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UTILIZING PRECISION TECHNOLOGIES TO VALIDATE A REAL-TIME LOCATION SYSTEM FOR DAIRY CATTLE AND MONITOR CALF BEHAVIORS DURING HEAT STRESS

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UTILIZING PRECISION TECHNOLOGIES TO VALIDATE A REAL-TIME LOCATION SYSTEM FOR DAIRY CATTLE AND MONITOR CALF BEHAVIORS DURING HEAT STRESS

DISSERTATION

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

By

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ABSTRACT OF DISSERTATION

UTILIZING PRECISION TECHNOLOGIES TO VALIDATE A REAL-TIME LOCATION SYSTEM FOR DAIRY CATTLE AND MONITOR CALF BEHAVIORS DURING HEAT STRESS

With the increase in on-farm precision dairy technologies (PDT) utilization, large quantities of information are readily available to producers. A more recently available technology for use in livestock species is the real-time location system. These technologies offer dairy producers the opportunity to monitor and track real-time locations of cows, track locomotion patterns, and summarize specific area usage. However, the usefulness of these insights is heavily dependent on the performance of the technology. Therefore, the first objective of this dissertation was to assess the positioning recording performance and the usefulness of the data recorded of a real-time location system (Smartbow GmbH; Zoetis Services LLC., Parsippany, NJ, USA) for use in freestall-housed dairy cattle on a commercial farm. The first objective evaluated a technology's positioning abilities under static and dynamic conditions. The system was able to accurately determine locations while under both static and dynamic conditions. Furthermore, PDT are also utilized to monitor the behaviors and activity of dairy calves. The second objective of this dissertation was to investigate the effects of heat stress on the behaviors of dairy calves using information gathered by PDT. Information recorded from automated milk feeders and pedometers were used to investigate the effects of an elevated temperature-humidity index on dairy calf behaviors. The changes in behavior recorded suggest that PDT can detect behavioral patterns changes of calves during heat stress.

KEYWORDS: indoor positioning, location accuracy, thermal stress, calf behavior

Emily Rice

07/21/2023

Date

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DEDICATION

To Mom

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This dissertation represents the past several years of my life, though words fall short on describing life’s experiences this is my attempt at acknowledging those who made this moment possible. I would like to express my utmost gratitude to my committee, Dr. Agouridis, Dr. Anderson, Dr. Jackson, and Dr. Vanzant. The support, guidance, and knowledge each of you have provided me throughout my PhD journey has been invaluable. I would also like to acknowledge the funding and data provided by Smartbow that supported my PhD journey.

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will never be able to express how lucky I am to have you as my biggest supporter. I love you and thank you endlessly.

To the rest of my family, your love and support have gotten me through the toughest times of my life, and I cannot thank all of you enough. Jimmy, I can always count on you to be there for me and I am so grateful that I have the best brother in the world. Aunt Lizzy and Aunt Judy, I always look forward to your random letters of encouragement that I get in the mail. I am so lucky to have been blessed with such an amazing family, without you I could not have accomplished this. I love you all so incredibly much, you make the 18-hour drive home worth it.

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Emily Rice

PREFACE

This dissertation was completed under the supervision of Dr. Joao H.C. Costa and co-supervision of Dr. Eric S. Vanzant. My supervisory committee also included: Dr. Joshua J. Jackson, Dr. Les H. Anderson, and Dr. Carmen T. Agouridis. All projects were approved by the University of Kentucky's Animal Care Committee. The research validating the real-time location system in chapters 2 and 3 was under (2018: 3105) and the calf behavior research in chapter 3 was conducted under 2018: 2864.

Chapter 1 is a review of the literature. I conducted all background research and wrote the original content of this chapter. Dr. Costa served as the supervisory editor and content manager for this work. Dr Vanzant and other committee members reviewed the completed chapter.

Chapter 2 describes my original research formatted for submission to the Journal of Dairy Science Communications and titled: Real-time location system for livestock dairy cattle: Validation of static positioning in a commercial facility. This research focused on validating the static positioning performance of a precision dairy technology. All data collection, analyses, writing, and interpretation of this research were conducted by me under the supervision and editing of Dr. Costa. Dr Vanzant and other committee members reviewed the completed chapter.

Chapter 3 discusses my original research formatted for submission to the Journal of Dairy Science Communications and titled: Real-time location system for livestock dairy cattle: Evaluation of dynamic positioning in a commercial facility. This research focused on evaluating the dynamic positioning abilities of a precision dairy technology. All data collection, analyses, writing, and interpretation of this research were conducted by me under the supervision and editing of Dr. Costa. Dr Vanzant and other committee members reviewed the completed chapter.

Chapter 4 consists of my original research formatted for submission to the Journal of Dairy Science and titled: Heat stress effects and dairy calf behavioral patterns: Association of an elevated temperature-humidity index with changes in daily behavioral patterns during the preweaning period. This research focused on behavioral patterns changes in preweaned dairy calves associated with heat stress. All data collection, analyses, writing, and interpretation of this research were conducted by me under the supervision and editing of Dr. Costa. Dr. Melissa Cantor supervised the data collection, curation and analyzes, and reviewed the manuscript. Dr Vanzant and other committee members reviewed the completed chapter.

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1.1 Introduction

The dairy industry is under growing demands for automation and increased efficiency, in the last decades a plethora of technologies have been developed and evolved for utilization in livestock species. Commonly referred to as precision livestock farming (PLF) or specifically precision dairy technologies (PDT), these technologies serve as tools that continuously measure and record behaviors and physiological variables of dairy cattle. There is a great deal of information that can be gathered on-farm via PDT such as milk yield and components, activity and idle time, position, feeding and ruminating behaviors, and real-time locations (Eastwood et al., 2012, Borchers et al., 2016). Implementing PDT on farms can improve efficiency and productivity by providing producers with the resources to make informed and timely management decisions. Additionally, as consumer demands continue to emphasize animal welfare in the livestock sector, the automatic monitoring of animals with PDT offers the opportunity for producers to improve welfare through PDTs early detection of behavioral changes associated with health concerns.

1.2 Precision Dairy Technologies: Current Status and Validations

Precision dairy technologies exist in several forms, such as automated milking systems, automated feeders, or wearables (collars, ear tags, or leg tags) (AlZahal et al., 2009, Borchers et al., 2016, Costa, 2019). For dairy calves, Costa et al, 2021 published a review of PDTs that have been validated for use in monitoring the health, performance and welfare of preweaned dairy calves. The review discussed the utilization of wearable PDTs to track activity levels and play behaviors of calves and suggested that changes in these behaviors can indicate disease or welfare concerns. Additionally, they reported that information obtained from automated milk feeders such as milk or starter intake, drinking speed, or visits to the feeder could alert producers to illness or distress in individual calves. Another comprehensive review of PDTs, produced by Stygar et al. (2021), reported on the wide array of available PDTs that are validated for application in adult dairy cattle. They discussed wearable PDTs that quantify activity with measures such as step count, lying and standing times, and movement indices. Additionally, they reported PDTs that accurately measure feeding behaviors including rumination and eating times, chewing and rumination bouts, and bout durations.

