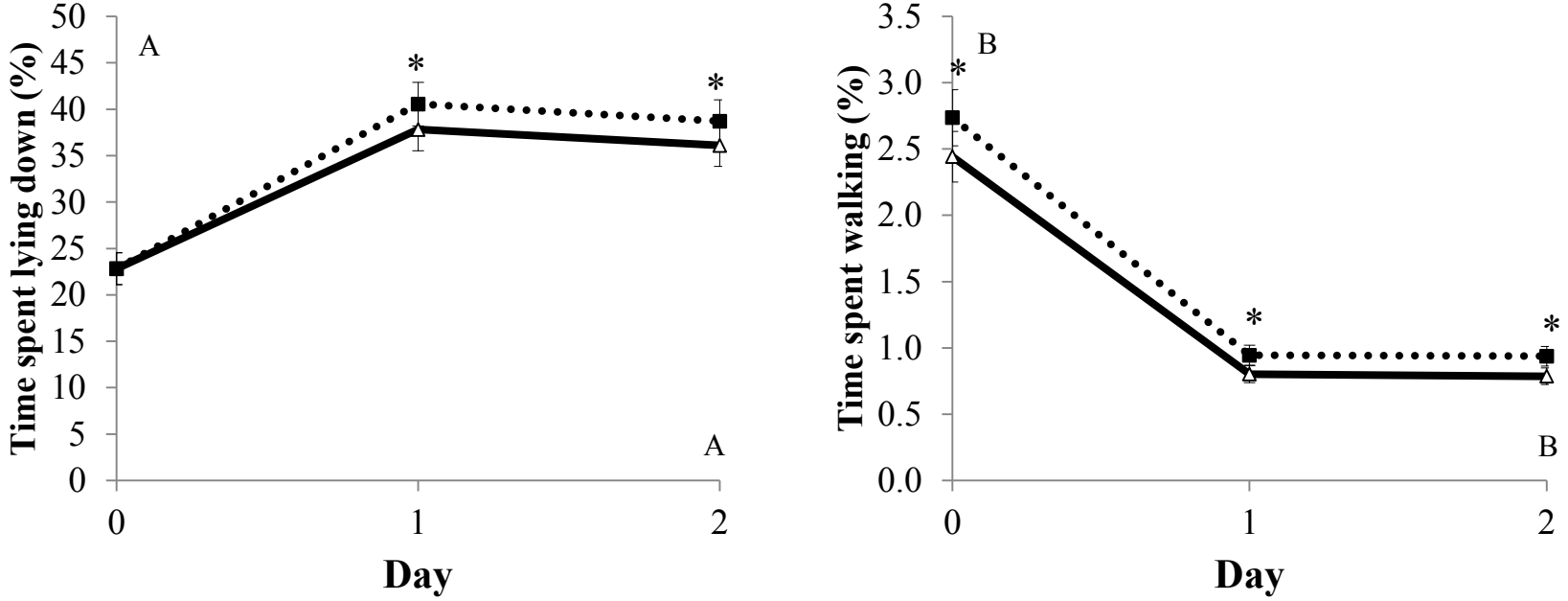


Figure 5.5. Mean ± SE number of steps traveled the day of (day, 0) and for the 2 days after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C).
Data were obtained via pedometers that were applied to all heifers on the lateral aspect of the right hind limb just proximal to the metatarsophalangeal (fetlock) joint. Interaction between trial day and transit status was significant ($P < 0.01$). *Within an observation day, values for heifers during transportation and control periods differ significantly ($P < 0.01$). *See* Figure 5.1 for remainder of key.

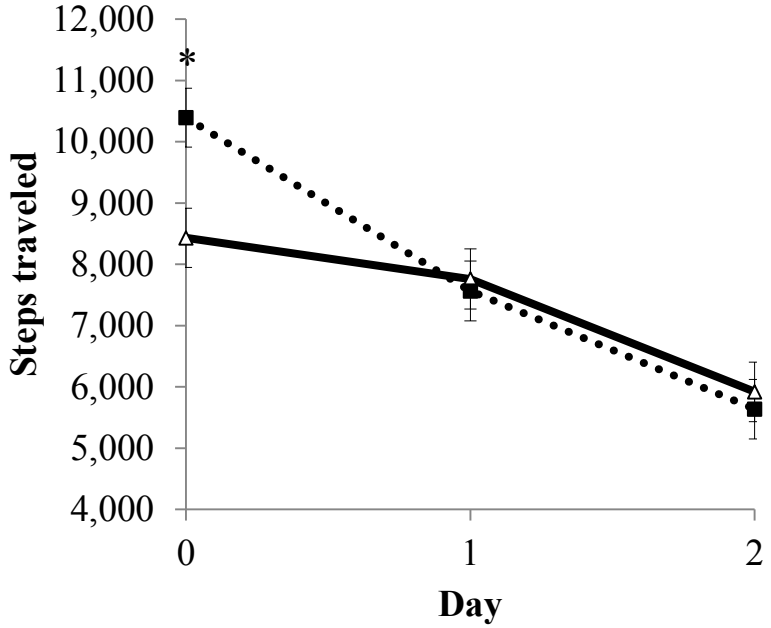


Figure 5.6. Mean ± SE percentage change in body weight immediately prior to (hour, 0) and at 4 (transit midpoint), 8 (transit end), and 48 hours after initiation of transportation for 20 beef heifers when they were (black squares with dotted line) and were not (white triangles with solid line; control) transported 518 km during periods of high ambient temperature (≥ 32.2°C). *Within an hour, values for heifers during transportation and control periods differ significantly ($P < 0.01$). *See* Figure 5.1 for remainder of key.

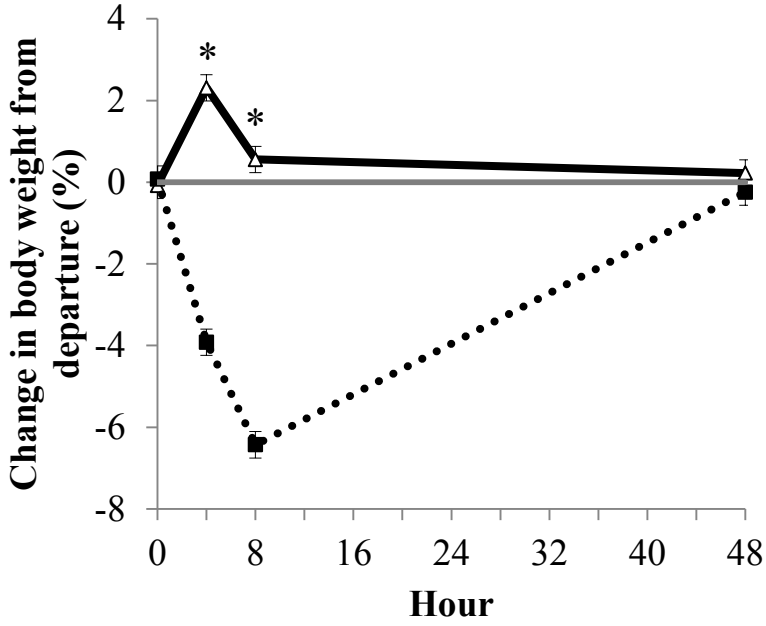


Figure 5.7. Mean and 95% confidence intervals for serum cortisol (A) and plasma substance P (B) concentrations immediately prior to (hour, 0) and at 4 (transit midpoint), 8 (transit end), 24, 36, 48, and 56 hours after initiation of transportation for 20 beef heifers that were transported 518 km during periods of high ambient temperature (≥ 32.2°C).
Plasma substance P concentration was not determined at 36 and 56 hours after initiation of transportation. Serum cortisol concentrations were determined via a solid-phase competitive chemiluminescent enzyme immunoassay and plasma substance P concentrations were determined via a commercial immunoassay. *Value differs significantly ($P < 0.01$) from that at hour 0. *See* Figure 5.1 for remainder of key.

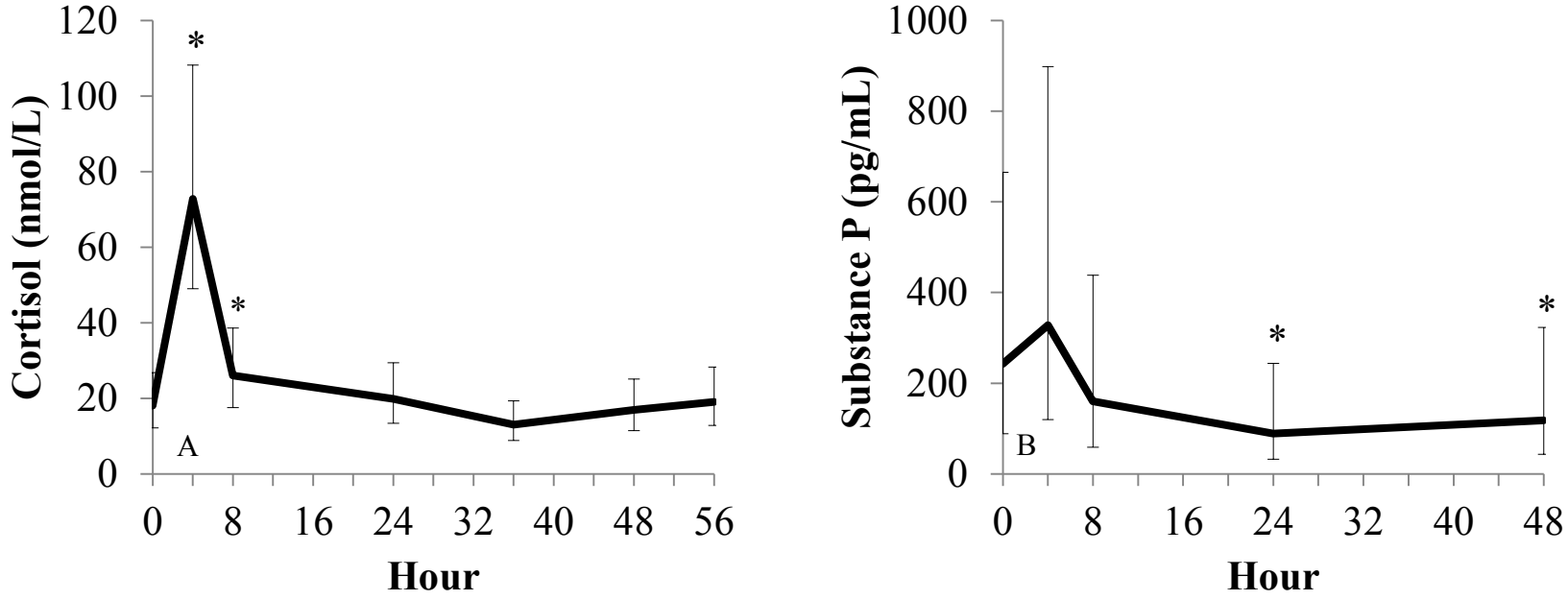


Table 5.1. Mean, maximum, and minimum temperature and humidity and maximum heat index for each of 4 days during which 10 beef heifers were transported 518 km while another 10 heifers served as untransported controls.

Variable	**Transportation day**			
	1	**2**	**3**	**4**
Mean temperature (°C)	33	30	35	36
Maximum temperature (°C)	40	38	42	43
Minimum temperature (°C)	24	24	25	29
Mean humidity (%)	54	67	47	39
Maximum humidity (%)	83	83	69	78
Minimum humidity (%)	22	27	23	27
Maximum heat index (°C)	40	41	41	42

Prior to study initiation, each of 10 heifers was matched to another heifer on the basis of weight to form a block of 2 heifers; then each heifer within a block was randomly allocated to 1 of 2 groups such that each group contained 10 heifers. The study had a double-crossover design such that each group of heifers was transported twice and served as controls twice. There were 3 days between transportation days 1 and 2 and transportation days 3 and 4 and 1 week between transportation days 1 and 3.

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Chapter 6 - Effect of *Mannheimia haemolytica* pneumonia on behavior and physiologic responses of calves during high ambient environmental temperatures

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Abstract

The objective of this study was to determine the effect of pneumonia during conditions of high (maximum ≥ 32°C) ambient temperatures on physiological and behavioral responses of calves. Eighteen black beef heifers averaging 240 kg were blocked by weight and randomly assigned to one of two treatment groups:1) pneumonia induced by bronchoselective endoscopic inoculation with *Mannheimia haemolytica* (MH; n=10) and 2) non-inoculated controls (CN; n=8). Nasal passage and rectal temperatures were measured every 2 h for 24 h after challenge and then twice daily for 9 d. Accelerometers, pedometers, and positioning devices monitored cattle behavior within the pen for 9 d after challenge. Blood samples were collected on trial d 0, 0.5, 1, 2, 3, 7,

and 9, and analyzed to determine concentration of substance P, cortisol, haptoglobin, and metalloproteinase. All calves in the MH group were euthanized and necropsied on trial d 9. All MH calves became clinically ill post-challenge. A treatment x time interaction ($P < 0.05$) was evident for nasal and rectal temperatures, behavior, weight, and blood analysis. Rectal temperatures in MH were greater ($P < 0.01$) than CN during the period from 6 to 24 h after challenge. Conversely, nasal passage temperatures were less in MH calves compared to CN at 12 to 22 h after challenge. Calves in MH spent less time at the grain bunk, less time at the hay feeder, and more time lying down during the early pneumonia period compared to CN calves. Also, MH calves had significantly higher levels of blood biomarkers of pain (substance P) on d 0.5 ($P < 0.01$); stress (cortisol) on d 0.5 and 1 ($P < 0.01$); haptoglobin on d 0.5, 1, 2, 3, 7 ($P < 0.01$); and metalloproteinase on d 1, 2, and 3 ($P < 0.01$) compared with CN calves. At necropsy, all MH calves had right cranioventral bronchopneumonia (median lung lesions = 6.8%). *Mannheimia haemolytica* pneumonia caused significantly more changes in behavior and increased biomarkers during high ambient temperatures as compared to control calves. The results of this study may guide research in the development of objective assessment tools for management of cattle affected with BRD during extreme summer conditions.

Key words: behavior, bovine respiratory disease, endocrinology, heat stress, *Mannheimia haemolytica*, physiology

Introduction

Bovine respiratory disease (BRD) complex is a common issue affecting feedlot cattle economic and performance outcomes (Galyean et al., 1999; Lechtenberg et al., 2011). *Mannheimia haemolytica* is the most common bacterial pathogen associated with BRD (Purdy et al., 1997). Improvement of the clinical case definition of BRD may lead to a more accurate disease diagnosis resulting in improved management strategies and treatment (Apley, 2006; Hanzlicek et al., 2010a). Current methods for identification of calves with BRD are based on visual observations, but these methods have low sensitivity (61.8%) and specificity (62.8%) (White and Renter, 2009). Behavior monitoring has been suggested for monitoring health of cattle (Weary et al., 2009). Remote monitoring tools have been utilized to detect changes in health status of calves (Buhman et al., 2000; Sowell et al., 1999).

Heat stress has been associated with increased body temperature, respiratory rate, and decreased activity (Fuquay, 1981). High ambient temperatures result in decreased dry matter intake (Hahn, 1999; Mader, 1999). Heat stress has adverse effect on the animal wellness status (Silanikove, 2000). Cattle affected with BRD are expected to suffer more severely during periods of heat stress.

The behavioral and physiological responses of calves suffering from *M. haemolytica* pneumonia during periods of heat stress have not been described. The objective of this study was to determine the effect of *M. haemolytica* pneumonia on biothermal regulation, behavior, and physiological responses during extreme ambient temperatures. Our hypothesis was that calves challenged with *M. haemolytica* would have greater body temperatures, lethargic behavior, and increased cortisol, substance P, haptoglobin, and metalloproteinase compared to control calves. Conclusions of this study will be useful in improving diagnostic capabilities of identifying morbid animals in situations with high ambient temperatures.

Materials and Methods

All study procedures were conducted with a protocol approved by the Institutional Animal Care and Use Committee (IACUC # 3039). Cattle were humanely handled throughout the research project and observed twice daily during the trial to monitor their health status. A protocol was in place stating that if a calf became severely ill (clinical illness score [CIS] = 4 – see CIS definitions below) at any point during the study, the calf would be immediately humanely euthanized to alleviate unnecessary suffering.