Raw information gathered by PDTs can be processed and interpreted by company-derived algorithms for use in detecting deviations from normal behaviors. Subsequently, alerts can be generated for potential health events such as estrus, calving, or disease (Saint-Dizier and Chastant-Maillard, 2012, Eckelkamp, 2019). These alerts serve as an early warning signal to producers thus improving intervention rates on-farm, supporting increased efficiency. These health insights can further be used to supply management recommendations to farm personnel, reducing the need for skilled laborers with extensive dairy knowledge (Lazarus et al., 1990). To successfully utilize PDTs on-farm, they should be validated for their performance in correctly measuring the parameters or behaviors of interest. Validation studies assess the precision and accuracy of PDTs by comparing the technology-reported data to the gold standard measurement of the same parameter or behavior. Validation studies compare PDTs to visual observations or another validated

PDT with well-defined performance (Schirmann et al., 2009, Gómez et al., 2021, Stygar et al., 2021).

When conducting validation studies on PDT, it is important to understand the type of technology being assessed. One of the most common PDTs for dairy cattle are devices based on tri-axial (3-dimensional; 3D) accelerometers which can be attached to the ear, neck, or leg of the cow (Eckelkamp, 2019). These PDTs record the tilt and the acceleration of the device in relation to the gravitational pull. These data can be translated into movements of the cow and classify specific activity-based behaviors (Schirmann et al., 2009, Burfeind et al., 2011, Pereira et al., 2018). Accelerometer-based PDTs have been validated for their use in quantifying lying time and lying bouts, standing time (Ledgerwood et al., 2010), and ruminating, eating, resting and active behaviors (Bikker et al., 2014) in adult dairy cattle. The HOBO Pendant Data Logger (Onset Computer Corporation, Poasset, MA, USA) was reported to accurately (predictability, sensitivity, and specificity >99%) measure the lying and standing behaviors of dairy cattle when technology-recorded data were compared to data generated by video recordings (Ledgerwood et al., 2010). In addition to activity behaviors, accelerometers have been validated for their ability to accurately classify rumination and feeding behaviors. An assessment of CowManager SensOor (Agis Automatisering, BV Harmelen, the Netherlands) by Bikker et al. (2014) reported very high agreement (Pearson correlation and CCC: 0.90-1.0) for rumination and resting behaviors between the technology and direct visual observations. Schirmann et al. (2009) reported that rumination time measured by Hi-Tag (SCR Engineers Ltd., Netanya, Israel) was highly correlated (Pearson correlation coefficient = 0.96) with visual observations. An ear tag sensor (SmartBow GmbH, Zoetis

Services LLC, Parsippany, NJ, USA) was highly correlated (Pearson correlation coefficient >0.99) with visual observations for measuring rumination times, chewing cycles and number of rumination bouts (Reiter et al., 2018). Additionally, algorithms have been developed that interpret the PDT-recorded behaviors and can detect and alert to underlying conditions (McGowan et al., 2007, Rutten et al., 2015).

One such application for PDT is for the detection of lameness which is a major contributor to poor health and economic loss on dairy farms. Mazrier et al. (2006) investigated a relationship between step activity measured by the AfiTag and clinical lameness. Per example, for the lameness cases identified cows showed a greater decrease in step activity. Another research group using IceTag accelerometers reported that lame cows had increased lying times and number of lying bouts and decreased total number of steps and activity index (Thorup et al., 2015). Using daily activity information obtained from IceQube accelerometers and an automatic milking system, De Mol et al. (2013) developed a detection model for lameness. The model had a high specificity with the inclusion of the following variables: lying time, number of lying bouts, number of steps, and the average length of time for lying and standing bouts. Another method to detect lameness used data from an automatic milking system (AMS), which found a significant association between decreased feeding time and lameness (Miguel-Pacheco et al., 2014).

In addition to automated lameness detection, many PDTs have been validated for estrus and calving detection. Dolecheck et al. (2015) evaluated several PDTs for their performance in estrus detection and reported that cows in estrus had increased activity (Hi-Tag, IceQube, CowManager, Track a Cow), lying times (min/h; IceQube, Track a Cow), and number of lying bouts/h (IceQube) compared to those not in estrus. They also found cows in estrus had decreased rumination times (min/h; Hi-Tag, CowManager) and feeding times (min/h; CowManager) when compared to those not in estrus. Vázquez Diosdado et al. (2015) developed a decision tree algorithm to classify lying, standing, and feeding behaviors recorded from an accelerometer (Omisense Series 500 Cluster Geolocation System, Omnisense Ltd., Elsworth, UK). The researchers suggested the possibility to improve estrus detection by incorporating position information into their algorithm. In addition to validating technologies for use in adult dairy cattle, PDTs have also been evaluated for use in dairy calves and heifers.

Similar to works investigating PDT use in adult dairy cattle, Costa et al. (2021) reviewed the various PDTs that have been validated for use in dairy calves. They reported on three main categories of PDTs available for use in calves: accelerometers, automated feeding systems, and other PDT such as infrared imaging and camera technologies. A leg-attached accelerometer (AfiTag II) was assessed for use in preweaned dairy calves, finding that step activity, lying bouts and lying times measured by the technology were highly correlated with visual observations (Swartz et al., 2016). Bonk et al. (2013) reported the HOBO Data Logger was highly correlated with visual observations for total lying time and lying bout frequency. Burfeind et al. (2011) validated the Hi-Tag for its ability in measuring rumination time and found the PDT was highly correlated for behaviors of postweaned heifers compared to visual observations. Lastly, the IceQube accelerometer (IceRobotics, Edinburgh, Scotland) was evaluated by several groups for activity behaviors finding the PDT was highly correlated for lying and standing times, number of lying bouts, step counts, and activity index with visual or video observations (Trénel et al., 2009, Finney et al., 2018, Gladden et al., 2020).

1.3 Real-time Location Systems

Real-time location systems (RTLS) enable producers to ascertain on-demand positions of their animals in real-time. New to the livestock industry, RTLS have been widely used in human healthcare systems and product manufacturing facilities for their reliable indoor positioning capabilities (Kamel Boulos and Berry, 2012). Sometimes referred to as indoor positioning technologies, RTLS can provide immediate positions, track movements through the barn and summarize area usage. These technologies remove the need for tedious visual observations and assist producers in swiftly finding animals that require attention, improving breeding times, fetching times, and overall efficiency on the farm (Bewley, 2010). A variety of RTLS technologies have been used in the livestock sector, with each having unique advantages and disadvantages.

The most common RTLS technologies used are Wi-Fi, BlueTooth, RFID, and ultra-wideband (UWB). Ultra-wideband systems are favored for their high positioning accuracy and reduced energy consumption, which is attributed to the inherent nature of the technology (Liu et al., 2007). These technologies operate on very large bandwidths of 500 MHz and greater, with frequencies ranging from 3.1 to 10.6 GHz (Karunaratne, 2010). Contrastingly, BlueTooth and Wi-Fi operate on much narrower bandwidths with frequencies of 2.4 or 5.0 GHz (Otis, 2005). Because UWB technologies transmit signals through short impulses across wider bandwidths, the received signal is more easily distinguished from interferences (Yavari and Nickerson, 2014). Oppermann et al. (2004) reported that because of the wider range, the true signal will appear as an exaggerated peak against “noise” in UWB systems when compared to signals sent on a narrower range where the peaks are less apparent. They concluded that these short-pulse signals contribute to improved energy efficiency of the system and support signal transmission through obstructions.

Although RTLS have variable characteristics, the main components of UWB systems remain relatively consistent. There are three primary elements: sensors, receivers, and a server. Sensors are mobile technologies attached to the target or object of interest (Zhang H., 2020). It is typical in livestock systems that these sensors are attached to the legs or ears of animals, with the latter being more common for RTLS (Oppermann et al., 2004). Ultra-wideband sensors transmit information at short-duration pulses (within 0.1 ns) through the environment to receivers or anchors that are fixed at known locations (Alarifi et al., 2016). Ultra-wideband sensors broadcast data only during pulse transmissions which lead to their reduced power consumption and extended battery life (Monica and Bergenti, 2019). Characteristics such as transmission frequency, battery life, and size can vary based on the sensor's application and position determination methods (Michaelsson and Quiroga, 2016). Sensors act as either transponders or transceivers, where both relay signals to receivers. However, sensors performing as transceivers are equipped with more power to receive signals back from contacted anchors (Yavari and Nickerson, 2014).

The position estimation methods employed by RTLS have an impact on their susceptibility to inaccurate measurements and subsequent reporting of incorrect positions of sensors. When determining the 2D position of an object, common methods include: Angle or Arrival (AoA), Time of Arrival (ToA), Time Difference of Arrival (TDoA), and Received Signal Strength Indicator (RSSI) (Giummarra, 2014). Angle of Arrival (AOA) sometimes referred to as Direction of Arrival (DoA) estimates the position of an object using angle measurements from several reference points or receivers in a method known as angulation (Mitilineos et al., 2010). An advantage to this method is that time synchronization across receivers is not required, but the hardware used for angulation is costly (Yavari and Nickerson, 2014). Most UWB technologies utilize time-based position determination methods, which requires synchronized clocks across receivers that are responsible for calculating the time intervals (Oppermann et al., 2004). The Time of Arrival (TOA) method is one of the more accurate methods used in determining object locations indoors. This method uses the exact time a signal is sent from a target, the time the signal arrives at the reference point, and the speed at which the signal travels (Mautz, 2009). With these known times and speed, the distance the object is from the reference point can be determined. The distance calculated can then be used in an equation to yield a circle, using multiple reference points the intersection of the calculated circles will reveal the estimated position of the object (Alarifi et al., 2016). When using the ToA method, it is essential that devices have synchronized clocks which requires more hardware and increases the cost of the technology (Linde, 2006). A method similar to ToA used for positioning with UWB technologies is Time Difference of Arrival (TDoA). This method estimates the object location through calculating the differences in arrival time of transmission signal from the sensor to multiple reference points. A major advantage to this method is that it does not require time synchronization between receivers and transmitting sensor (Liu et al., 2007). Lastly, Received Signal Strength Indicator (RSSI) is another popular method for localization that measures received signal strength to determine sensor location. The sensor transmits a signal to reference points or receivers, which are equipped with technology that measures the strength of the signal received and returns an estimated location of the sensor

(Deak et al., 2012). The utility of a RTLS is dependent on how accurately and reliably the technology can report information to the producer, therefore, it is essential to validate the performance of the technology before releasing it commercially.

1.4 Validation of Real-time Location Systems

Animal management based on PDTs data are successful only when the data are reliable; thus, validating RTLS performance is crucial. Validation studies with RTLS commonly report performance using the term positioning or location accuracy, which is a measure of how close the technology-reported locations are to the true ("gold standards") locations. Additionally, validation studies will oftentimes report the precision of a system, which is a measure of closeness between the technology-reported locations of that measurement in that condition (Kuusniemi and Lachapelle, 2004). Positioning accuracy can be assessed under static and dynamic conditions. Most studies have assessed RTLS under static conditions where the sensors are left undisturbed and are fixed at locations of known coordinates (Kanter, Linde, 2006). However, static testing does not reflect the effects of movement or speed, therefore, dynamic positioning accuracy serves as a more powerful measure for evaluating performance of mobile sensors (Delamare et al., 2020). The following section describes the methodologies and results of other RTLS validation studies.

A common UWB system (Ubisense, Omnisense Series 500 Cluster Geolocation System, Omnisense Ltd., Elsworth, UK) has been assessed for its positioning performance by several research groups. Frondelius et al. (2014) evaluated the system for static and dynamic accuracy in a freestall dairy cow barn, without the presence of cows. For static testing, ten sensors were brought to 120 barn locations with known *x, y* coordinates; remaining undisturbed for one min at each reference point. During this period, a total of 465 technology-reported locations were recorded and then compared to their respective reference point locations to determine accuracy. For dynamic testing, two sensors were moved forwards and backwards along a wooden plank attached to the top of the stalls. Sensors were moved along eight specified lines (3.45 – 3.63 m) resulting in a total of 343 technology-reported locations. The coordinates of the beginning and end of each line were known and used as reference points for dynamic accuracy calculations. Based on the manufacturer's promised accuracy of 30 cm, the research group established a desired accuracy of less than 1.0 m. Under both conditions the system met the accuracy requirements, with mean distance errors of 0.17 m (SD= 0.17 m) and 0.10 m (SD= 0.09 m) for static and dynamic positioning, respectively. Nearly 95% of all technology-reported locations were found within the manufacturers' reported accuracy of 30 cm. This work was followed up by several other groups that evaluated the system's performance when implemented on-farm for commercial use. As an example, Porto et al. (2014) evaluated the system under static and dynamic conditions in a semi-open freestall dairy facility. During both phases sensors transmitted signals at a frequency of 1 Hz and positions were reported by the RTLS as *x*, *y* coordinates. For static testing, a functioning sensor was secured to a barn pillar of known position coordinates which served as the reference point. For dynamic testing, eight cows were fitted with functioning ear tag sensors and data were collected when cows were feeding or lying. Video cameras were installed throughout the barn to capture panoramic top-view images of freestall and feedbunk areas. Data used in the analysis came from two periods of approximately 27 min; the first was characterized by the period where the cow was located at the feedbunk engaging in feeding activity and the second period where the cow was lying in the freestall area of the barn. Each image was processed via software developed using Microsoft Visual C# Express. The software then created a visualization of each tag location and used graphic elements to calculate the position coordinates of the sensor. Accuracy was reported by distance error, which is the absolute distance difference between the true position coordinates and those reported by the RTLS. Results showed that the RTLS had improved accuracy when the sensor was fixed at a location compared to when sensors were attached to moving cows. Static accuracy was reported as mean distance error of 0.11 m (SD= 0.05 m) and dynamic accuracy was reported with a mean distance error of 0.51 m (SD= 0.36 m). The authors reported the RTLS performed with high accuracy because distance error remained within 1.0 m and would be considered small when compared to the dimensions of a cow. Additionally, they suggest that the system performance during dynamic testing is comparable to static testing results of other RTLS technologies.

The Ubisense RTLS was further evaluated by Barker et al. (2018) for use in freestall-housed dairy cattle under both static and dynamic conditions with particular interest in validating the system while cows were performing specific behaviors such as feeding, lying, and standing. For the static experiment, sensors (n= 18) were attached to fixed objects at known reference point locations throughout a freestall barn and remained undisturbed for the 10 min data collection period. This process was repeated two more times, resulting in 4050 technology-reported locations to be compared to the known reference locations. Accuracy was described by a mean distance error of 2.70 m (SE= 0.24 m) and precision was reported with a mean circular error of probability of 1.10 m (SE=

0.08 m). For dynamic testing, sensors were attached to the neck collars of 9 cows (n= 9) that were moving freely within the pen. Trained researchers continuously observed each cow, recording behaviors (standing, feeding, lying) and locations until at least 2 bouts of each behavior were observed. There was great variability in system performance when sensors were attached to the cows. Mean distance error for cows while standing was 2.80 m (SE= 0.56 m) compared to 4.40 m (SE= 0.92 m) and 5.60 m (SE= 0.85 m) for cows feeding and lying, respectively. Precision values were similar to the static testing, where standing cows had a lower mean circular error of probability at 1.90 m (SE= 0.28 m) versus 2.70 m (SE= 0.32 m) and 2.70 m (SE= 0.41 m) for feeding and lying, respectively. These results suggest that the positioning performance of the Ubisense system may be affected by cattle performing certain behaviors.