Calf Selection and Trial Design

Eighteen black, beef heifers averaging 240 kg (±13.1 kg) owned by Kansas State University were selected for this study. The heifers were observed twice daily for 30 days prior to study d 0 for clinical signs of illness. Calves were commingled and group housed throughout the trial in a 12.2 m by 24.4 m pen and fed a receiving ration that included 2.3 kg of corn/d, 0.9 kg alfalfa/d, and ad libitum access to brome hay. A remote weather station (WS-2812, La Crosse Technology, La Crosse, WI) was placed at the research station in order to continuously monitor the environmental conditions where the calves were housed throughout the trial. Criterion for study initiation were forecasted high ambient temperature conditions including maximum daily temperature $\geq 32°C$, average daily humidity $\geq 40\%$, and sun exposure.

Calves were blocked by weight and group participation from previous transportation stress trial (Theurer et al., Accepted, In Press), then randomly allocated to a *Mannheimia haemolytica* (MH; n=10) treatment group or control group (CN; n=8). Endoscopic inoculations were conducted using bronchoselective endoscopy. This procedure allows selective placement of the endoscope and media into the right apical lung lobes via the tracheal bronchus. Calves in the CN were endoscopically challenged with 70 mL of phosphate buffer solution in the tracheal bronchus using a fiberoptic endoscope (length, 110 cm; diameter, 6.6 mm; biopsy channel, 2.0 mm; VetVu Flexible Endoscope, Swiss Precision Products, Spencer, MA) as previously described (Hanzlicek et al., 2010a). Calves in the MH treatment group were challenged with 10 mL of *M. haemolytica* serotype A1 at a dosage rate of 1 x 10^9 cfu/mL and then flushed with 60 mL of phosphate buffered solution to give a total dosage volume of 70 mL deposited in the tracheal bronchus with a fiberoptic endoscope. *Mannheimia haemolytica* was prepared for inoculation as previously described (Corrigan et al., 2007; Hanzlicek et al., 2010a; Mosier et al., 1995). Challenge began at 0800 h on trial d 0. Calves challenged with *M. haemolytica* were necropsied 9 d after inoculation.

Temperature Monitoring

Radiofrequency biothermal sensors (Biothermal RFID Chip, Destron Technologies, Round Rock, TX) were implanted submucosally in the nasal passages of the left and right nares approximately 2 mm deep and 100 mm caudal to the alar cartilage. The radiofrequency transponders were initiated by an electronic reading device (Pocket Reader, Destron Technologies, Round Rock, TX) which recorded temperature within ± 0.1°C. A rapid equilibration thermometer (Pavia Rectal Temp, Pavia Sales Group, Inc., Plymouth, MN) was used to measure rectal temperatures. A high definition thermal sensor camera (ThermaCAM S65, FLIR Systems, Wilsonville, OR) was used to record surface temperature of the right and left nares, nasal planum, and cornea at trial h 0, 4, 8, 12, 24, and 48. The left and right nares temperatures were then averaged to yield a single reading per calf per time point for temporal analysis.

Behavior Monitoring

Triangulation positioning transponder tags (Ubisense Series 7000 Compact Tag; Ubisense, Denver, CO) were placed in the left ear of all calves to monitor behavior activity. These tags are a component of a remote triangulation monitoring system that evaluates calf

position within the pen based on relative position compared to the sensors (Steggles and Gschwind, 2005). The compact design of the tags allowed attachment of each tag to a conventional ear tag button and placed with the sensor on the ear. The X and Y coordinates of the tag location were recorded and compared with the known X and Y locations of the previously mapped locations of the grain bunk, hay feeder, water, and shed using a data mining software program (Insightful Miner; Insightful Corporation Seattle, WA). A time stamp was documented by the computer every time the tag location was transmitted (average signal frequency, 1 s). Using criterion previously established, the amount of time at each location was calculated by subtracting the time stamp from the previous reading and then classifying the calf as being at the previous reading location (Theurer et al., 2012; White et al., 2012).

Accelerometers (GP1 SENSR; Reference LLC, Elkader, IA) and pedometers (NL-800; New-Lifestyles Inc., Lees Summit, MO) were placed within a protective neoprene sleeve which was attached to the lateral aspect of the metatarsus immediately proximal to the fetlock (Robert et al., 2009). The accelerometers use tri-axial measurements, have an axis range of ± 10g, and record 100 samples per second (Reference LLC, 2007). The accelerometers were initialized with previously validated settings to measure cattle behavior (Robert et al., 2009) including 5 s recording intervals and recording X, Y, and Z acceleration, vector magnitude average, and vector magnitude maximum data. Vector magnitude and average force of gravity values were calculated by summing the *g* values and calf acceleration movement recordings and then dividing by 5 s intervals. The vector magnitude maximum is the highest combined acceleration during the 5 s span.

Accelerometers were removed, downloaded to a computer, and reattached to the calf on trial d 6. Postural data were then processed using a data mining software program (Insightful Miner; Insightful Corporation Seattle, WA). The variables were used to classify the amount of time each calf spent standing, lying, or walking per 5 s epochs. The data from the accelerometers were then aggregated by hour.

Pedometers contained an accelerometer inside of them that monitored the number of steps each calf took based on the up and down movement of the calf leg. The pedometer data were recorded at trial d 6 when the accelerometers were taken off and downloaded. All behavioral activity was continuously monitored for a period of 1 d before and for 9 d after

challenge. Trial day consisted of a 24-h period beginning from the time the respiratory challenge initiated (0800 h).

Body Weight

All calves were individually weighed on trial d 0, 2, 3, 5, 7, and 9 to calculate percent change in body weight. For statistical comparisons, changes in body weight were analyzed using percent change in body weight calculated by comparing current weight to the individual animal weight at the beginning of the trial prior to challenge.

Initial 24-hour Monitoring Period

All calves were monitored prior to challenge (trial d 0, trial h 0) and then every 2 h for 24 h post-challenge. Rectal and nasal temperatures were recorded every 2 h for 24 h following endoscopic challenge (trial h 0).

Daily Monitoring Period

Following the 24 h post-challenge period, calves were assessed twice daily at 0800 h and 1600 h during the following 9 d. Rectal temperature, nasal temperature, heart rate, respiration rate, and clinical illness scores were collected from all calves twice each day. Clinical illness (CIS) scores were assigned by the same individual trained in detection of clinical illness using an established classification method (White et al., 2012). This CIS system ranged from 1 to 4 with the following criteria used for each level:1 = normal behavior, 2 = slight illness, mild depression and/or a cough, 3 = moderate illness, severe depression, labored breathing, and/or cough, and 4 = severe illness, animals may be moribund or have little response to human approach. All calves in the MH were humanely euthanized on trial d 9 by captive bolt (Koch Magnum 0.25 Stunner, KOCH Supplies Inc., Kansas City, MO) in accordance with the American Veterinary Medical Association guidelines (American Veterinary Medical Association, 2007).

Lung Lesion Scores

Lungs were harvested immediately following euthanasia and evaluated by a board certified pathologist experienced in BRD assessment. This investigator assigned values to the extent of lung that was consolidated in each lung lobe. Lung scores were calculated by determining the total amount of lung that was infected and dividing it by the total lung volume using a previously described scoring system (Fajt et al., 2003). The total percent lung involvement was calculated with the following formula:

(0.06*right caudal apical/100) + (0.063*right cranial apical/100) + (0.053*left cranial apical/100) + (0.049*left caudal apical/100) + (0.319*left diaphragmatic/100) + (0.043*intermediate/100) + (0.352*right diaphragmatic/100) + (0.061*accessory/100) = total lung score.

Blood Samples and Immunoassays

Blood samples were drawn from the jugular vein of all calves prior to challenge (trial d 0), at 12 h after challenge, and on trial d 1, 2, 3, 7, and 9. Samples were transferred to 6 mL serum clot activator and potassium EDTA tubes and centrifuged for 10 min at 1,500 x *g*. Serum and plasma were harvested, placed in 2 mL cryovials, and frozen at -80°C until analyzed. Appropriate samples were assayed for cortisol, substance P, haptoglobin, and haptoglobin-metalloproteinase-9 complex.

A solid phase competitive chemiluminescent enzyme immunoassay and automated analyzer system (Immulite, Siemens Medical Solutions, Los Angeles, CA) were used to analyze serum cortisol concentration as previously described (Coetzee et al., 2007). All samples that had a serum cortisol concentration less than 5.5 nmol/L (the sensitivity of the machine was 5.5 nmol/L) were transformed to 5.5 nmol/L prior to statistical analysis.

Plasma substance P concentrations were analyzed by use of a commercial immunoassay kit (SP Correlate-EIA, ELISA kits, Assay Designs Inc, Ann Arbor, MI) that has been validated for use in bovine plasma (Coetzee et al., 2008). Samples were extracted by use of C-18 cartridges (Sep-Pak Vac 3cc C_{18} SPE, Waters Corp, Milford, MA). The immunoassay utilized a polyclonal antibody against substance P in the test sample. The substance P concentration in the sample was inversely proportional to the intensity of the color generated after incubation as determined at 405 nm on a microplate reader. All samples that had a substance P concentration outside of the standard curve were removed prior to analysis.

Total haptoglobin (Hp) concentrations in serum were determined using a commercially available bovine haptoglobin ELISA kit (Life Diagnostics, West Chester, PA) Briefly, all serum samples from calves were diluted 1:2,000 in sample buffer prior to aliquoting 100 μL to each well for Hp analysis. The serum was aliquoted into individual wells of the Hp ELISA plate pre-coated with anti-bovine Hp antibody. The plates were covered with plastic film and allowed to incubate at room temperature on an automated plate shaker. After incubation, the plates were washed with pre-diluted Hp ELISA wash buffer (3 times, 5 min each) and the pre-diluted

horseradish peroxidase conjugated anti-bovine Hp antibody was added and incubated under plastic film for 30 min on the plate shaker at room temperature. The plates were washed again (as above) and 100 µL of tetramethylbenzidine (TMB) substrate was added to each well. Color development was monitored on an automated plate reader at 630 nm until the highest standard concentration (250 ng/mL) achieved an absorbance of > 0.500 AU. After development, the enzyme reaction was stopped by addition of 100 µL of stop solution (1 N hydrochloric acid) and the well absorbance determined at 450 nm. Serum concentrations were determined by first defining the concentration vs. absorbance relationship based upon the standards (7.8, 15.6, 31.25, 62.5, 125, 250 ng/mL) and using the slope and intercept of the line to calculate serum concentrations based upon their individual absorbance values. All serum sample concentrations were corrected for dilution (2,000 fold dilution).

The ELISA for bovine haptoglobin matrix metalloproteinase 9 complex (Hp-MMP 9) was performed as described previously with a few changes (Bannikov et al., 2011). The ELISA is designed to exploit the binding of bovine MMP 9 to the well coated with anti-bovine MMP 9 monoclonal antibody. The serum samples were each diluted 1:5 with sample diluent (TBS + 1% Bovine serum albumin [BSA] + 0.05% Tween 20). Non-conjugated bovine MMP 9 MAb 10.1 as a capture antibody (100 µL, containing 2 µg per well in TBS + 0.1% BSA; Bovine Serum Albumin, Fraction V, Thermo Fisher Scientific, Pittsburgh, PA), was allowed to bind to the wells of a 96 well ELISA plate and incubated overnight at 4°C. Plates were blocked with TBS containing 2% bovine serum albumin at 4°C for 120 minutes. After blocking the wells, calf serum samples and serum containing known concentrations of Hp-MMP 9 at a standard dilution were added (120 min at 21°C). Samples were washed 3 times for 5 minutes each with 300 µL TBS+0.05% Tween 20, and rabbit anti-bovine Hp-HRP conjugate (100 µL, containing 0.1 µg per well in TBS + 0.1% BSA) was added. Wells were washed as before and 100 µL of TMB was added to each well for detection of bound Hp-MMP 9. The wells were incubated for 20 min and the reaction stopped by addition of 100 µL of 1N hydrochloric acid to stop the color reaction. The concentrations of Hp-MMP 9 were determined using the linear slope of the graphed line generated from an equation of the absorbance of the calibrators at 450 nm and the known concentration of these calibrators.

Statistical Analysis

Data were analyzed using statistical software packages (JMP Version 9. SAS Institute Inc., Cary, NC; SAS 9.2, SAS Institute, Cary, NC). Descriptive statistics were used to evaluate the percentage of calves receiving a CIS 2 by treatment group and trial day. Generalized mixed models were used to evaluate the potential relationships between continuous variables (rectal temperature, nasal passage temperature, heart rate, respiratory rate, number of steps, percentage change in body weight, cortisol, substance P, haptoglobin, and Hp-MMP 9 complex concentrations), treatment group (MH or CN), trial hour and day, and the potential interaction between these variables. All analyses included random effects on each calf to account for repeated measures. Descriptive analysis was performed on rectal temperatures to determine the frequency of rectal temperatures above 39.5°C during the initial 24-hour monitoring period and frequency of nasal temperatures above 39°C by treatment group during the entire trial (Mills et al., 1971; Radostits, 2001).

Analysis of behavioral data used model effects including treatment (MH and CN), trial day (d -1 through 9), and the potential interaction between treatment group and trial d. Statistical models were constructed in a stepwise procedure by including all potential effects and removing non-significant ($P > 0.05$) effects. A first-order autoregressive correlation structure was defined to account for the repeated measures on calves over time in all behavioral analyses (Agresti, 1996). Type 3 likelihood-ratio statistics were used to test for associations of effects and comparisons with a P value < 0.05 were considered statistically significant. Potential differences between treatment groups within individual trial days were evaluated using t-tests. To account for multiple comparisons, a P value < 0.01 was considered statistically significant for all comparisons between CN and MH calves within hour or day for all data.

Results

Environmental conditions met the desired thresholds throughout the study period except for 1 day (day 4) on which maximum ambient temperature was 31.8°C (Table 1). At trial initiation, the mean (±SD) body weight of calves in the CN were 241.3 ± 13.6 kg and in the MH were 242.1 ± 12.4 kg. All MH calves had a CIS of 2 at 24 h after challenge; however, only 2 calves had a CIS of 2 at 12 h after challenge. The percentage of MH calves with CIS of 2 varied between 10% and 70% on subsequent evaluation periods (Figure 1). No calf was classified greater than CIS 2 during the trial. One CN calf was classified as a CIS 2 on trial day 5 and a

different CN calf was classified as a CIS 2 on trial d 6. Otherwise, CN calves were clinically normal at all other time points.

Analysis of the average nasal passage temperature and average rectal temperature determined a significant interaction ($P < 0.05$) between trial hour and treatment group during the intensive monitoring period with MH being greater than CN beginning 6 h post-challenge (Figure 2). Calves in the MH had greater average rectal temperatures at trial h 6 through 24 and lower average nasal temperatures at trial h 14 and 18 compared to CN calves. Five of the calves in the CN exceeded the 39.5°C rectal temperature threshold and all 10 calves in the MH group exceeded the 39.5°C cutoff value during the initial 24 hour monitoring period. Rectal temperatures had a significant ($P < 0.05$) interaction between treatment group and trial day with calves in the MH group having a greater average rectal temperature on trial d 0 and 1 compared to CN calves (Figure 3). Average nasal passage temperature did not have any significant ($P > 0.10$) interaction by trial day and treatment group but the main effect of trial day was significant ($P < 0.05$; Figure 3). Nine (90%) of the calves in the MH group and five (62.5%) calves in the CN group exceed 39°C nasal temperature during the trial. An interaction ($P < 0.05$) was found between treatment group and trial hour for average nasal planum surface temperature as measured by thermography with MH greater than CN throughout the monitoring period. Calves in the MH had greater average nasal planum temperature compared to the CN at trial h 8 and 48 (Figure 4). A treatment group effect ($P < 0.05$) was found for average nares surface temperature with MH calves having a greater average surface temperature (32.0 ± 0.4°C) compared to CN calves (31.2 ± 0.4°C). No significant relationships were identified between treatment group and the physiological variables of heart rate and average respiratory rate.

There was a significant ($P < 0.05$) interaction between treatment group (CN or MH) and trial d for the amount of time calves spent within 0.3 m of the grain bunk, hay feeder, water, and shed. Calves in the MH spent less time near the grain bunk on trial d 1; less time at the hay feeder on trial d 0, 1, and 2; more time at the water on trial d 4 and less time near the water on trial d 5; and less time near the shed on trial d 4 compared to CN (Figure 5). After challenge (study d 0), MH calves spent less time near the grain bunk and within the shed compared to CN. There was an interaction ($P < 0.05$) between treatment group and trial d for posture behavior and the number of steps taken daily. Calves in MH spent more time lying down on d 0 through 8 compared to CN (Figure 6). Pedometer analysis determined a significant ($P < 0.05$) interaction

between treatment group and trial d (Figure 7). Evaluation of the average percent change in body weight determined a significant interaction ($P < 0.05$) between treatment group and trial day. Calves challenged with *M. haemolytica* had significantly more body weight loss post-challenge when weighed on trial d 2, 3, 5, 7, and 9 compared to CN (Figure 8).