A similar UWB localization technology (Smartbow GmbH, Zoetis Services LLC, Parsippany, NJ, USA) that tracks individual animal positions and movements showed promising results when evaluated for use in group-housed sows and dairy cattle. Will et al. (2017) evaluated the system in gestational stalls of sows under static conditions and reported moderate accuracy values. Three ear tags were mounted onto a stake and brought to 34 reference locations of known *x, y* coordinates. Stakes remained at each location for 5 min undisturbed and ear tags sent data at a frequency of 1 Hz. Distance errors between technology-reported locations and reference point locations were calculated to report static accuracy. Prior to applying filtering techniques; there was a median distance error of 2.70 m with a minimum value of 1.20 m and maximum value of 5.20 m. They found that 35% of technology-reported data points fell within 2.0 m of the reference point locations. The precision of technology-reported locations prior to filtering was measured, reporting a median distance error of 1.40 m with a minimum value of 1.10 m and maximum value of 2.80 m. Roughly 90% of technology-reported data points were precise within 2.0 m. Upon applying filtering methods, accuracy was improved with a median distance error of 2.0 m with a minimum value of 0.60 m and a maximum value of 4.50 m. They found that 50% of technology-reported data points fell within 2.0 m of the reference point locations. Additionally, precision was improved with filtering methods, reporting a median distance error of 0.40 m.

The same UWB localization technology (Smartbow GmbH, Zoetis Services LLC, Parsippany, NJ, USA) was also assessed by Wolfger et al. (2017) for static and dynamic positioning performance within a dairy barn. The validation took place in a series of 4 steps, with steps 1 and 2 used for static testing and steps 3 and 4 for dynamic. For all testing, ear tag sensors transmitted location data at a frequency of 1 Hz. For step 1, two ear tag sensors were mounted onto a freestanding wooden stake at heights of 76 and 152 cm to represent Holstein dairy cow heights when lying down and standing, respectively. The stake was brought to 30 reference point locations of known *x*, *y* coordinates and remained undisturbed at each location for 3 min. In step 2, one haltered dairy cow equipped with a functioning ear tag sensor was brought to each of the 30 reference point locations. The cow remained at each location for 2 min with minimal movements. For dynamic testing, trained observers utilized laser measurements to determine cow locations while cows were moving freely through the freestall barn. Cows were not observed during milking or when located in holding areas. Laser measurers were used to measure the distance from the cow to reference walls, resulting in *x*, *y* coordinate values to be used as true reference point locations. In step 3, fifteen observers measured the hourly locations of 15 lactating Holstein cows for a period of 48 h. In step 4, one trained observer measured the hourly locations of 20 cows for eight hours per day over a 3 day period.. Accuracy was reported by calculating the root mean square error (RMSE) or distance error between technology-reported locations and true reference locations. For static positioning accuracy, there was a median distance error of 1.50 m (IQR= 0.85 - 2.24 m) for step 1 and a median distance error of 1.30 m (IQR= 1.06 - 3.02 m). For dynamic testing prior to outlier removal, mean distance error was 1.80 m (n= 386; SE= 1.11 m) and 1.22 m (n= 334; SE= 1.32 m) for step 3 and step 4, respectively. After outlier removal, dynamic positioning accuracy was improved with mean distance errors of 1.55 m (n= 367; SE= 1.09) and 1.09 m (n= 322; SE= 1.08 m) for step 3 and step 4, respectively. Across all four validation steps, the system had a mean distance error within 1.22 m and 1.80 m suggesting the RTLS would be sufficient in locating animals indoors, however, as distance errors increase past one cow-length (approximately, 2 m)there may be limitations of what inferences can be made with RTLS technology. Moreover, the system was further evaluated by others to determine its applicability for use in grazing systems.

Another group evaluated the static positioning performance of the UWB localization technology (Smartbow GmbH, Zoetis Services LLC, Parsippany, NJ, USA) system on a pasture-based dairy operation (Byrne et al., 2019). Twenty functioning ear tag sensors were mounted individually to poles (height= 1.50 m). Researchers randomly selected a total of 318 locations across several paddocks that served as reference points. Poles were brought to reference point locations where they remained undisturbed for 10 min. Reference point locations (*x*, *y* coordinates) were determined via GPS system (Leica Geosystems, St. Gallen, Switzerland) that had been validated for use as the gold standard prior to the start of this study. The GPS position data were converted to the same coordinate system as the RTLS technology, so they could be compared on the same coordinate system. Distance error values were then calculated between technology-reported locations and reference point locations; reporting a mean distance error of 0.67 m. Additionally, 99% of data points fell within 4.97 m of true reference point locations and 95% of data points fell within 2.75 m. Authors reported high static positioning accuracy with only 2.8% of data points found to have distance errors greater than 3.0 m. In addition to determining real-time locations, the system has been further validated in detecting locations and measuring specific area usage by individual animals.

The system was assessed by Chapa et al. (2021) for its ability to accurately predict cow locations within distinct zones in the barn. Using proprietary algorithms and farm coordinate information, the system produces a digital map of the barn layout with virtual boundaries associated with certain areas. For this study, the areas of interest were alleys, feedbunks, and cubicles. The system software interpreted location information and classified cow location to be in one of the three defined areas each minute. Cameras (n= 9) were installed throughout the barn such that clear video recordings of all cows and areas of the barn were obtained. Thirty-five dairy cows were randomly selected for video observation over a period of three days. After recordings were obtained, a 1 h period was randomly selected for each cow/d for a total of 105 h of recordings. One trained observer classified cow locations (alley, feedbunk, or cubicle) each minute through visual observation. The cow locations that were classified through visual observations were then compared to their respective technology-reported locations. Using the correlation coefficient there was good (0.80-0.90) to strong (0.90-0.99) agreement between visual observations and technology-reported classifications of locations; with values of 0.82, 0.98, and 0.92 for time spent in alleys, feedbunks, and cubicles, respectively. The group noted that the technology had an overall accuracy of 87.6% in correctly classifying cow locations when located in alleys, feedbunks, or cubicles. Results from this research show the potential of improving the predictive ability of precision technologies through the integration of real-time location data with information collected by other technologies.

The UWB localization technology (Smartbow GmbH, Zoetis Services LLC, Parsippany, NJ, USA) sensor technology is also equipped with a tri-axial accelerometer, which has been validated for its ability to accurately classify feeding behaviors and activities. This capability in addition to RTLS information was investigated by Shane et al. (2016) to determine the probabilities of calves engaging in certain behaviors when located in specific areas. They observed that when calves were located at waterers and feeders, the system was able to estimate drinking and eating behaviors with median probabilities of 54 and 88%, respectively. Several groups have investigated the ability to quantify health or disease events via integration of RTLS information with other animal-based factors. Alterations in the movement patterns or behaviors can alert to health events, earlier detection of these deviations would improve intervention times and overall performance and welfare (Tullo et al., 2016). However, the data provided by the RTLS alone cannot estimate the time spent engaging in certain behaviors. Rather, they provide information that the animal is located within the area where these behaviors are expected to occur. These results suggest the potential for integrating location information with other technology-reported information to better identify animal behaviors.