Average cortisol, substance P, haptoglobin, and Hp-MMP 9 concentrations revealed a significant ($P < 0.05$) interaction between treatment group and trial day (Figure 9). Calves challenged with *M. haemolytica* had greater average serum cortisol concentrations at trial d 0.5 and 1. Substance P was significantly increased in MH calves on trial d 0.5 but had lesser average concentrations at d 7 compared to CN. Calves in the MH had greater average haptoglobin concentrations on trial d 0.5, 1, 2, 3, and 7 compared to CN. Average HP-MMP 9 concentration was greater in MH calves on trial d 2 and 3 compared to CN.

At necropsy, all lung sets from calves challenged with *M. haemolytica* contained cranioventral gross lesions consistent with *M. haemolytica* bronchopneumonia. Pathological lesions were present in the right apical and intermediate lung lubes and consisted of dense fibrinous to immature fibrous adhesions, enlarged interlobular septae, parenchymal necrosis, and atelectasis. Pulmonary lung scores ranged from 5.04% to 9.71% (median lung score of 6.8%; Table 2). Bacterial cultures of lung at necropsy were positive for *Mannheimia haemolytica* in 9 out of 10 calves.

Discussion

The results of this study augment the clinical case definition, physiological, and behavioral changes that occur in calves exposed to *M. haemolytica* during conditions of high ambient temperature conditions. The challenge model to induce *M. haemolytica* disease has been previously described and successfully established BRD as confirmed by post-challenge CIS and necropsy examination (Hanzlicek et al., 2010a). The challenge model used here was successful in establishing BRD in calves based on the lung lesions at necropsy. This allowed us to focus on extreme environmental conditions as the primary stressor. In our model, bronchoselective inoculation of the tracheal bronchus is effective at confining the developing pneumonia to the right apical lung lobes. Similar to field cases of BRD, all calves had cranioventral bronchopneumonia.

Increased rectal temperature in MH calves 6 h after challenge was expected and similar to published literature (Burciaga-Robles et al., 2010b; Confer et al., 2009; Corrigan et al., 2007).

Calves challenged with *M. haemolytica* had rectal temperatures greater than 42°C during the first 24 h after challenge. However, after d 1 MH rectal temperatures were not different from CN. Others have shown that rectal temperatures return to normal by 1 to 3 d post-infection (Ames et al., 1985; Hewson et al., 2011; Vestweber et al., 1990). These transient responses may be a result of endotoxin, or other pyrogenic features of *M. haemolytica*. The CN calves also had rectal temperatures that exceeded the upper limit of the normal rectal temperature reference range (39.5°C) (Radostits, 2001). This provided evidence of heat stress within these cattle and the need for reference range correction when using rectal temperature as a diagnostic tool for BRD during summer conditions.

Nasal mucosal temperature was measured to determine how ambient and body temperature effect nasal mucosal temperature. One possible explanation for interaction in nasal passage temperature between treatment group and trial hour may be contributed to endotoxin release and subsequent peripheral vasoconstriction in nasal mucosae. Also, we were able to show that nasal mucosal temperature exceeds the critical thermal limit (39°C) for temperature sensitive vaccines in MH calves (Mills et al., 1971; Pastoret et al., 1980). Contrary to studies evaluating nares and nasal planum temperature that reported that nares temperature are considerably and consistently below this limit (Mills et al., 1971), we were able to document nasal passage mucosal temperature exceeded the critical thermal limit in 5 out of 10 calves suffering BRD during high ambient temperatures. This may need to be considered when administering intranasal modified live vaccines as these temperatures may inactivate the MLV vaccine and result in vaccine failures.

Previous research has shown that *M. haemolytica* administration caused increases in both heart rate and respiratory rate (Friend et al., 1977), and that respiratory rate is expected to be associated with the extent of lung consolidation (Reeve-Johnson, 2001). In the current trial heart rate and respiratory rate were not different between treatment groups. Calves in this experiment were housed in extremely high ambient temperatures throughout the trial. Therefore, increases in heart rate and respiratory rate in response to heat stress may have obscured our ability to use these physical examination tools as discriminators of BRD (Fuquay, 1981; Hahn, 1999).

Although infrared thermography has been suggested as an effective animal welfare monitoring tool in cattle, extreme ambient conditions likely diminish the effectiveness of surface thermography for detection of BRD (Gomez et al., 2011; Stewart et al., 2005). In this study, we

were only able to detect an interaction between treatment group and time in nasal planum surface temperature. These thermography temperatures differed from nasal passage mucosal temperatures in that calves challenged with *M. haemolytica* had greater surface temperatures throughout the trial.

Behavior of animals is commonly analyzed to determine animal well-being (Gonyou, 1994). Behavioral changes lasted longer than temperature differences in our study. The lethargy associated with BRD was evident in grain bunk and hay feeder activity of MH compared to CN calves. Similar to previous studies, MH calves spent less time near the grain bunk early on during the pneumonia period (Hewson et al., 2011; Sowell et al., 1998; Sowell et al., 1999). However, while we were able to detect a difference between treatment groups on d 1, calves spend a relatively small amount of time per day at the grain bunk.

Accelerometers were useful in detecting differences in MH and CN calves based on the percent of time lying down. The percent of time calves spend lying may be correlated with lethargy or depression. Lying behavior is expected to be one indicator of animal well-being. Previous trials indicated that calves challenged with *M. haemolytica* spent more time lying down (Hanzlicek et al., 2010a; Hanzlicek et al., 2010b). In MH calves, lying time was inversely related with grain bunk and hay feeder behavioral activity. In our study, pedometers were not useful for discrimination between MH and CN calves. This differs from previous research in which pedometers detected decreased number of steps traveled after challenge with *M. haemolytica* (Hanzlicek et al., 2010a). The increase in percentage of time spent walking and number of steps traveled on day 0 may be attributed to the frequent movement that occurred during the intensive monitoring period.

Change in body weight in calves suffering BRD is of short-term economic importance for the cattle industry. Burciaga-Robles *et al*. determined that calves challenged with *M. haemolytica* had less average daily gain up to 4 days after challenge, but they did not demonstrate a difference in body weight between calves challenged with *M. haemolytica* and non-challenged calves (Burciaga-Robles et al., 2010a). We detected a difference between treatment groups, possibly due to the larger sample size of calves challenged with *Mannheimia haemolytica* and control calves and the analysis of percent change in body weight rather than average weight.

Serum biomarkers may be effective diagnostic tools for early diagnosis of BRD or for use in assigning risk categories for calves moving to feedlots. Cortisol is widely used as an indicator

of stress in livestock. Serum cortisol concentrations increased rapidly in the MH group after challenge with MH. This was paralleled by increases in rectal temperatures. Surprisingly, serum cortisol concentrations rapidly declined despite the progressing pneumonia. This differs from previous research in which cortisol concentrations did not change following challenge with *M. haemolytica* (Corrigan et al., 2007). Hewson *et al.* demonstrated highly variable concentrations of cortisol after challenge and could not determine a difference between control calves and calves challenged with *M. haemolytica* until 5 days after challenge when control calves had higher concentrations (Hewson et al., 2011).

Substance P has been used as a specific biomarker to quantify pain in cattle (Coetzee et al., 2008; DeVane, 2001). The rapid increase in substance P concentrations after challenge was expected, but the increased in substance P in control calves after trial day 2 was not anticipated and cause of the change is unknown. Substance P is a volatile neuropeptide that may be influenced by a wide variety of conditions, including the heat stress that both MH and CN groups encountered; however substance P may not be a specific indicator for disease.

Haptoglobin was the most specific and reliable biomarker for discriminating MH and CN calves as CN had near 0 μg/mL haptoglobin concentrations throughout the trial whereas MH had significantly higher haptoglobin concentrations. These results agree with previous reports where haptoglobin was shown to increase after exposure to *M. haemolytica* (Burciaga-Robles et al., 2010b; Ganheim et al., 2003). More research needs to be performed to establish more precise reference ranges for calves with BRD to use haptoglobin concentrations as a diagnostic tool in the field.

Rapid recruitment and accumulation of white blood cells, especially neutrophils, occurs with the onset of BRD (Slocombe et al., 1985). Metalloproteinases are expressed by alveolar macrophages within 12 h of lipopolysaccharide treatment. Degranulated neutrophils release preformed matrix metalloproteinase and Hp-MMP 9 complex into tissue or blood (Lakritz et al., 2004; Lemjabbar et al., 1999; Lubbers et al., 2007). In a previous trial using endotoxin to initiate an inflammatory response, calves showed increased changes in both haptoglobin and Hp-MMP 9 (Lakritz et al., 2004). In our study, *M. haemolytica* was deposited directly into the lung allowing exact temporal relationships to be assessed during the period from insult to onset of pneumonia. This model of naturally occurring disease allows assessment of the immune response to localized injury as opposed to that typically seen in a systemic response when administering endotoxin.

The haptoglobin concentrations in the CN were well below the reference range established by Young *et al.* (1996) for clinically healthy calves. The increase in Hp-MMP 9 but not an increase in haptoglobin concentrations in CN calves indicates there was only a local inflammatory response from the pulmonary macrophages and not a systemic response since haptoglobin concentrations remained low in the CN calves. The frequent handling of the calves during the first 24 h post challenge may have caused a transient increase in Hp-MMP 9 complex and thus not allowing us to detect a difference between treatment groups at 12 h after challenge. These results warrant future research analyzing the effectiveness of Hp and Hp-MMP 9 as effective diagnostic tools for diagnosing BRD.

Limitations of this trial include the use of a challenge model to induce BRD in calves rather than evaluating naturally occurring animals affected by BRD as in a feedlot situation. Lungs from the CN were not evaluated at necropsy because this was not the primary objective and there were no clinical signs in the CN indicating the need for euthanasia of the CN calves. None of the MH calves had a CIS > 2, but calves with a CIS 2 would have been deemed morbid enough to treat in production systems and our goal was to look at changes relative to early BRD. Using a challenge model to induce BRD allowed the calves to not have other confounding stressors and the challenge model may be less virulent than naturally occurring BRD. However by using a challenge model, it allowed us maximum temporal control for when calves became infected, thus allowing controlled monitoring along an established timeline for the development of BRD. The challenge model also puts a conservative estimate on the difference between the treatment groups during periods of high ambient temperature.

Conclusions

In this study, we evaluated cattle with and without experimental *M. haemolytica* infection during high ambient environmental conditions. The influence of increasing temperature during the day may be related to increased rectal temp in the CN group during the initial 24-h monitoring period. These effects were magnified in the MH group. Evaluating a combination of behavioral, physiological, and clinical parameters provides a more comprehensive look at the host and its response to these conditions compared to using single parameters.

Results of this study may guide future research on management techniques to mitigate risk for disease in cattle during extreme summer conditions. The tools used herein demonstrate the value of multimodal assessment in disease research. Multimodal tools can enhance our ability

to determine the effect of treatments with regard to changes in behavior and physiology. Development of practical interpretation of these tools may be utilized as a diagnostic tool for detection of BRD. This study further demonstrates the ability of *M. haemolytica* to be used in BRD challenge models where control of temporality, severity, and extent are desired. Results from this study demonstrate the ability of behavioral detection systems to identify morbid calves and augment clinical case definition of BRD caused by *M. haemolytica*.

Figure 6.1**.** Percentage of calves by treatment group of control calves (n=8) and calves challenged with *Mannheimia haemolytica* (n=10) receiving a clinical illness score of 2 (mild illness) by trial day after endoscopic inoculation on day 0. Calves were observed twice daily (am and pm) throughout the trial.

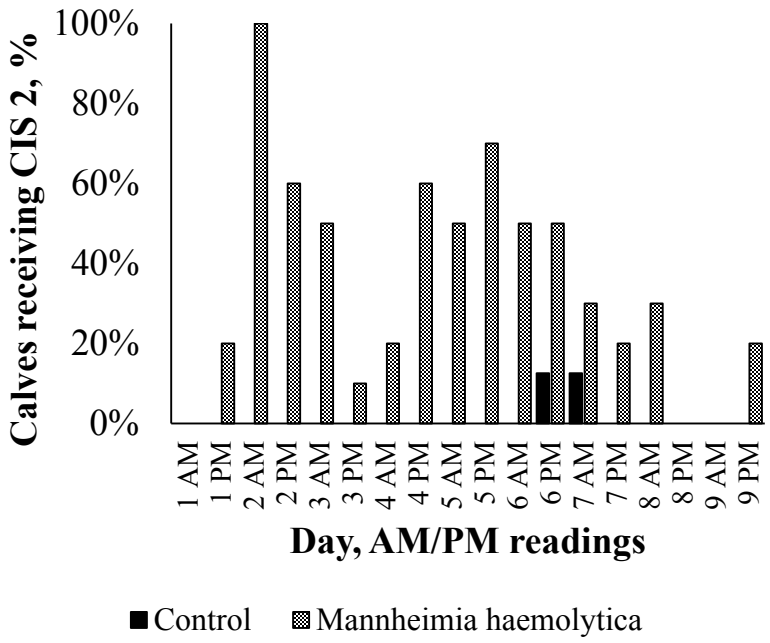

Figure 6.2. Model adjusted least square means (±SE) rectal temperature (A) and nasal temperature (B) in beef heifers by trial hour and treatment group of control calves and calves challenged with *Mannheimia haemolytica* during initial 24-hour monitoring period. Model included effects for trial hour and repeated measures on individual calves. Significant differences ($P < 0.01$) between treatment group within trial hour are denoted by *. Interaction between trial hour and treatment group was significant ($P < 0.05$).

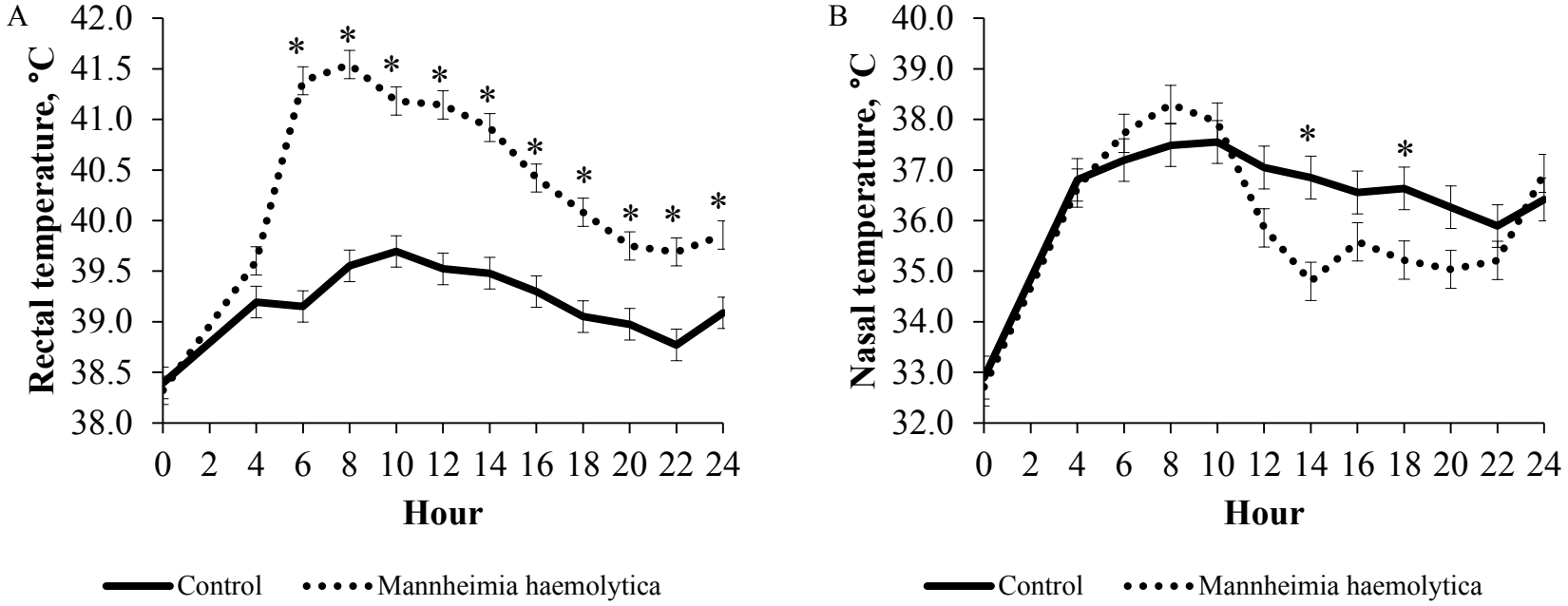

Figure 6.3**.** Model adjusted least square means (±SE) rectal temperature (A) and nasal temperature (B) in beef heifers by trial day and treatment group of control calves and calves challenged with *Mannheimia haemolytica* during daily monitoring period.
Model included effects for trial day and repeated measures on individual calves. Significant differences ($P < 0.01$) between treatment group within trial day are denoted by *. Rectal temperature had a significant ($P < 0.05$) interaction between trial day and treatment group. Interaction between trial day and treatment group was not significant ($P > 0.10$) in nasal temperature.

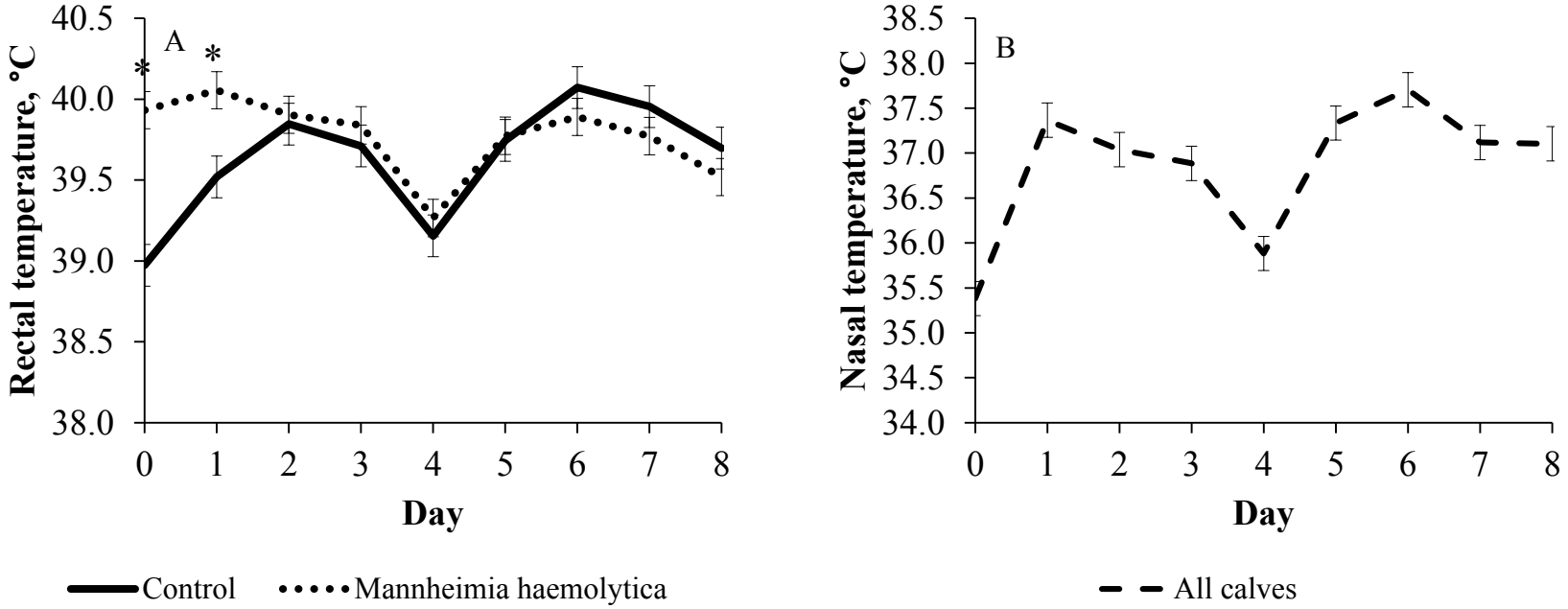

Figure 6.4**.** Model adjusted least square means (±SE) nasal planum surface temperature in beef heifers by trial hour and treatment group of control calves and calves challenged with *Mannheimia haemolytica*.
Model included effects for trial hour and repeated measures on individual calves. Significant differences ($P < 0.01$) between treatment group within trial hour are denoted by *. Interaction between trial hour and treatment group was significant ($P < 0.05$).

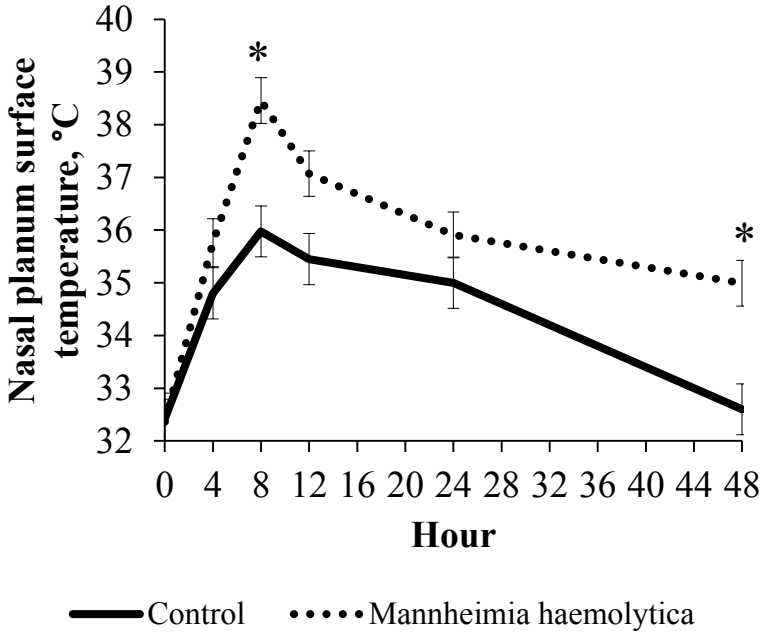

Figure 6.5**.** Model adjusted least square means (±SE) percent of time calves spent within 0.3 meters of the grain bunk (A), hay feeder (B), water (C), and shed (D) in beef heifers by trial day and treatment group of control calves and calves challenged with *Mannheimia haemolytica*.
Model included effects for trial day and repeated measures on individual calves. Significant differences ($P < 0.01$) between treatment group within trial day are denoted by *. Interaction between trial day and treatment group was significant ($P < 0.05$).

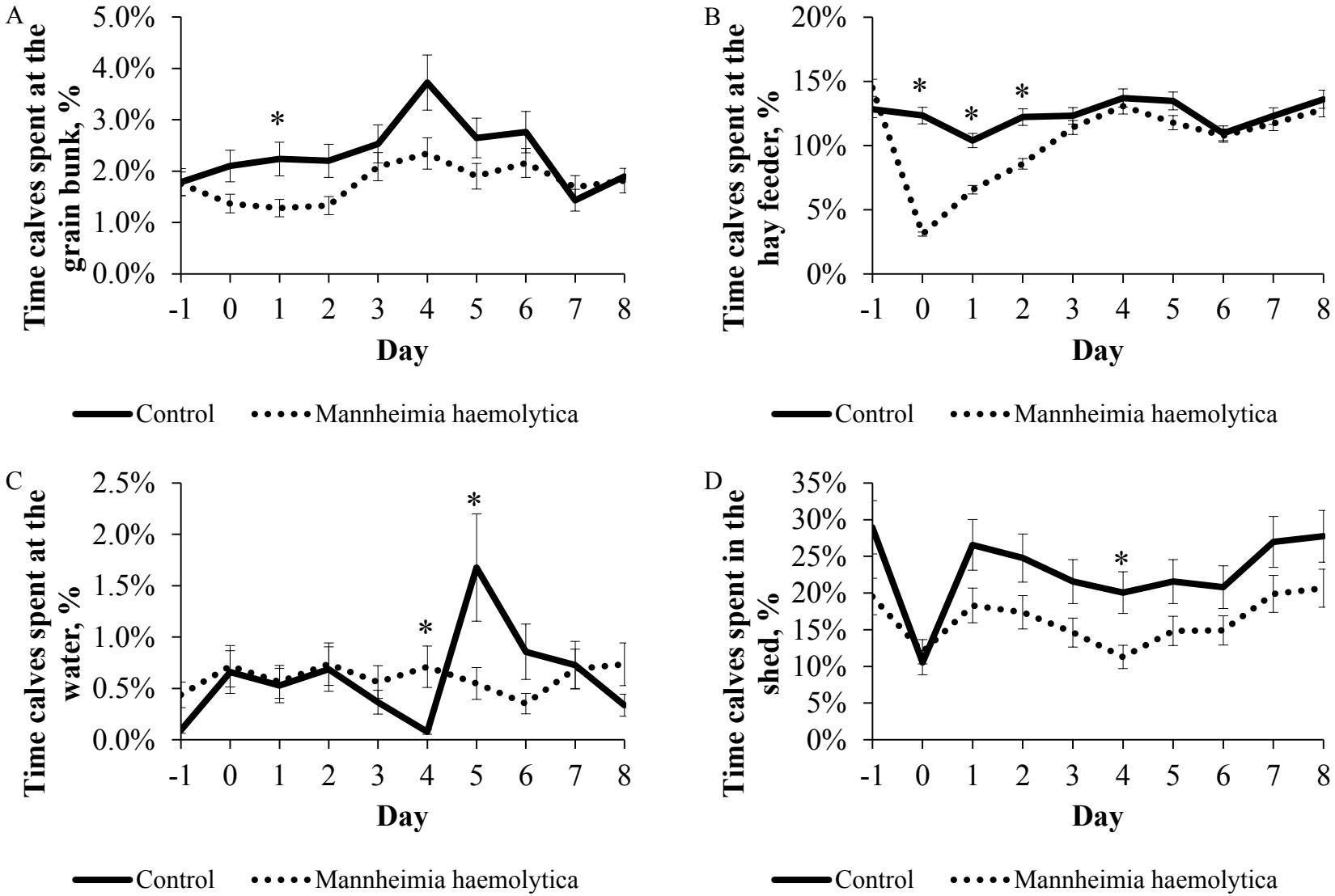

Figure 6.6. Model adjusted least square means (±SE) percent of time calves spent lying down (A) and walking (B) in beef heifers by trial day and treatment group of control calves and calves challenged with *Mannheimia haemolytica*.
Model included effects for trial day and repeated measures on individual calves. Significant differences ($P < 0.01$) between treatment group within trial day are denoted by *. Interaction between trial day and treatment group was significant ($P < 0.05$).

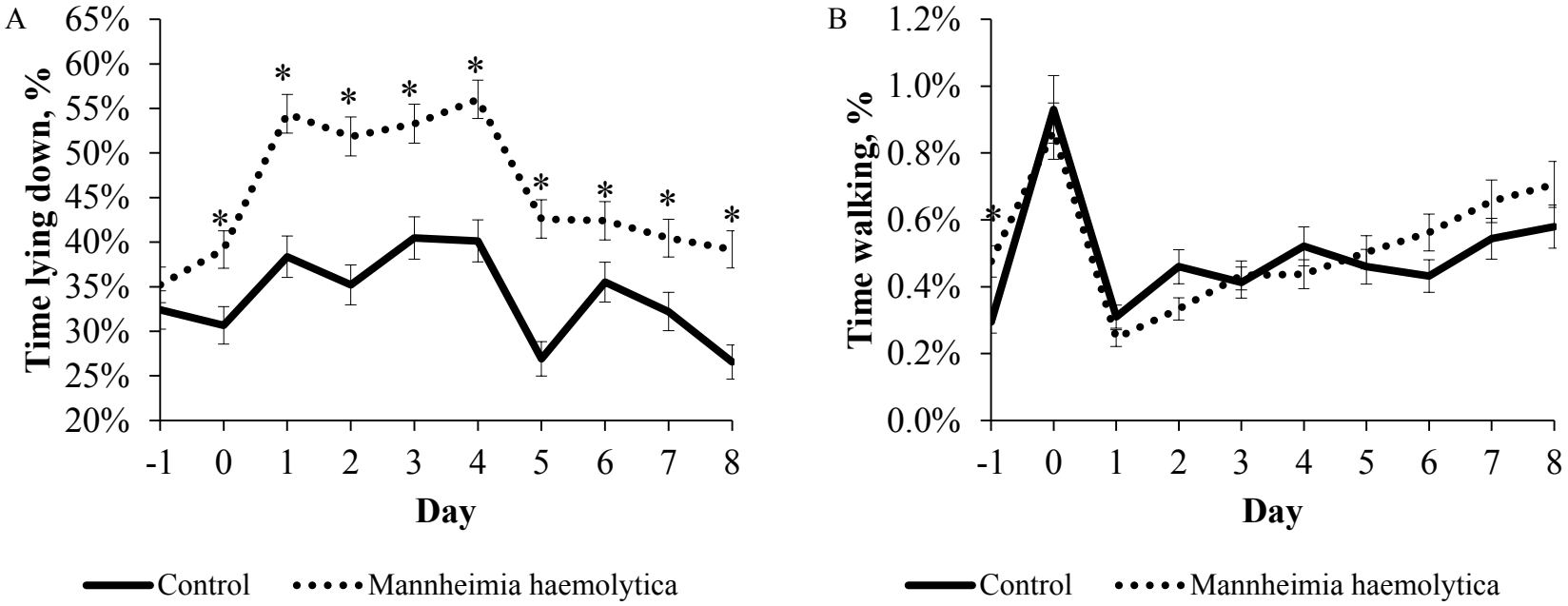

Figure 6.7. Model adjusted least square means (±SE) number of steps traveled in beef heifers by trial day and treatment group of control calves and calves challenged with *Mannheimia haemolytica*.
Model included effects for trial day and repeated measures on individual calves. Significant differences ($P < 0.01$) between treatment group within trial day are denoted by *. Interaction between trial day and treatment group was significant ($P < 0.05$).

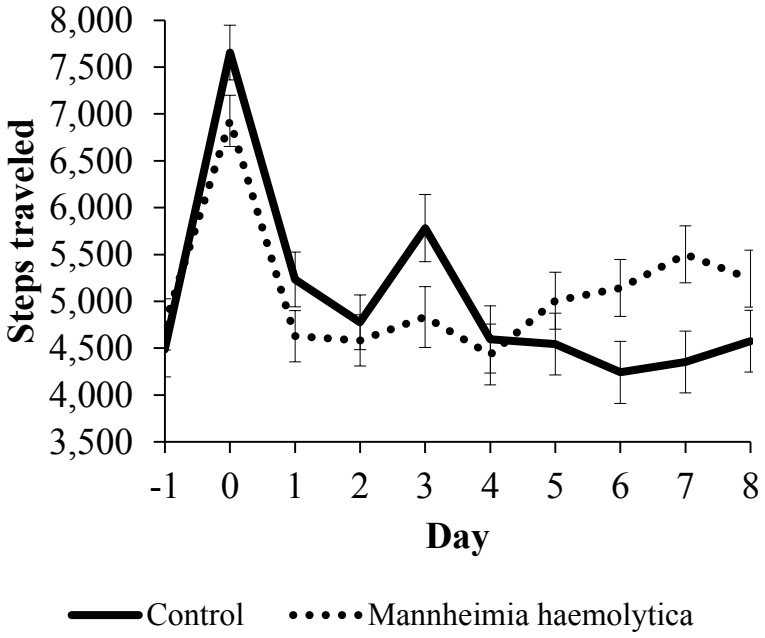

Figure 6.8**.** Model adjusted least square means (±SE) percent change in body weight in beef heifers by trial day and treatment group of control calves and calves challenged with *Mannheimia haemolytica*. Model included effects for trial day and repeated measures on individual calves. Significant differences ($P < 0.01$) between treatment group within trial day are denoted by *. Interaction between trial day and treatment group was significant ($P < 0.05$).

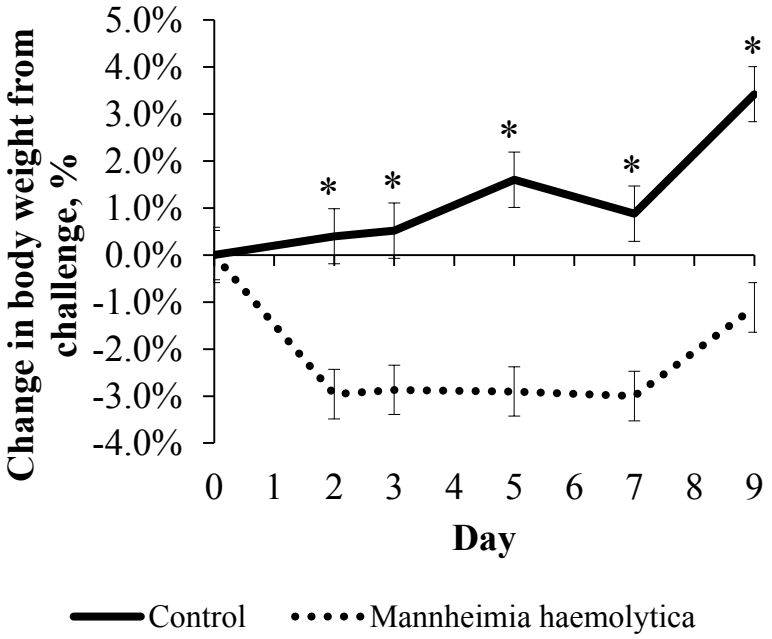

Figure 6.9**.** Model adjusted least square means (±SE) cortisol (A), substance P (B), haptoglobin (C), and matrix metalloproteinase-9 (D) concentrations in beef heifers by trial day and treatment group of control calves and calves challenged with *Mannheimia haemolytica*.