Information on the specific area usage of livestock can indicate barn design flaws that may impact animal welfare. For example, if dairy cows within a pen environment show avoidance behaviors to a specific area of the barn (i.e., a set of headlocks, an area of freestalls, or a particular waterer) the producer may be able to draw conclusions that something specific to that region of the barn is causing the altered behavior. Cattle may avoid certain areas of the barn that are not well ventilated or lack adequate stall size. Regarding overcrowding and heat stress-related bunching, location tracking can be used to determine occupancy level and gain insight into animal welfare (Fregonesi et al., 2007).

In addition to assessing technology systems for their ability in determining positions of animals, many researchers have investigated the potential of incorporating these location data with other technology-recorded variables to indirectly monitor animal behavior and assess welfare. For example, detecting lameness is of particular interest in both cattle and swine species due to the widespread negative impacts it can have if left untreated. Traulsen et al. (2016) used accelerometer and position data to describe activity patterns of sows affected by lameness. True integration of location information with data from other technologies has not yet been fully accomplished. However, Fogarty et al. (2021) showed promising results when they integrated data from GNSS positioning technology and accelerometers into an algorithm. This algorithm was able to detect up to 91% of lambing events. The information provided by RTLS has the potential to provide powerful insight into the social behaviors of cattle which could further understanding of social hierarchy within herds and improve the ability to track disease transmission. Additionally, location data can be used to assess bunching behaviors seen during heat stress and in the presence of high fly populations (Lefcourt and Schmidtmann, 1989). Producers can use information gathered by RTLS to improve their heat abatement methods and improve fly control practices, which will ultimately improve the welfare and performance of their herd. Thus, more studies should investigate the ability of the system to work in commercial facilities and to detect changes in animal behavioral patterns. One of the major challenges in the dairy industry lately has been heat stress.

1.5 Heat Stress: Mechanisms and Impacts

Heat stress is a major contributor to reduced performance in dairy cattle, impacting milk production, reproductive ability, and welfare (Collier et al., 2017). The thermoneutral zone for dairy cattle is between 0°C and 20°C for ambient temperature (AT) and below 45% for relative humidity (RH). These two measurements are also combined into a temperature-humidity index (THI), which is commonly used to characterize varying levels of heat stress (McDowell, 1972). When conditions remain within thermoneutrality, cattle maintain a stable core body temperature (CBT) and, therefore, do not expend energy on heat dissipation efforts. However, as temperatures rise above 25°C, CBT will increase above homeostatic levels (hyperthermia) stimulating physiological and behavioral changes (Bernabucci et al., 2010).

Common physiological responses to hyperthermia in cattle are altered blood flow, sweating, panting, increased respiration rates, and reduced dry matter intake (Gaughan et al., 2000, Farooq et al., 2010). Elevated ambient temperatures will increase skin surface temperature which disrupts the temperature differential hindering the flow of heat away from the core via conduction (McDowell, 1972). Ruminants overcome this hindrance by increasing blood flow to the skin surface and restricting flow to the core (Kadzere et al., 2002). With blood circulation increasing to the surface, heat can be lost through evaporation. However, if this is not effective the cow will employ other thermoregulatory responses, such as behavioral patterns changes.

Panting is an effective means of reducing body temperature via evaporation through the lungs. The increased respiration rates become most pronounced when ambient temperatures exceed 29°C but this threshold varies based on other environmental factors such as humidity, air movement, and ventilation of the barn (McDowell, 1972). Increasing relative humidity levels inhibits respiratory heat loss because evaporative cooling is dependent on a vapor or pressure gradient. Berman (2006) investigated the impacts of varying levels of AT and RH on respiratory evaporation. At a temperature of 40°C and relative humidity of 15% the evaporative loss of water via the respiratory tract was 33% greater than at a relative humidity of 45%. If increased respiration rates and subsequent hyperventilation continue for a prolonged period, the cow will experience respiratory alkalosis. Respiratory alkalosis causes a reduction in the CO_2 combining capacity of the blood and increases blood pH which increase the susceptibility of the cow to metabolic issues (McDowell, 1972). Another common thermoregulatory response stimulated by elevated core body temperature is appetite suppression.

When core body temperature exceeds that of thermoneutral range, the hypothalamus transmits signals to suppress appetite which, in turn, reduces feed intake (Baile and Della-Fera, 1981). Due to less feed being ingested the heat produced via ruminal fermentation is reduced, further contributing to heat alleviation. Coinciding with feed intake, a decrease in rumination activity will occur as maximum THI exceeds 76 (Soriani et al., 2012). Because of this, there will be a reduction of salivary buffer which puts the cow at risk of developing ruminal acidosis and other metabolic disorders (Baumgard et al., 2014). In addition to reduced feed intake and rumination, other behavioral patterns changes are observed when dairy cows are subjected to heat stress.

In response to heat stress, dairy cows exhibit signs of increased restlessness, increased standing time, decreased lying time and shade-seeking behaviors. Time budget measurements can be useful in assessing behavioral pattern changes associated with heat stress conditions. Cook et al. (2007) created daily time budgets for a group of lactating dairy cows using mean daily times of feeding, lying, and standing behaviors obtained through video analysis. As mean THI values increased from 56.2 to 73.8, mean lying times decreased from 10.9 h/d to 7.9 h/d. Additionally, time spent standing in alleys increased from 2.6 h/d to 4.5 h/d with increasing THI levels. They also reported increased time spent drinking water from 0.3 h/d to 0.5 h/d as THI levels increased. Nordlund et al. (2019) further evaluated the effects of heat stress on the standing and lying behaviors of lactating dairy cow using leg-attached accelerometers (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Bourne, MA, USA) to record behaviors. As daily THI increased from 68.5 to 79.0 cows exhibited reduced daily lying times. Daily number of lying bouts were no different; however, lying bout durations decreased from 49.7 min on the coolest day to 32.8 min on the hottest days. Not only do these behavioral changes reflect decreased cow comfort, but they also suggest decreased production performance and increased risk of lameness.