Model included effects for trial day and repeated measures on individual calves. Significant differences ($P < 0.01$) between treatment group within trial day are denoted by *. Interaction between trial day and treatment group was significant ($P < 0.05$).

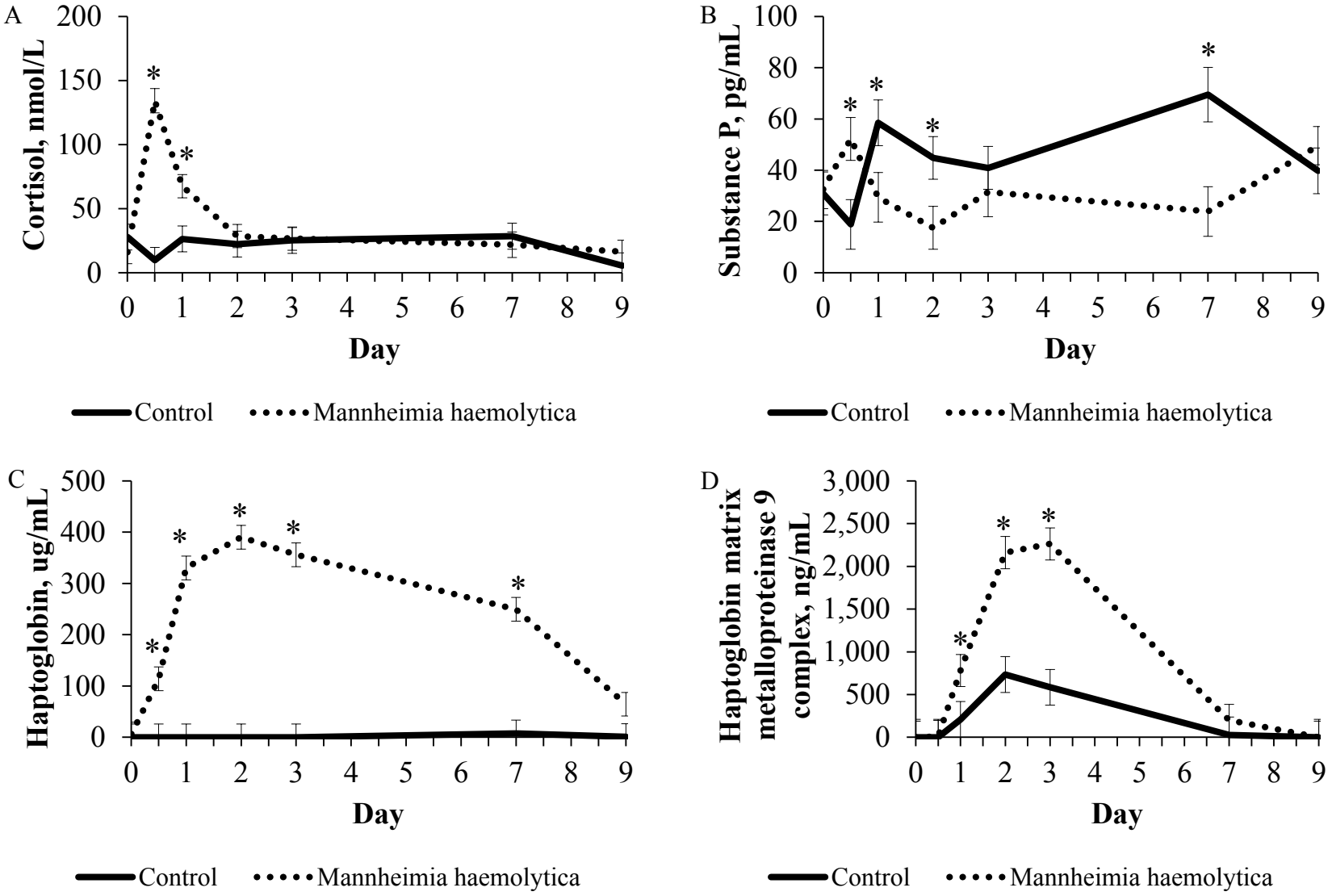

Table 6.1. Environmental conditions beef heifers were exposed to by trial day.

Day	Avg temp, °C	Max temp, °C	Min temp, °C	Avg humidity, %	Max humidity, %	Min humidity, %	Max heat index, °C
0	28.9	34.8	21.0	67.4	92	46	34.9
1	32.0	39.7	22.9	60.9	92	37	40.5
2	35.0	45.1	29.0	42.8	61	22	46.6
3	30.7	36.0	25.3	59.0	77	41	36.2
4	26.0	31.7	22.6	78.6	89	59	31.6
5	28.6	36.0	22.4	75.8	94	49	36.3
6	31.9	39.7	24.6	68.0	92	39	40.2
7	35.1	45.1	23.9	50.2	85	22	45.7
8	34.4	43.9	27.0	45.9	76	20	45.8
9	30.0	33.8	26.1	71.1	85	41	36.9

Table 6.2**.** Pulmonary pathology as percentage of each lung lobe and total long volume from calves challenged with *Mannheimia haemolytica*.[1]

Calf ID	Right cranial apical lobe	Right caudal apical lobe	Right diaphragmatic lobe	Accessory lobe	Intermediate lobe	Left cranial apical lobe	Left caudal apical lobe	Left diaphragmatic lobe	Total lung score
1	20	90	5	0	30	0	0	0	9.71
2	40	90	0	0	10	0	0	0	8.35
3	0	100	0	0	0	0	0	0	6.00
4	0	100	0	0	10	0	0	0	6.43
7	80	0	0	0	0	0	0	0	5.04
9	20	90	0	0	10	0	0	0	7.09
11	10	90	0	0	20	0	0	0	6.89
18	80	5	0	0	0	0	0	0	5.34
19	100	0	0	0	10	0	0	0	6.73
20	100	10	0	0	5	0	0	0	7.12
Avg	45	57.5	0.5	0	9.5	0	0	0	6.87

[1]Percentage pulmonary lesions were calculated by the following formula based on the percentage each lung lobe to the total lung volume:(0.06*right caudal apical/100) + (0.063*right cranial apical/100) + (0.053*left cranial apical/100) + (0.049*left caudal apical/100) + (0.319*left diaphragmatic/100) + (0.043*intermediate/100) + (0.352*right diaphragmatic/100) + (0.061*accessory/100) = total lung score.

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Chapter 7 - A stochastic model to determine the economic value of changing diagnostic test characteristics for identification of cattle for treatment of bovine respiratory disease

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Abstract

Bovine respiratory disease is an economically important syndrome in the beef industry, and diagnostic accuracy is important for optimal disease management. The objective of this study was to determine whether improving diagnostic sensitivity or specificity was of greater economic value at varied levels of respiratory disease prevalence by using Monte Carlo simulation. Existing literature was used to populate model distributions of published sensitivity, specificity, and performance (average daily gain, carcass weight, yield grade, quality grade, and mortality risk) differences among calves based on clinical respiratory disease status. Data from multiple cattle feeding operations were used to generate true ranges of respiratory disease prevalence and associated mortality. Input variables were combined into a single model that calculated estimated net returns for animals by diagnostic category (true positive, false positive, false negative, and true negative) based on the prevalence, sensitivity, and specificity for each iteration. Net returns for each diagnostic category were multiplied by the proportion of animals in each diagnostic

category to determine group profitability. Apparent prevalence was categorized into low (<15%) and high (≥15%) groups. For both apparent prevalence categories, increasing specificity created more rapid, positive change in net returns than increasing sensitivity. Improvement of diagnostic specificity, perhaps through a confirmatory test interpreted in series or pen level diagnostics, can increase diagnostic value more than improving sensitivity. Mortality risk was the primary driver for net returns. The results from this study are important for determining future research priorities to analyze diagnostic techniques for bovine respiratory disease and provide a novel way for modeling diagnostic tests.

Key words: bovine respiratory disease, diagnostic tools, economic modeling, Monte Carlo simulation

Introduction

Bovine respiratory disease (BRD) is the most common and economically significant disease affecting cattle in the United States (Galyean et al., 1999; Lechtenberg et al., 2011). Diagnosis of BRD is commonly performed by observing clinical signs including depression, anorexia, coughing, nasal discharge, and lack of rumen fill (Smith et al., 2001). Current methods used to diagnose BRD based on visual inspection have poor sensitivity and specificity (Amrine et al., 2013; Leruste, 2012; White and Renter, 2009). Poor sensitivity and specificity cause frequent erroneous diagnoses by not identifying and treating truly diseased cattle (low sensitivity) and unnecessarily treating disease-free animals (low specificity). Treatment efficacy is evaluated and wellness assessments are commonly performed using these low sensitivity and specificity measures demonstrating economic losses and misrepresentation of the effect BRD has on the industry.

Performance differences between calves diagnosed with BRD compared to clinically healthy calves vary in published literature. The objective of this study is to determine the economic value of improving BRD diagnostic sensitivity or specificity values in a production feedlot system using Monte Carlo simulation. This model allows the use of the expected range of responses to BRD and accounts for the variability of published results and uncertainty where data are lacking. We hypothesized that it would be more beneficial to improve diagnostic specificity in pens with low prevalence of BRD and to improve sensitivity in high prevalence pens. For diseases that are present at a low apparent prevalence within a population, diagnostic

specificity is expected to have a greater impact on the number of cattle mis-classified compared to diagnostic sensitivity; however, to determine whether improving sensitivity or specificity has the greater economic impact requires the additional knowledge of the cost of each mis-classification.

Materials and Methods

The general research approach was to create an economic framework for comparison of relative changes in estimated cattle feeding net returns based on characteristics of BRD diagnostic testing in low (< 15%) and high (≥ 15%) BRD feeding period apparent prevalence classification. The division into low and high apparent prevalence categories was based on the authors' opinion and supported by 15% apparent prevalence being the 75th percentile for cohort-level feeding period apparent BRD in cohorts with greater than 0% feeding period apparent BRD prevalence in the databased used in the study (i.e. 75% of lots in the database reported BRD apparent prevalence less than or equal to 15% and 25% of lots reported BRD apparent prevalence greater than 15%). The 10-year average net returns to feeding cattle in each diagnosis category: true positive (TP), false positive (FP), false negative (FN), and true negative (TN) were estimated conditional on performance parameters of ADG, carcass weight, yield grade, and quality grade for cattle associated with each diagnosis category. A range of sensitivity and specificity estimates were developed based on existing literature and combined with disease prevalence for each iteration to determine the proportion of animals within each management group classified as TP, FP, FN, and TN in order to determine overall net returns for the group. Monte Carlo simulations estimated the performance parameters for each category and generated multiple iterations based on performance and health outcome data from a large feedlot dataset. The outcome of interest was net returns per head for each cohort while accounting for the proportion and economic net returns impact of TP, FP, FN, and TN calves based on changes in prevalence, and BRD diagnostic sensitivity and specificity. Figure 1 provides an overview of the model structure used.

Model inputs

Published literature: diagnostic sensitivity and specificity

Differences in BRD diagnostic sensitivity and specificity were considered the primary variables of interest of the study; therefore, a literature search was performed to determine realistic ranges for sensitivity and specificity of using visual observation to diagnose BRD

(Amrine et al., 2013; Leruste, 2012; White and Renter, 2009). In one study, the sensitivity and specificity values of clinical illness scores changed as the amount of lung consolidation varied (Amrine et al., 2013). One study reported two separate data points for sensitivity and specificity of BRD diagnosis by clinical signs using greater than 5% lung consolidation for one gold-standard outcome classification for being BRD-positive, and greater than 30% lung consolidation as the other outcome classification for cattle being truly BRD-positive (Amrine et al., 2013). For all other studies, a single case-definition for BRD was used and therefore, one data point was obtained from each study. The minimum and maximum estimated sensitivity and specificity values determined from these past studies were selected as the minimum and maximum values respectively. The median value from these four data points was used as the most likely sensitivity and specificity value for the distributions (Table 1).

Published literature: performance reduction and mortality risk due to BRD

A literature search was performed to determine the range of health and performance impacts of calves visually diagnosed with BRD at any time during the feeding phase compared to calves never diagnosed with BRD. Study inclusion criteria from the literature search consisted of diagnosing BRD based on visual, clinical signs, measuring performance data to closeout, and a non-treated, clinically healthy control group. Challenge models of induced BRD were excluded as data sources. Performance parameters evaluated from published literature included: ADG, carcass weight, percent yield grade 1 or 2, percent yield grade 4 or 5, percent quality grade prime or choice, percent quality grade standard, and mortality risk, as these are important profit drivers (Table 1). We utilized past research to help parameterize the values of these factors in our simulation model. A study was included if it measured any of these parameters. In studies that compared the number of treatments for BRD, results from calves treated only a single time and calves never treated were included to provide a conservative estimate of the effects of BRD.

For each study, the percentage change among performance parameters of interest was calculated between calves identified with BRD compared to calves identified as clinically healthy. The lowest value of the percentage change was selected for the minimal value for a triangular distribution, the highest value of percentage change was selected for the maximal value for the triangle distribution, and the most likely value was determined from a weighted average using the number of animals in each study (Table 1).

Pivotal pharmacological studies: drug efficacy

For each iteration of the submodel, the health parameters of lots from the feedlot health database of feeding period apparent prevalence and mortality risk were selected. Relative risk of death for TP, FP, FN, and TN animals were calculated based on the distributions of mortality in published literature (Table 1). The relative risk for TN calves was set at 1.00 for a baseline as the TN calves should be the least likely to die. The FP was set to have the same relative risk as TN to keep the model consistent with the performance parameter calculations. True positive relative risk was established from the percent mortality in test positive divided percent mortality in test negative calves for diagnosis of BRD based on published literature (Table1). The FN relative risk was calculated using pivotal control pharmacological studies from the U.S. Food and Drug Administration (FDA, 2014). The FN relative risk of mortality was determined for each iteration of the stochastic model by dividing TP relative risk by 1-apparent reduction mortality risk from pivotal pharmacology studies (Michael Apley, Department of Clinical Sciences, Kansas State University, personal communication; Table 1).

Death loss was then calculated for each TP, FP, FN, and TN category based on the relative risk calculated and the number in each category given the sensitivity, specificity, and apparent prevalence values for each iteration. The total death loss was summed and a ratio used to calculate the number in each category (TP, FP, FN, and TN) were based on the proportion of animals in that category and the total death loss for that iteration.

Study feedlot database

Retrospective data from pen-level animal performance and carcass traits collected from Midwestern United States feedlots from 2000 through 2008 were used as baseline performance distributions for this study and included 49,480 cohorts of cattle (Table 2). Cohorts were defined as animal groups that were purchased, assembled, managed, and marketed similarly, but not necessarily housed in the same pen throughout the entire feeding period (Babcock et al., 2010). Cohorts were included for analysis only if all performance parameters of interest were complete. Cohorts were excluded from analysis if performance parameters were incomplete or biologically infeasible (e.g. incorrectly recorded data such as negative ADG or % yield grade 1 and 2, % yield grade 3, and % yield grade 4 and 5 does not total 100 %).

Model structure

Performance calculations for each diagnostic category

Literature describing animal performance relative to BRD status is based on an imperfect test (visual appraisal) generating performance impact for apparent positives (TP, FP) being compared to apparent negatives (TN, FN). The objective of this phase of the study was to estimate actual performance parameters for cattle based on each diagnostic category (TP, FP, FN, and TN) using a submodel. A Monte Carlo performance model was created to calculate the actual impact of BRD by incorporating performance impacts in combination with the estimated distributions of diagnostic sensitivity and specificity at varied levels of disease apparent prevalence. The Monte Carlo simulation was performed with a commercial program (Oracle Crystal Ball, Fusion edition, release 11.1.2.2.000, Oracle Corp., Redwood Shores, CA) as an add-in for a commercial software package (Microsoft Excel 2010, Microsoft Corp., Redmond, WA). Each simulation included 100,000 iterations by using a fixed random number seed. The number of iterations was determined by evaluating the outcomes of interest and determining the outcomes were biologically feasible with appropriate distributions. The number of iterations were increased until performance parameters had normal distributions for each diagnostic category.