Although heat stress in adult dairy cows has been widely investigated, research regarding the impacts of heat stress on calves and heifers has been lacking. Several groups have reported that in utero heat stress impacts the growth, health, and behaviors of neonatal calves. Tao et al. (2012) assessed the effects of late gestation in utero heat stress on immune function and growth of calves from birth to weaning. Dams were subjected to heat stress without cooling or provided cooling in the form of sprinklers and fans from approximately 45 d prepartum until calving. Calves from cooled dams had higher mean birth weights when compared to calves from non-cooled dams. No differences were found between groups for body weight gain (birth to weaning) and body weight and withers heights after weaning (3-7 mo of age). However, body weight at weaning was greater for calves from cooled dams when compared to calves from non-cooled dams. Regarding immune function, calves from cooled dams have greater levels of total plasma protein, total serum IgG, and apparent efficiency of absorption (AEA). These results provide evidence that heat stress during late gestation can impact the body weight of calves at birth and weaning, as well as reduced immune function. Other studies (Laporta et al., 2017) presented similar results with calves from cooled dams having higher birth weights than calves of non-cooled dams. During the first week of life, calves from cooled dams had a tendency for greater standing times versus calves from non-cooled dams which was attributed to longer standing bout durations for calves from cooled dams. Monteiro et al. (2016) followed calves from cooled or non-cooled dams subjected to heat stress in utero through their first lactation. They reported a higher morbidity and mortality rate in calves from non-cooled dams where a greater percentage of calves from non-cooled dams left the herd before puberty due to sickness, malformation, or growth retardation when compared to calves from cooled dams. They also found that milk production up to 35 wk in lactation was higher for calves from cooled dams compared to calves from non-cooled dams with no differences in milk components. Skibiel et al. (2017) reported that calves born to cooled dams had a more responsive immune system when compared to calves born to heat stressed dams. Calves from cooled dams had higher lymphocyte counts, which the authors suggest indicates greater cell-mediated immunity. The findings described above and from others provide evidence that heat stress during late gestation resulted in calves with lower birth weights, compromised immune function, and reduced lactation performance. With the understanding that heat stress can impact calves in utero, there is validity in investigating the effects of heat stress on calves during early life.

Several groups evaluated the effects of seasonality and THI on physiological and behavioral patterns changes of calves during preweaning, weaning, or postweaning periods of life. Holstein heifers (6 mo) exhibited significant differences in lying, drinking, and feeding behaviors during summer and winter months (Tripon et al., 2014). Mean daily lying and resting times decreased from winter to summer months. Moreover, calves during summer months had increased mean daily feeding and drinking times with reduced rumination times when compared to calves in winter. Another group reported similar findings when evaluating lying behaviors in Holstein bull calves (7 wk) that were either in shaded or non-shaded hutches during the summer (Kovács et al., 2018a). Calves that were not provided shade structures exhibited decreased average daily lying times and increased lying bout frequency, suggesting more restlessness or posture changes in non-shaded calves. These results were supported by similar findings from Montevecchio et al. (2022) that investigated behavioral changes of male Holstein calves during preweaning under various heat abatement strategies. They found that calves provided shade and fans had increased lying times and fewer lying bouts than calves provided shade with no fans or calves kept in hutches. In summary, when subjected to heat stress during the preweaning period, calves exhibited behavioral changes with decreased lying times and increased mean daily standing times (Tripon et al., 2014, Kamal et al., 2016, Kim et al., 2018).

Young animals are more thermotolerant due to their reduced metabolic heat production and increased surface area to body mass ratio (West, 2003). Heat stress dramatically altered behavioral patterns of mature dairy cows, thus, heat abatement strategies such as shade (Tucker et al., 2008), soakers (Grinter, 2019), sprinklers (Tresoldi et al., 2018), and fans (Anderson et al., 2013) have been shown to be effective in reducing heat stress effects in dairy cattle. Although effective, these strategies focus on alleviating heat stress at the herd level, rather than on an individual basis. Evidence exists that individual animals experience different degrees of heat stress. Factors such as genetics (Aguilar et al., 2009), body size, and milk production (Stone et al., 2017) have all been shown to affect individual animals' responses to heat stress. Therefore, utilizing automation to detect changes associated with heat stress in individual animals would be a useful management tool in combating heat stress which can further be used to apply heat abatement strategies while accounting for individual animal's needs. The information gathered by precision technologies can also be used to determine the effectiveness of the farm's current heat abatement strategies.

1.6 Dissertation Objective

The first objective of this dissertation was to assess the positioning performance of an ear-attached precision technology for use on a commercial US dairy farm. Because the technology can detect cow positions while standing still and in motion, the system was evaluated under both static and dynamic conditions. Static testing was conducted with sensors remaining motionless in a pen absent of cows. Dynamic testing was carried out while sensors were attached to freely moving cows throughout the barn with minimal human interference. To determine the performance of the system, true locations were compared to those reported by the technology system. It is expected that static testing results will be more accurate than those of dynamic testing due to the nature of the technology. When assessed under a controlled environment, we expect less disturbances and therefore signal transmission from the technology is expected to experience fewer obstructions. After the technology has been validated, there is potential to integrate location data with information from other technologies to provide more powerful insight into the health and welfare of dairy cows.

The second objective of this dissertation was to investigate the relationship between heat stress and dairy calf behavior patterns during preweaning. Under elevated temperature-humidity levels or heat stress conditions, dairy calves exhibit behavioral patterns and physiological changes that can lead to impaired health, performance, and welfare. This dissertation focuses on feeding behaviors and activity levels that are recorded by precision dairy technologies, specifically an automated calf feeder and leg-attached accelerometer. The automated feeder monitors average daily milk intake and drinking speed while the pedometer records average daily number of lying bouts, lying time, number of steps, and activity index. A relationship between elevated THI and daily behavioral pattern changes in calves was investigated. My hypothesis is that behavioral pattern changes recorded by technologies will serve as early indicators of heat stress. This information can further be utilized to develop thresholds based on behavioral pattern changes that could alert producers to heat stress conditions and additionally provide insight into their heat abatement strategies. Producers would be able to assess their heat abatement strategies and make decisions regarding the health and welfare of their herd.

CHAPTER 2. REAL-TIME LOCATION SYSTEM FOR DAIRY CATTLE: VALIDATION OF STATIC POSITIONING IN A COMMERCIAL FACILITY

2.1 Introduction

With the emergence of positioning technologies, such as real-time location systems (RTLS), producers are granted remote access to real-time positions and the ability to track movements of individuals or groups of animals. The information provided by RTLS technologies can improve labor efficiency by reducing the amount of time spent searching for specific animals, allowing personnel to reallocate their time to tasks requiring more immediate attention (Frost et al., 1997). Additionally, incorporating localization technologies into the management of livestock production systems offers the possibility of optimizing animal health and welfare. For example, the integration of location and behavioral data was used to describe the activity patterns of sows affected by lameness (Traulsen et al., 2016). A similar data-driven approach was used to compare movement patterns of lame versus non-lame cows within the barn environment (Vázquez Diosdado et al., 2018). While these findings are insightful, the true potential of RTLS technologies is limited by the accuracy and precision of the system and, therefore, assessment of the technology prior to its application on-farm is crucial.

Ultra-wideband systems have become one of the more common RTLS utilized in livestock due to their desirable accuracy and relatively low operating costs. Barker et al. (2018) reported that an UWB system accurately determined locations of sensors at fixed positions with 95% of all technology-reported locations found within 2.00 m of reference point locations. In a study conducted by Huhtala et al. (2007), the performance of a WLAN technology had an average distance error of 1.00 m for static positions. Another RTLS technology showed promising results for use in group-housed sows and dairy cattle. Static positioning accuracy was reported with a median distance error of 2.70 m which was improved to 2.00 m through filtering of the location data (Will et al., 2017), suggesting filtering methods can increase the robustness of positioning technologies. Evaluation of the same system in dairy cattle reported similar results, with mean distance errors between 1.22 m to 1.80 m (Wolfger et al., 2017). These examples indicate that different localization technologies can successfully determine real-time locations of livestock in different facilities.

To maximize the potential of RTLS technologies, the system should be able to reliably determine locations and convey this information to producers in an accurate and precise manner. Thus, the goal of this study was to assess the performance parameters (precision and accuracy) of a commercially available, RTLS (Smartbow, Smartbow GmbH, Weibern, Austria) for use in freestall-housed dairy cattle under static positions on a commercial farm.

2.2 Materials and Methods

This study was conducted in May 2019 on a commercial dairy farm in New York (USA). All animals utilized were approved by the Institutional Animal Care and Use Committee (2018: 3105).

2.2.1 Animals and Housing

This validation was carried out in the sand-bedded freestalls of the two on-site barns (barn 1= 100 m × 33 m; barn 2= 117 m × 32 m; Figure 2.1). Stalls were deep-bedded with sand and groomed twice daily; sand was replaced as deemed necessary by farm personnel.

Concrete alleys were cleared of manure 2-3 times daily, dependent on milking schedule. All animals were grouped in pens based on breed (Holstein or non-Holstein) and stage in lactation. A total of 950 milking dairy cattle housed in the two barns were used. Cows were allowed free access to water and fed twice daily with ad libitum total mixed ration (TMR) formulated following current nutritional recommendations. During data acquisition, cows were in the milking parlor and not present in the pen.

2.2.2 Real-time Location System

The ultra-wideband (UWB) localization system (Smartbow, Smartbow GmbH, Weiburn, Austria) functions through three vital components: ear tag sensors, receivers (wallpoints), and the system server. Installation of system hardware and server software was carried out following the system's user manual. Following manufacturer's instructions, ear tags were placed on the proximal region of the ear at the side which does not contact the moveable portion of the feeding fence. The ear tag (52 mm × 36 mm × 17 mm; 34 g) consists of an internal battery and integrated components used for animal monitoring. Power was supplied by a 3.0 V battery with an approximate 2-yr lifetime. The functioning components of the ear tag sensor, the accelerometer and integrated microchip continuously collect 2-D (x, y coordinates) position and 3-D accelerometer data. The sensors communicate and exchange data with receivers by radio frequency on the 2.4 GHz ISM band. Position data from the ear tag were transmitted at a frequency of 1 Hz (1/s) to receivers located throughout the barns.

Within the barn, receivers were evenly distributed and anchored at fixed locations. The corresponding position information of each receiver was based on the Cartesian coordinate system established during initial installation of the RTLS hardware within the barns. A total of 30 receivers were located throughout the barns with 14 and 16 receivers in barn 1 and barn 2, respectively. Average distance between receivers was 19.1 m which was deemed acceptable because the manufacturer-recommended maximum distance between receivers was 25.0 m. For proper position determination, ear tag data must be received by at least three receivers of close proximity. The receivers exchange the position data between the individual ear tags and the location server system. The RTLS location server was equipped with specific software to analyze the received data using company-derived algorithms. The server uses the Time Difference of Arrival (TDoA) technique to trilaterate the ear tag positions. This method requires that receivers generate and record timestamps when signals are transmitted by ear tags and when they arrive at the receivers. This information is then used by the server to measure the time difference of the received signal between each of the receivers that collected the data. The time differences of signal arrival are then used to calculate the distance the ear tag was from the relevant receivers. The calculated distances are then used as the radius of the circle centered at known x, y coordinates of each respective receiver. The server then calculates the ear tag position from the point at which the circles intersect. The real-time position of the ear tag is reported by the server as x, y coordinates relative to the pre-established coordinate system of the RTLS.

2.2.3 Determination of Static Positions

Prior to the start of the validation, eight Smartbow ear tags were randomly selected and placed in a secure location in the barn for 24 hr. After this time, the system was used to ensure that each of the tags was transmitting location data. Two ear tags were excluded from use in the study because of hardware malfunctions. Researchers constructed three individual, freestanding wooden stakes (2.00 m) by drilling a screw through the bottom of a flat plywood board (0.61 m × 0.61 m) into the stake. Two holes were then drilled into each stake at heights of 0.76 m and 1.52 m from the ground to mimic the heights of a Holstein dairy cow when lying and standing, respectively. Using rubber bands, one ear tag was attached to the stake at 0.76 m height and a second ear tag was attached at 1.52 m height (Figure 2.2). The attachment process was repeated for each of the three stakes, utilizing a total of six ear tags.

One researcher was responsible for using a laser measuring device (Bosch GLM 50C; Robert Bosch LLC, Farmington Hills, MI, USA) to determine barn dimensions, receiver x, y coordinates in relation to a point zero, distance between receivers, and reference point measurements. A self-leveling 90° laser device (Bosch GPL 5; Robert Bosch LLC, Farmington Hills, MI, USA) was used for all measurements to ensure that each measurement was obtained at a 90° angle from the X and Y axes and parallel with the flooring. For barn dimensions and receiver coordinates, the distance from each receiver to the two neighboring receivers was measured. If the receiver was in one of the four barn corners, the distance from the receiver to the neighboring receiver and the distance to the receiver in the opposite corner were measured. Each distance measurement was repeated, and the average of the two measurements was used in data analysis. The known receiver coordinates and barn dimensions were then plotted in AutoCAD to generate a virtual display of the barn layout.

Researchers randomly selected 138 locations throughout the two barns to act as static reference points in this experiment. For each measurement, the same researcher as before was responsible for taking the distance measurements. The laser leveling device was used to measure the distance each wooden stake or reference point was from the X and Y axes of the barn. The self-leveling 90° laser device was used to orient the laser measurer at a 90° angle between the reference point and X or Y axis being measured. Each was measured to a specific fixture or receiver located along the axes that was within the barn dimensions previously measured and of known *x, y* coordinates. Subsequently, the distance each point was from the relative receivers and/or barn wall were plotted in the barn layout generated in AutoCAD. The reference point locations were reported as the *x*, *y* coordinates of the point where the plotted lines intersected. The coordinate data of each reference point were later transposed onto the AutoCAD-generated barn layout (Figure 2.3).

After reference points were measured, researchers moved at least 3.00 m away from stakes. The stakes remained in the reference point locations undisturbed for a total of 10 min. The first three min allowed for system adjustments and were not included in statistical analyses. The final one min was also not included in analyses to remove any disturbance that may occur from premature human interference. The exact time (hh:mm) that each positioning began was recorded. Each reference point had a total of six min of data collection that were divided into individual time points (hh:mm) that were used in the subsequent analyses by comparing the true location data to the technology-reported data of the respective time points.

2.3 Statistical Analysis

Distance error, distance root mean squared (DRMS), and circular error probable (CEP) were used as parameters to validate system accuracy and precision. Distance error was reported as the absolute distance difference between the technology-reported positions and the actual or true reference point positions. This value reflects the magnitude at which the technology-reported positions differ from the true positions. For each data point reported by the technology, distance error was calculated for *x, y* coordinates as well as the total distance error. Equations for the calculations are found below:

$$distance\ error_x = \sqrt{(x_{actual} - x_i)^2} \tag{1}$$

$$distance\ error_y = \sqrt{(y_{actual} - y_i)^2} \tag{2}$$

$$distance\ error_{total} = \sqrt{(x_{actual} - x_i)^2 + (y_{actual} - y_i)^2} \tag{3}$$

Distance root mean squared (eqn. 4) calculates the standard deviation of sample *x, y* coordinates from the true *x, y* coordinates and takes the sum square of the two and ultimately takes the square root of the calculated value (Zelenkov et al., 2008). Equation shown below:

$$DRMS = \sqrt{\frac{\sum_{i=1}^{n}(x_{actual} - x_i)^2}{n} + \frac{\sum_{i=1}^{n}(y_{actual} - y_i)^2}{n}} \tag{4}$$

In the equation above, derived from Maalek and Sadeghpour (2013) n is the number of technology-reported data points for a specific reference point, (x_i and y_i) are the coordinates of the technology-reported point from the *i*th reading, and (x_{actual} and y_{actual}) refer to the true reference point coordinates. The true probability level of this error measure depends on the ratio of standard deviations and therefore, ranges from 65 % to 68% (Kuusniemi and Lachapelle, 2004, Lachapelle, 2004). A variation of this accuracy measure that improves the probability level is twice the distance root mean square (2DRMS) which reports accuracy at a probability level of 95% (Lachapelle, 2004). Both are powerful when evaluating performance of positioning technologies and are even more robust when reported in conjunction with precision parameters.