To identify the performance effect of BRD, this submodel used cohorts in the database with high BRD feeding period apparent prevalence (40-75%) to allow all iterations to contain at least one animal in each of the TP, FP, FN, and TN categories to determine performance parameters for each category. Cohorts were assumed to contain 300 animals, and based on the apparent prevalence, sensitivity, and specificity for each iteration, the number of calves (rounded to the nearest whole number) in each category (TP, FP, FN, and TN) was determined. As the goal was to compare growth and carcass performance differences, death loss of calves was not included in this phase of the simulation; therefore, results are attributable to performance differences with death loss removed.

True prevalence for each iteration was calculated using the following formula (Dohoo et al., 2009):

True prevalence = (apparent prevalence + specificity - 1) / (sensitivity + specificity - 1)

Iterations were excluded from analysis if the calculated true prevalence of BRD in the pen was less than 0% or greater than 100%. Positive predictive values (PPV) and negative predictive values (NPV) for each iteration were calculated based on the feeding period apparent

prevalence, sensitivity, and specificity of the diagnostic test to determine the number of TP, FP, FN, and TN with the following formulas (Dohoo et al., 2009):

PPV = (true prevalence*sensitivity) / [true prevalence*sensitivity + (1 - true prevalence)*(1 - specificity)]

NPV = [(1 - true prevalence)*specificity] / [(1-true prevalence)*specificity + true prevalence*(1 – sensitivity)]

TP = apparent prevalence*PPV

FP = apparent prevalence*(1 - PPV)

FN = (1 - apparent prevalence)*(1 - NPV)

TN = (1 - apparent prevalence)*NPV

The study feedlot database was used to provide the distribution of true baseline performance values for each iteration. Each cohort in the feedlot database was assigned a unique, identifiable number in which a uniform distribution was established causing each cohort having an equal probability of being selected. Each time a cohort was selected, actual production performance values were used to calculate estimated production performance of cattle in each diagnostic category (TP, FN, FP, and TN). The performance parameters calculated for each cohort included: ADG, CW, percent of calves yield grade 1 or 2, percent yield grade 4 or 5, percent of calves quality grading prime or choice, and percent of calves quality grade standard. The same lot of cattle could be selected multiple times in the simulation, but have imposed different performance values calculated based on stochastic performance variables parameterized from the published literature. In addition, the number of animals in each diagnostic category could change based on sensitivity and specificity values from the established distributions. The percent yield grade 3 and percent quality grade select were adjusted to balance the proportion of cattle that moved in to or out of yield grade 1 or 2, yield grade 4 or 5, percent prime or choice, and percent standard.

Each iteration resulted in an estimated number of animals in each diagnostic category (TP, FP, TN, and FN). For the initial performance analysis, any iteration that resulted in having no calves in one of the TP, FP, FN, or TN categories was filtered out of the analysis. The proportion of animals that were clinically diseased (TP, FP) and those not diagnosed with disease (TN, FN) were calculated from the initial database. For each performance parameter, the estimated performance reduction for cattle diagnosed with BRD was selected from a distribution

based on past literature (Table 1) and a performance estimate was determined for clinically diseased and clinically healthy cattle. The performance parameters for truly diseased (TP, FN) and truly healthy (TN, FP) were then determined by using known values to determine the unknown values solving algebraically using the following function in commercial software package (Microsoft Excel 2010, Microsoft Corp., Redmond, WA): Mmult(miniverse) using two equations to solve two unknown variables described by:

$$(\%TP)X + (\%FP)Y = a \text{ and}$$

$$(\%FN)X + (\%TN)Y = b$$

where X represents the performance of disease positive; Y, the performance of disease free; a, the performance of test positive (apparent prevalence); and b, the performance of test negative (clinically healthy) animals. Array 1 of Mmult(miniverse) was set as the portion of calves each category represented of the row for the 2 by 2 table. Row 1 was the clinically diseased animals containing TP and FP individuals based on sensitivity, specificity, and feeding period apparent prevalence values. Row 2 was the clinically healthy calves containing FN and TN individuals based on sensitivity, specificity, and feeding period apparent prevalence values. Array 2 was set as the performance determined for test positive animals (apparent prevalence) and clinically healthy animals. There was no expected performance difference among FP and TN calves. The range of effect of treating TP calves with antibiotics to regain performance lost due to being diseased was set as a minimum value of 0%, most likely 20%, and maximum value of 60%, as literature comparing the effect of treating TP compared to non-treated FN animals for performance parameters is unavailable. Utilizing these methods, each performance parameter was estimated for calves in each category (TP, FP, FN, and TN) for all iterations.

Net returns for each diagnostic category

Net returns per animal were calculated for TP, FP, FN, and TN within each iteration from the estimated performance parameters established above with the following formula:

$$NR = DP * CW - SP * IW - FC - TC - YD - IN$$

where NR represents the net returns; DP, dressing price; CW, carcass weight; SP, stocker price; IW, in weight; FC, feed costs; TC, treatment costs; YD, yardage; and IN, interest. Prices were averaged over a period of 10 years from September 2002 to August 2012 to provide a long-term market estimate of returns. Dressed price was determined from a five-market weighted average

dressed price for steers and heifers adjusted for carcass quality grade and yield grade premiums or discounts. Stocker price was a continuous slide-adjusted price for each weight and sex averaged across Kansas, Nebraska, Oklahoma, and Texas markets. Feed costs were calculated based on a ration that was 85% corn, 12% alfalfa, and 3% supplement and averaged over a period of 10 years from September 2002 to August 2012 to provide a long-term market estimate of costs. Treatment costs were determined to incorporate the entire feeding period, the range in treatment cost is based on an estimate of the cost of a single treatment ($11.09), in addition to costs for processing the animals as well as the potential to retreat a portion of the animals (USDA, 2000). For TP and FP categories, treatment cost were set as a triangle distribution with minimum value of $15.00/animal, maximum value of $25.00/animal, and most likely value at $20.00/animal. Yardage was assumed as $0.40 per animal per d on feed (Adams et al., 2010; Belasco et al., 2009). Interest was calculated using a 5% annual rate for the entire purchase price of the stocker calf times the proportion of a year the cattle were fed plus one-half of the time on feed for FC, treatment cost, and YD. Average daily gain for each diagnostic category was inputted into the model with the assumption that greater ADG cattle would take fewer days to reach the same end weight the cohort had at harvest from the initial performance analysis. Net returns were separated into male and female distributions for final analysis. Each iteration resulted in a calculated net returns for cattle in each category (TP, FP, TN, and FN) and the result of the simulation model was a distribution of net returns for each category. Returns can be compared within each distribution to estimate the true costs of BRD and misdiagnosis.

An economic value was determined for death loss for each iteration. The death loss value was determined using the following formula:

$$D * (PP + ND*Yd + FC)$$

where D represents the number that died; PP, initial purchase price; ND, number of d to death; Yd, yardage per day; and FC, feed costs. The initial purchase price was extracted from each net returns distribution. The number days to death was set at 50 d (Edwards, 1996). Yardage was established at $0.40 per animal per d (Adams et al., 2010; Belasco et al., 2009). The feed cost consumed was calculated based on a feed conversion of males converting 6 kg of DM to 1 kg of gain and females converting 6.25 kg of DM to 1 kg of gain. Price per kg dry matter was set at $0.1646 per kg of DM. All of these values are the same values used in the economic model to determine profitability of TP, FP, FN, and TN.

Value of changing diagnostic sensitivity and specificity at varied feeding period prevalence levels

The objective of this phase was creation of a Monte Carlo simulation model to estimate the economic implications of changes in sensitivity and specificity at varied feeding period prevalence levels. The simulation model was based on the existing feedyard database, the net returns estimates for each classification of cattle (TP, FP, FN, and TN) generated above, and estimated distributions of sensitivity and specificity. The same feedyard dataset was used as in the first phase of the study; however, all estimates of net returns were gender specific and cohorts recorded as mixed sex were excluded, resulting in 42,267 cohorts of cattle for the final analysis. The simulation model selected cohorts from the feedyard database randomly using a uniform distribution, and the apparent feeding period apparent prevalence and mortality (from all causes) from the cohort was used to populate the model. Each simulation included 500,000 iterations and used a fixed random number seed for this model.

Output analysis

Sensitivity and specificity distributions

For each iteration, sensitivity and specificity were selected from defined distributions. Sensitivity and specificity values have been suggested to not change among varying true prevalence distributions (Dohoo et al., 2009). However, inferences can be made related to observed specificity based on the number of animals classified as clinically diseased. For example, at feeding period apparent prevalence of 0%, the actual specificity is 100% because every animal was classified as healthy (no opportunities for any FP animals in this instance). As long as the distribution of potential test sensitivity is limited to be 50% or greater and apparent feeding period apparent prevalence is 50% or less, mathematic constraints require that the minimum test specificity be 1 – apparent prevalence. For example, if feeding period apparent prevalence was 10%, the minimum specificity would be 90% because even if, in a worst-case scenario, for test specificity with the entire 10% being FP then specificity would then be 90/100=90%. In our model, for pens with a feeding period apparent prevalence of 38% or less, specificity distributions were set to triangle distributions for each feeding period apparent prevalence where the minimal value was 1-apparent prevalence and the maximum value was set to 100%. The most likely value for specificity based on literature (Table 1) was established at 77.9%, and this value was used as most likely when the specificity range included this number;

otherwise, the minimum value was used as most likely. For database cohorts with feeding period apparent prevalence ≥ 38%, the minimum specificity was 61.5%, maximum 100%, and most likely 77.9%. Sensitivity distributions were limited in the same manner as specificity estimates for high feeding period apparent prevalence pens. For all feeding period apparent prevalence pens ≤ 61%, the minimum sensitivity value was 61.5%, maximum 100%, and most likely 71.8% for the distribution.

Final net returns submodel

The proportion of dead animals for each category was subtracted from the initial calves classified into each category, respectively. The final proportion of living animals from each category was then multiplied by the respective distribution of net returns for each category to determine the dollar amount each TP, FP, FN, and TN cattle provided to overall pen profitability. The death loss cost per group determined above was then subtracted from each diagnostic category's profitability. Total pen profit was determined by summing the net profitability of TP, FP, FN, and TN calves. Net returns per animal for the cohort in each iteration were then determined by dividing total pen profit by initial animal units.

Feeding period apparent prevalence from the study feedlot database, feeding period apparent prevalence calculated by the Monte Carlo simulation, and true prevalence frequencies were determined during using the final economic model. The prevalence distributions from the study feedlot database and the Monte Carlo simulation were compared to determine accuracy of the model to represent field data. These distributions were established using histogram analysis and determining proportion of iterations at each frequency level. The entire model was reanalyzed using economic input values from November 2013 to October 2014 to determine if cattle inventory prices during this time period had different effects on the model results compared to the time period of September 2002 to August 2012.

Statistical Analysis

Distribution of all parameters modeled were evaluated to detect if each were biologically feasible. Final economic data were extracted to a commercial software program (JMP, Version 9, SAS Institute, Cary, NC). Cohorts with a feeding period apparent prevalence >0% were categorized as either low feeding period apparent prevalence (<15%) or high feeding period apparent prevalence (≥ 15%) to determine how the value of improving sensitivity and specificity compared between these categories. Cohorts with 0% feeding period apparent prevalence were

excluded prior to analysis as sensitivity and specificity are fixed for these cohorts preventing variation in the values necessary to analyze the effect of change on net returns per animal. Linear relationships were evaluated for net returns per animal by sensitivity and specificity values using a commercial software program (Microsoft Excel 2010, Microsoft Corp., Redmond, WA) where trendlines were calculated for sensitivity and specificity values at the two feeding period apparent prevalence categories used in the study. Slopes for the trendlines were analyzed to determine the change in net returns per animal by changing the value of sensitivity or specificity by one percentage point. Standard deviations of the slopes of the trendlines were also determined using the Linest function (Microsoft Excel 2010, Microsoft Corp., Redmond, WA).

Sensitivity Analysis

The sensitivity analyses of the economic outcome variable, net returns per animal, to model inputs were analyzed for the final net returns model. Iterations from the final model were extracted to a commercial software program (JMP, Version 9, SAS Institute, Cary, NC). Diagnostic sensitivity, treatment efficacy, treatment costs, purchase price, in weight, d on feed, carcass weight, diagnostic specificity, yield grade, stocker price, ADG, sex, true prevalence, apparent prevalence, dressed price, quality grade, and mortality risk were the input parameters used for the sensitivity analysis. Pearson correlation between net returns and input parameters were calculated for sensitivity analysis (Hamby, 1994; Levine and Renelt, 1992). The sensitivity analysis determined the factors that were highly influential on the outcome net profit per animal by determining the amount of variation in net profit per animal contributed by the parameter. A positive correlation in sensitivity analysis illustrates the variable had an increasing effect on profit per animal; whereas, a negative correlation indicates as the variable increased, profit per animal decreased.

Results

The literature search revealed high variability in the range of diagnostic sensitivity, specificity, and performance parameters of calves visually diagnosed with BRD compared to calves clinically healthy (Table 1). The distribution of ADG, carcass weight, in weight, percent yield grade 1 and 2, percent yield grade 4 and 5, days on feed, percent quality grade prime and choice, percent quality grade standard, morbidity risk, and mortality risk are presented in Table 2.

Feeding period apparent prevalence from the final net returns model follows the feeding period apparent prevalence from the database. True prevalence from the final net returns model also followed a similar pattern as apparent prevalence. Sensitivity and specificity distributions for the final net returns model are displayed in Figure 2. 8,915 iterations were excluded from the initial analysis due to negative biological performance values and true prevalence values outside the boundaries of 0-100%.

The 75th percentile of the distribution of feeding period apparent prevalence in cohorts with greater than 0% feeding period apparent prevalence was 15% , which was used to distinguish high feeding period apparent prevalence (≥ 15%) from low feeding period apparent prevalence (< 15%) cohorts in the subsequent analyses. Net return trendlines increased as sensitivity and specificity values increased for both apparent prevalence categories; however, the linear slope was greater for specificity values compared to sensitivity values (Figure 3). Linear relationships were evaluated between sensitivity and specificity values by net returns per animal for low and high feeding period apparent prevalence using one-way analysis. Slopes and standard deviation of trendlines by feeding period apparent prevalence categories are displayed in Table 3.

Mean, median, quartile 1, and quartile 3 for net returns for each classification of calves (TP, FP, FN, and TN) by feeding period apparent prevalence categories are displayed in Table 4. Average percent of cattle expected to be represented in each BRD status classification by feeding period apparent prevalence categories are displayed in Table 4. Median values are appropriate to evaluate because profit distributions are not normally distributed. True negative calves produced the greatest net returns per animal, and false negative calves produced the lowest net returns per animal in both apparent prevalence categories. Sensitivity analyses were performed for low and high feeding period apparent prevalence categories and displayed in Figure 4. Analysis of the model with economic inputs from November 2013 to October 2014 had similar results to the 10 year average economic model values used. For the apparent prevalence <15% category, the value of improving sensitivity 1 percentage point was $0.01 per animal increase in net returns and the value for improving specificity 1 percentage point was $0.99 per animal increase in net returns. For the apparent prevalence ≥15% category, the value of improving sensitivity was $0.06 per animal increase in net returns and the value for improving specificity 1 percentage point was $0.95. The low correlation values for diagnostic sensitivity and diagnostic specificity indicate

that differences in BRD diagnostic characteristics are not the primary drivers of net returns. Mortality risk, feeding period apparent prevalence, true prevalence levels, dressed price, and quality grade were the dominant drivers of variability in net returns for the model.

Discussion

The results of this simulation model illustrate the economic value of changing sensitivity and specificity for BRD diagnosis varied between low and high feeding period apparent prevalence. To the authors' knowledge, this is the first study to evaluate the effects of changes in sensitivity and specificity values for BRD diagnostic tests on net returns by applying the performance effects of BRD from published literature to a production management setting. The similarity between modeled and actual feeding period apparent prevalence distributions provided evidence for the accuracy of the model. Improving either sensitivity or specificity increased net profit; however, at both feeding period apparent prevalence categories, the rate of increase in net returns was higher with improving diagnostic specificity compared to increasing diagnostic sensitivity.

The calculation to determine true prevalence of BRD using values for sensitivity and specificity that vary based on the mathematical constraints of apparent prevalence was a novel approach that we have not seen previously published. Feeding period apparent prevalence is the data available to use in production settings, and producers should be able to apply the results of this study to their current production setting. By applying the apparent prevalence limited test sensitivity and specificity values, we were able to utilize field data (feeding period apparent prevalence) to influence the true distribution of specificity values which resulted in calculated true prevalence values that appeared to accurately represent common BRD estimates from commercial feedlot data. In contrast, if one applies the broad range of sensitivity and specificity estimates from the published literature to pens regardless of apparent prevalence, abnormal distribution of true prevalence result with several of the calculations falling outside the boundaries of 0% and 100%. These instances of infeasible results occur when published values for BRD diagnostic specificity are applied to low feeding period apparent prevalence cohorts. We recognize that while BRD is one of the most economically significant diseases to affect beef feedlot cattle, the syndrome is still a low feeding period apparent prevalence disease because the majority of cohorts have less than 4.9% BRD cases (USDA, 2001). Therefore, the specificity estimates from published literature calculated from populations of feedlot cattle with high BRD

apparent prevalence are not applicable to the entire population of feedlot cattle (Amrine et al., 2013; Leruste, 2012; White and Renter, 2009).

Cattle are a prey animal which may cause them to avoid displaying overt signs of disease (Grandin, 2000). This instinctive behavior as well as other animal factors may result in FN classification when cattle truly are morbid. If high numbers of FN animals are present in a population, distributions of BRD diagnostic sensitivity will be skewed to the right. The high number of calves classified as clinically healthy results in a heavily negative skewed specificity distribution (Figure 2). Specificity values only relate to disease-free animals. Specificity values must be high in cohorts with low apparent feeding period apparent prevalence as there is a large percentage of the population classified as clinically healthy.

Lung lesions have been suggested as a more accurate method to assess BRD compared to clinical observations (Bryant et al., 1999; Gardner et al., 1999; Thompson et al., 2006). Timing of the identification of morbid animals relative to disease progression may impact the amount of lung pathology at harvest because if minor damage occurs to the lung tissue well before harvest, the lung is capable of replacing damaged tissue and thus displaying no lung lesions at harvest (Brumbaugh, 2007; Bryant et al., 1999; Hanzlicek et al., 2010). Lung lesion evaluation is only applicable at necropsy or harvest and may be helpful for evaluating the impact on the group retrospectively, but does not provide information regarding health status at the time the disease is impacting animal performance and ultimately producer profitability.

Improving diagnostic specificity was more valuable in in both low and high feeding period apparent prevalence pens compared to improving diagnostic sensitivity, as the slope of the net returns trendline for specificity was greater than the slope of net returns trendline for sensitivity. Improvement in diagnostic sensitivity values only has a potential impact on a few animals in cohorts with low feeding period true BRD prevalence, as there were few truly morbid animals. However, even using appropriate limitations on how low diagnostic specificity is actually possible, iterations that drew from the allowable low specificity values resulted in several animals classified as FP which reduced profitability for the pen because of unnecessary treatment costs applied to those cattle. Improving diagnostic specificity has a potential impact on more animals in low feeding period apparent prevalence cohorts compared to the number of animals potentially impacted by improving diagnostic sensitivity. While increasing diagnostic specificity was more profitable compared to increasing diagnostic sensitivity, it is important to

note there is a limited magnitude that specificity could be improved in the low feeding period apparent prevalence cohorts. There is more room for improvement in increasing sensitivity, but since there are few truly morbid animals in the lot there is limited value in improving diagnostic sensitivity to identify and treat animals based on net returns. Studies evaluating sensitivity and specificity of visual appraisal compared to lung lesions at harvest placed emphasis that sensitivity was more valuable to improve based on the number of animals with lung lesions present at harvest that were not treated for BRD (Gardner et al., 1999; Schneider et al., 2009; Wittum et al., 1996). The results from this simulation model demonstrate that it would be more profitable to improve specificity to diagnose BRD in a production setting.

The net returns projections based on sensitivity and specificity estimates are for the overall diagnostic system and not just an individual test's performance. These results support the use of a confirmatory test for BRD to be administered once cattle are confined to a squeeze chute after meeting the initial case definition for BRD based on observation for clinical signs of disease in the feedlot pen; and interpreting the two tests in series. The improvement of specificity by use of confirmatory tests needs to be greater than the cost of the test implemented in order to increase profitability. Cattle would need to test positive to both the initial test and the confirmatory test in order to receive an antibiotic treatment. The confirmatory test may be lung auscultation, rectal temperature, or other chute-side tests. These results illustrate it may be valuable to change rectal temperature cut-points of what is classified as a positive animal depending on the best estimate for eventual pen feeding period apparent prevalence. These results also support establishing specific criteria for the way cattle are identified as morbid at the pen level, by only selecting truly morbid animals to receive treatment.

Cattle marketing strategies and values used between 2013 and 2014 are different from the 10 year averages used to determine net returns in the study model (Schulz, 2014). To account for these changes in economic value, the stochastic model was re-analyzed using economic values from November 2013 to October 2014. Analysis of the value of improving diagnostic sensitivity and specificity by appearance prevalence category with the economic inputs from November 2013 to October 2014 show similar trends to the 10 year economic average model in terms that diagnostic specificity was more valuable to improve than diagnostic specificity in both feeding period prevalence categories.

Net returns for TP, FP, FN, and TN calves have not been reported before to the authors' knowledge. These values show a much larger difference between TP and TN compared to differences based on the number of treatments cattle received for BRD reported in previous studies (Brooks et al., 2011; Cernicchiaro et al., 2013). In those studies, FN and TN net profits are combined in the untreated calves, and TP and FP are combined into calves treated any number of times for BRD. The different methodologies for classifying calves in the models, and different sources for market prices and treatment costs explain reasons for differences from published studies. While FN calves have the lowest net returns per animal, it is important to note the low percentage of these calves in the overall feeding period apparent prevalence categories; 0.67% of calves in low feeding period apparent prevalence group and 5.28% of the calves in the high feeding period apparent prevalence group. The low number of calves classified as FN indicates there is relative little benefit in improving sensitivity, which also relates to the relative flat slope of the sensitivity trendline.

Comparing the net returns for TP, FP, FN, and TN calves also demonstrates the true cost of BRD, as we were able to separate the FP calves from the clinically diseased calves. The FP calves are artificially increasing the performance parameters of the clinically diseased animals as the FP calves are truly healthy and do not have performance detriments as a result of BRD. The FN calves are artificially decreasing the performance parameters of the clinically healthy animals as the FN calves are truly diseased. These two factors result in an underestimation of the true impact of BRD in feedlot cattle as all previous comparisons are based on clinically ill vs. clinically healthy animals. If we were more accurate in our clinical diagnosis of BRD, there would be an increase in the difference in performance between the test positive and test negative individuals. Treatment efficacy may decrease because there would be fewer FP animals receiving a treatment; therefore treatments would only be applied to truly diseased individuals. Increasing diagnostic specificity would result in fewer cattle treated for BRD which may reduce the selection pressure for antimicrobial resistance (Belongia and Schwartz, 1998; McEwen and Fedorka-Cray, 2002). The only negative effect of FP calves accounted for in this stochastic model were additional treatment costs as there was not any data available to quantify the negative relationship of reduction in ADG due to treatment or being housed in a hospital facility, to the authors' knowledge; however if a negative impact was included in the model, there would

be more value added to improving diagnostic specificity and specificity was already more valuable to improve compared to sensitivity.

The low correlation values for the diagnostic sensitivity and specificity trendlines for overall net returns at both feeding period apparent prevalence levels show that BRD diagnostic test performance is not a primary driver for net returns in feedlot cattle. Performance such as ADG, carcass weight, days on feed, yield grade, quality grade, feeding period apparent prevalence, and mortality risks have greater correlation to net profit than BRD test sensitivity and specificity estimates (Irsik et al., 2006; Schroeder et al., 1993). However, we were interested in determining whether improving sensitivity or specificity would be more beneficial from a profitability standpoint and thus we utilized economics as a metric for evaluating diagnostic capabilities. While mortality risk was the biggest driver of overall net returns in high feeding period apparent prevalence cohorts, diagnostic specificity was still more profitable to improve compared to sensitivity due to the frequency of FP and treatment costs.

Potential limitations of this study include that all data were generated by a simulation model; therefore, the external validity of this simulation model is dependent of the accuracy of the model inputs and the quality of the model. While careful consideration of all inputs was performed prior to acceptance of the model, it is important to acknowledge the wide range of the effects of BRD from the published literature. The study feedlot database used for this model represents standard feedlot production setting, but would be from a low to medium BRD mortality risk population. Treatment efficacies for reducing mortality risk for antibiotics were selected using the mortality results from pivotal pharmacological studies. These treatment efficacy values are performed with studies evaluating a short time interval after treatment minimizing the true death loss that may occur in treated calves and untreated control calves; however the sensitivity analyses demonstrated treatment efficacy had a relatively low impact on the overall profitability results. The economic determinants of profit were performed across a variety of types of cattle and may not accurately predict expected results in specific categories or descriptions of cattle. Feeding period apparent prevalence values were used to apply sensitivity and specificity values instead of individual feeding day apparent prevalence values; however, the use of feeding period apparent prevalence values will provide more of a conservative estimate on the value of improving specificity as there will be a higher percentage of calves classified as healthy if individual feeding day apparent prevalence values were utilized. The sensitivity and

specificity distribution were independently sampled. The model evaluates the overall sensitivity and specificity estimates of the system used to classify morbid and healthy calves in the production setting.

Conclusion

Diagnosticians will be able to use these results to estimate the net value of improved diagnostic tests for BRD. For both apparent prevalence categories, increasing diagnostic specificity created more rapid, positive change in net returns than increasing sensitivity. Improvement of diagnostic specificity, perhaps through a confirmatory test interpreted in series or improved pen-level diagnostics, can increase diagnostic value more than improving sensitivity. Efforts to reduce mortality risk and prevalence of BRD will have a greater impact than improvement of diagnostic capabilities based on sensitivity analyses of the stochastic model. Continued efforts to properly identify morbid and healthy animals will lead to earlier diagnosis of disease to provide treatment earlier and the continued judicious use of antibiotics. In addition, refinement of diagnostic and treatment protocols for BRD will result in economic benefit for the cattle producers.

Figure 7.1. Schematic outline of the structure of the model inputs used and the flow of each submodel into the final model used. BRD = bovine respiratory disease

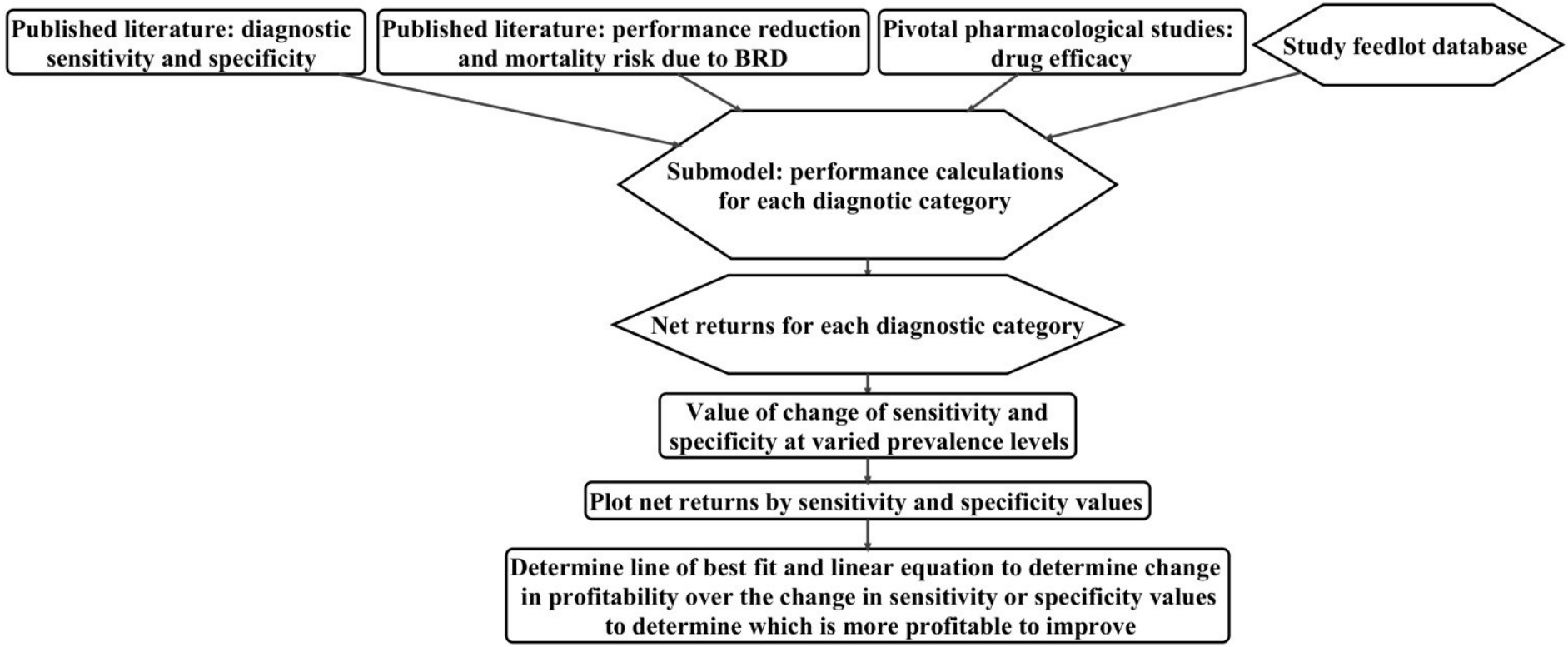

Figure 7.2. Sensitivity (A) and specificity (B) distributions from model output in simulated cohorts.

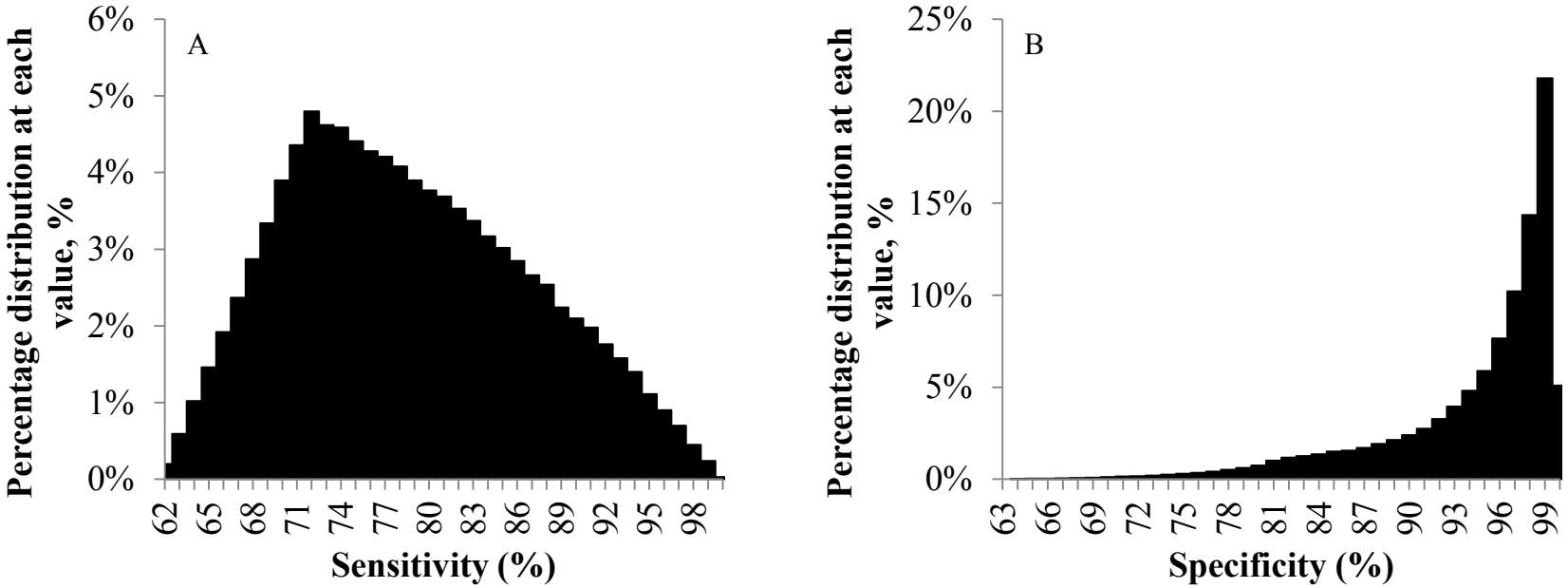

Figure 7.3. Mean net returns (US$/animal) trendlines by sensitivity and specificity values by feeding period prevalence level. Apparent prevalence was divided into low (<15%) and high (≥15%) apparent feeding period prevalence categories.
Sensitivity trendlines are the solid lines and specificity trendlines are displayed by the dashed lines. The black lines are the low apparent prevalence category and the gray lines are from the high apparent prevalence category. Separate models were used for each prevalence category.

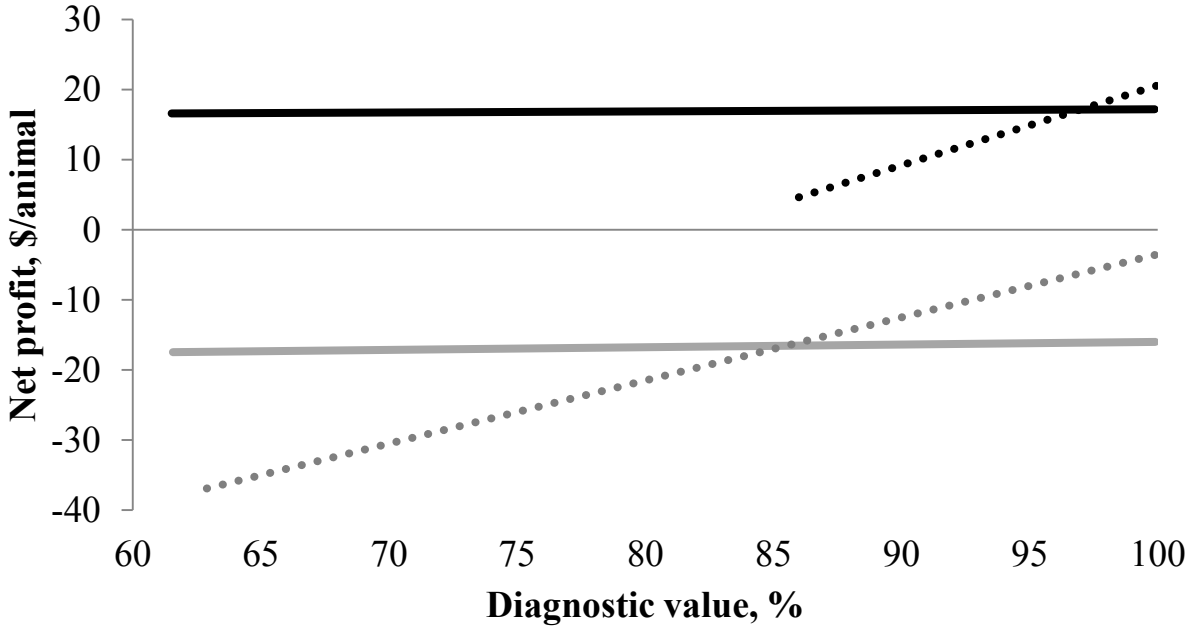

Figure 7.4**.** Sensitivity analysis for net profit per animal from final net returns calculations simulation model to quantify the value of changing sensitivity and specificity values for bovine respiratory disease diagnostics.
Diagnostic sensitivity, treatment efficacy, treatment costs, purchase price, in weight, days on feed, carcass weight, diagnostic specificity, yield grade, stocker price, ADG, sex, true prevalence, apparent prevalence, dressed price, quality grade, and mortality risk were the input parameters used for the sensitivity analysis. Pearson correlation between net returns and input parameters were calculated for sensitivity analysis.

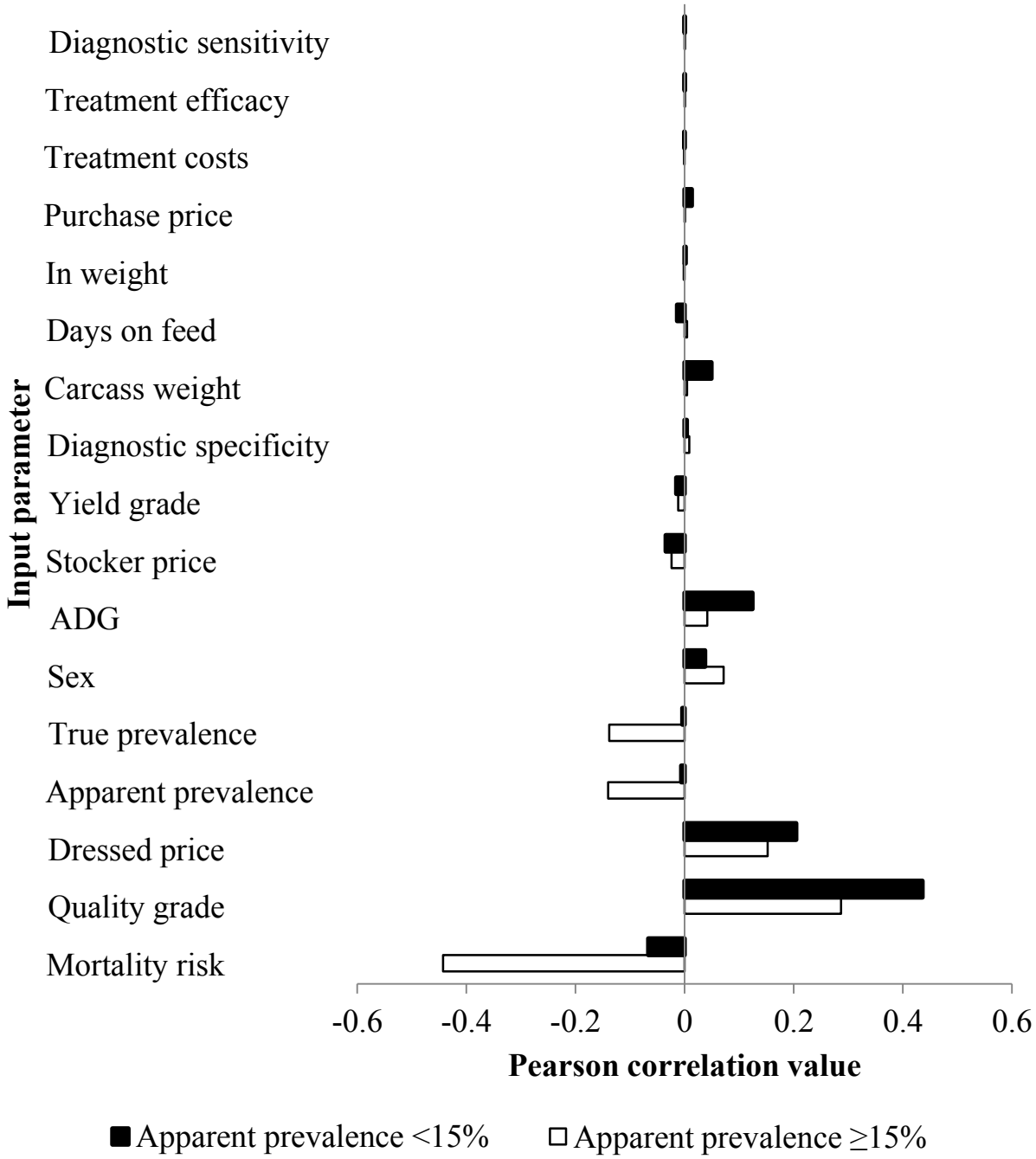

Table 7.1. Percentage input distributions describing health effects of calves infected with bovine respiratory disease compared to clinically healthy calves based on published literature.
The parameters needed to characterize the triangular distribution are indicated in the parentheses, triangular (A, B, C), where A is the minimum values, B is the most likely value, and C is the maximum value.

Parameter description	Distribution	Reference
Sensitivity	Triangular (0.615, 0.718, 0.989)	(Amrine et al., 2013; Leruste, 2012; White and Renter, 2009)
Specificity	Triangular (0.628, 0.779, 0.949)	(Amrine et al., 2013; Leruste, 2012; White and Renter, 2009)
Average daily gain reduction	Triangular (-0.037, 0.074, 0.148)	(Bateman et al., 1990; Brooks et al., 2011; Buhman et al., 2000; Cernicchiaro et al., 2013; Faber et al., 1999; Gardner et al., 1999; Holland et al., 2010; Jim, 1993; McNeill et al., 1996; Reinhardt et al., 2009; Reinhardt et al., 2012; Roeber et al., 2001; Schneider et al., 2009; Thomson et al., 2012; Wittum and Perino, 1995; Wittum et al., 1996)
Carcass weight reduction	Triangular (-0.010, 0.012, 0.025)	(Brooks et al., 2011; Cernicchiaro et al., 2013; Garcia, 2010; Gardner et al., 1999; Holland et al., 2010; Jim, 1993; Reinhardt et al., 2009; Reinhardt et al., 2012; Roeber et al., 2001; Schneider et al., 2009; Thomson et al., 2012)
% Yield grade 1 and 2	Triangular (-0.148, -0.141, 0.031)	(Gardner et al., 1999; Holland et al., 2010; Reinhardt et al., 2009; Reinhardt et al., 2012; Thomson et al., 2012)
% Yield grade 4 and 5	Triangular (-0.076, 0.333, 0.371)	(Gardner et al., 1999; Holland et al., 2010; Reinhardt et al., 2009; Reinhardt et al., 2012; Thomson et al., 2012)
% Choice	Triangular (0.040, 0.195, 0.307)	(Cernicchiaro et al., 2013; Garcia, 2010; Gardner et al., 1999; Holland et al., 2010; McNeill et al., 1996; Reinhardt et al., 2009; Reinhardt et al., 2012; Thomson et al., 2012)

% Standard	Triangular (-0.455, -0.394, -0.333)	(Gardner et al., 1999; Reinhardt et al., 2009)
% Mortality in test positive	Triangular (0.021, 0.038, 0.059)	(Faber et al., 1999; Reinhardt et al., 2009)
% Mortality in test negative	Triangular (0.001, 0.004, 0.015)	(Faber et al., 1999; Reinhardt et al., 2009)
Apparent reduction in mortality risk	Triangular (0.025, 0.170, 0.440)	Michael Apley, Department of Clinical Sciences, Kansas State University, personal communication
% Treatment efficacy on performance	Triangular (0.000, 0.200, 0.600)	Author opinion, (USDA, 2000)
Treatment costs	Triangular (15.00, 20.00, 25.00)	Author opinion, (USDA, 2000)

Table 7.2**.** Descriptive analysis of existing feedyard database used as baseline performance distributions for model simulation.

Parameter	Mean	Median	Standard Deviation	Quartile 1	Quartile 3
ADG (kg/d)	1.31	1.32	0.21	1.17	1.46
Carcass weight (kg)	351.81	354.09	31.84	327.27	376.93
In weight (kg)	314.90	322.05	50.65	283.04	352.90
Yield grade 1 and 2 (%)	61.60	62.00	15.12	52.00	572.07
Yield grade 4 and 5 (%)	4.28	3.00	4.76	1.00	6.00
Days on feed (d)	179.99	169.00	42.21	150.00	201.00
Quality grade prime and choice (%)	45.08	45.00	14.82	34.51	55.00
Quality grade standard (%)	7.18	6.00	5.19	3.93	9.00
Morbidity risk (%)	9.25	4.92	11.95	1.21	12.09
Mortality risk (%)	1.47	0.95	2.04	0.00	1.96

Table 7.3. Slope of trend line for sensitivity and specificity value by net profit by feeding period apparent prevalence category from stochastic model.
Model included estimated performance difference, mortality risk, and treatment cost differences between diagnostic categories and determined net returns for each iteration. Slope of line determined the increase profit per animal by increasing sensitivity and specificity value of diagnostic test 1%.

Feeding period apparent prevalence category	Sensitivity ($/% change)	SD of sensitivity slope	Specificity ($/% change)	SD of specificity slope
<15%	0.02	0.012	1.14	0.035
≥15%	0.04	0.025	0.90	0.031

Table 7.4. Net returns ($/animal) by category of true positive, false positive, false negative, and true negative based on performance parameters and mortality risks for each category by feeding period apparent prevalence category.
Model included estimated performance difference, mortality risk, and treatment cost differences between diagnostic categories and determined net returns for each iteration.

Feeding period apparent prevalence category	BRD status	Mean	Median	Quartile 1	Quartile 3	Avg % of each category at apparent feeding period prevalence level
<15%	True positive	(106.18)	(83.42)	(142.95)	(38.50)	2.23
	False positive	1.18	2.18	(34.88)	38.14	3.19
	False negative	(122.57)	(88.80)	(162.66)	(36.29)	0.67
	True negative	21.25	22.26	(14.76)	58.17	93.91
≥15%	True positive	(129.66)	(106.57)	(174.87)	(54.05)	17.89
	False positive	(2.32)	(0.31)	(37.41)	34.69	11.38
	False negative	(153.61)	(119.62)	(205.52)	(57.87)	5.30
	True negative	17.65	19.69	(17.48)	54.69	65.43

* Negative values are in parentheses.

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Chapter 8 - Dissertation Conclusions

Bovine respiratory disease (BRD) continues to be the most economic significant disease affecting feedlot cattle, despite all of the efforts to develop new vaccines, antibiotics, or diagnostics in order to reduce morbidity and mortality risks. The purpose of this dissertation was to evaluate a variety of behavior and biological indicators in various stress and diseased states in order to provide additional information for usefulness and practicality of these systems to be applied to the industry. These results also help further define case definition of disease progression and provide a baseline for industry comparisons.

Rectal temperature is commonly monitored in production systems, and there are a wide variety of things that can affect rectal temperature including environmental conditions, time of day, and severity and timing of disease state. Rectal temperature at first treatment for BRD had limited value as a prognostic indicator whether calves would finish the production cycle normal. As rectal temperature increased, the probability that a calf did not finish the production cycle increased; however, the relationship was not linear. Environmental conditions have an effect on thermoregulation of calves exposed to extreme heat conditions as well monitored by rectal temperatures and nasal submucosal temperatures.

Cattle are routinely transported long distances to feedlots located in the central portion of the United States. We transported stocker heifers to Hays, Kansas and back during periods of high ambient temperature to evaluate the effect of transportation on physiological and behavioral differences compared to non-transported control calves. Calves that were transported had lower nasal and rectal temperatures for 24 hours after transportation and spent more time lying down for 2 days after transportation compared to non-transported control calves. After 48 hours of initiation of transportation procedure, there were no differences in change in body weight, rectal temperature, or time spent at various locations within the pen. Overall, results indicated transportation of calves for 8 hours during high ambient temperatures caused transient changes in physiologic and behavior parameters.

The use of a challenge model allows the researcher to know the exact timing of disease to evaluate physiologic, behavioral, and pathologic changes to provide improvement in case definition for disease. *Mannheimia haemolytica* is one of the primary bacterial pathogens involved with BRD. We endoscopic inoculated 10 black beef heifers with *Mannheimia*

haemolytica and compared behavioral and physiological responses to non-inoculated control calves. *Mannheimia haemolytica* challenged calves had greater rectal temperatures, spent less time at the grain bunk, less time at the hay feeder, and more time lying down compared to control calves. These results provide further information to identify affected calves with BRD during high ambient environmental temperatures.

Stochastic models are able to include variability and uncertainty in a wide range of situations to evaluate outcomes. These stochastic models are able to combine known range of input parameters with range of other parameters for variables where information is lacking to determine outcomes of interest. New modalities are being developed in order to improve diagnosis of BRD through behavior or diagnostic tests, but we evaluated whether it was more economically profitable to improve diagnostic sensitivity or specificity using ranges of outcome from published literature and diagnostic outcomes for BRD. Mortality risk had the greatest impact of net returns. Improvement in specificity resulted in more rapid, positive change in profitability compared to improvement in sensitivity. Improvement in specificity can be made through more specific pen-level diagnostics or confirmatory chute side test. These results are important for determining future research priorities for identification of BRD. Restricting the minimum and maximum distributions to apparent prevalence is a novel approach for evaluating diagnostic tests.

The preceding chapters evaluated multiple different outcomes in a variety of settings. Retrospective database analysis for rectal temperature at first identification of BRD, use of weather patterns on rectal and nasal submucosal temperatures, randomized controlled trials for evaluating effects of transportation and *Mannheimia haemolytica* infection, and stochastic modeling to evaluate the economic value of changing diagnostic test characteristics for BRD diagnosis. Hopefully these results provide some information to validate and improve production practices for the beef cattle industry. More research is needed in these areas to further evaluate methodologies to improve beef production practices, but these results provide objective ways to monitor and evaluate outcomes.

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