The circular error probable (CEP) reports the radius of a circle centered at the true reference point within which a specified percentage of technology-reported data points are found (Grisso et al., 2005). This measure assesses the precision of the system. To calculate CEP, distance errors are calculated, and a radial error is assigned to each and sorted, and the magnitude of the radius is determined based on percentile (50 or 95%). This measure explains the likelihood of a sample coordinate being found within a circle based on a predetermined radius. Typically, when obtaining precision measures of GPS or localization technologies, a 50% probability measure is less favorable than a 95% probability. Therefore, a CEP based on a radius that encompasses 95% of data points is often preferred.

Below is the equation for calculating precision for 2-D positioning, sometimes referred to as CEP50. This value is calculated from the standard deviations of the technology-reported data points to the average value of the technology-reported data points.

$$CEP_{50} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{mean})^2}{n} + \frac{\sum_{i=1}^{n}(y_i - y_{mean})^2}{n}} \qquad ((5)$$

In this equation (derived from Maalek and Sadeghpour (2013)), n is the number of technology-reported data points for a specific reference point, (x_i and y_i) are the coordinates of the technology-reported data point from the *i*th reading, and (x_{mean} and y_{mean}) refer to the average value of the technology-reported data points. Low values are favored in this precision calculation.

The statistical software SAS (SAS 9.4, SAS Institute Inc., Cary, NC, USA) was used for extraction of data and calculations of accuracy and precision for performance assessment of the system. Researchers obtained the technology-reported data from the software and directly from the company responsible for the system. The data output included: time stamp (year, month, day and hour, minute, second, and millisecond), farm-assigned animal ID, ear tag (sensor) ID, and *x, y* coordinate information. The data files were imported into SAS and all components of each time stamp were extracted into separate fields. As mentioned previously, each reference point had six time points associated with the data collection process. The extracted time stamp information was further combined into a time point variable (hh:mm). Using the time point variable, technology-reported data were then assigned to their respective reference points resulting in a total of 6,091 data points. Using distance error values, precision and accuracy were first calculated for all data points (n= 6,091) and subsequently averaged by reference point (n= 138). These parameters were further assessed by determining the percentage of technology-reported data points (n= 6,091) that fell within circles of specified radii of 0.25, 0.50, 1.00, 2.50, 5.00, and 10.0 m centered about true reference point locations. The distribution of points around the true reference point location was plotted in SAS using the distance errors of the *x, y* coordinates of the technology-reported data points as the *x, y* coordinates values with the origin (0,0) acting as the true reference point location.

2.4 Results

This research evaluated the performance of a localization technology for use in dairy cattle housed indoors. The study assessed the technology's ability to determine static positions in a freestall barn environment. The technology uses TDoA method for determination of ear tag sensor locations. This system showed promising results for its use in commercial dairy facilities with high accuracy and moderate precision. Of the 138 reference points, there was a total of 6,091 technology-reported data points with an average of 44.14 (SD= 1.43) technology-reported points for each reference point. Technology-reported locations were plotted in AutoCAD for visual representation (Figure 2.4).

2.4.1 Precision

Precision was described by the circular error probable (CEP) calculated from the technology-reported data points. Typically, CEP is reported as the radius of a circle, centered about the mean location for each reference point of which 50% of the technology-reported points are found. For all 6,091 data points, we found a mean precision of 1.04 m with a median of 0.87 m (SD= 0.73 m; Q1= 0.51 m; Q3= 1.40 m). When summarized by reference point, we found a mean precision of 1.99 m with a median of 1.81 m (SD= 0.88 m; Q1= 1.33 m; Q3= 2.61 m). Precision results depicted in Table 2.1.

2.4.2 Accuracy

Accuracy was described by the distance root mean squared (DRMS) and reported in meters. The DRMS is considered as the radius of a circle, centered about a true reference point location or of which 65-68% of the technology-reported points are found. This accuracy parameter is different from the average distance between true and technology-reported points because it is dependent on the probability distribution of the data set. Therefore, when reporting accuracy as DRMS it is important to note that it is more reliability-based because it uses a single value to report the accuracy of the data within a determined probability distribution .

For the 6,091 technology-reported data points, we found a mean accuracy of 1.99 m with a median of 1.74 m (SD= 1.24 m; Q1= 1.07 m; Q3= 2.60 m) within the probability distribution of 65-68%. Within 95% probability distribution, there was a mean accuracy of 3.98 m with a median of 3.48 m (SD= 2.48 m; Q1= 2.15 m; Q3= 5.20 m). Values were determined by DRMS of individual technology-reported location data points compared to their respective true reference point locations. The accuracy of the system, when averaged by reference point, was similar to what was found above with a mean accuracy of 1.99 m and median distance error of 1.81 m (SD= 0.88 m; Q1= 1.33 m; Q3= 2.60 m). Accuracy results shown in Table 2.2. Distance error values for accuracy were not affected by stake, sensor, or the height of the sensors (0.76 m and 1.52 m) on the stakes.

Further assessing distance error of the data, the percentage of technology-reported data points that fell within the specified radii of the true location were calculated for each reference point. Subsequently, these percentages were then averaged across all reference points and overall median percentages of data points falling within the specified radii were reported. Mean percentages of data points found within circles with radii of 0.25, 0.50, 1.00, 2.50, 5.00, 10.0 m were 1.64, 5.96, 22.18, 72.53, 97.14, and 100.0%, respectively (Table 2.3).

The *x, y* coordinate differences that were calculated between the technology-reported coordinates and their respective reference point coordinates were then plotted against the origin (0,0) to visually assess the distribution of points found within the specified radii (Figure 2.5 a-b). The frequency distribution of total technology-reported data points (n= 6,091) by distance error range (m) is depicted in Figure 2.6.

2.5 Discussion

The objective of this research was to determine the performance parameters of a commercially available, ear-attached RTLS technology for use in freestall-housed dairy cattle in a commercial barn. The results show that this technology can estimate the positions of ear tags under static conditions with a mean accuracy of 1.99 m and mean precision of 1.04 m. The distance error values found for accuracy reflect accuracy values reported by others that validated the same RTLS technology. Researchers assessed the system with dairy cattle on a research facility and found median distance errors or 1.30 m and 1.50 m under two static conditions (Wolfger et al., 2017). Additionally, Will et al. (2017) evaluated the system in group-housed sows and reported a median accuracy of less than 3.0 m with 35% of technology-reported data points falling within 2.0 m of the reference points. The decreased accuracy seen when the RTLS is validated in a swine facility compared to a dairy facility is notable and may be attributed to the different facility structures. Swine facilities are made up of a lot of metal pen equipment; which disrupt signal transmissions (Maalek and Sadeghpour, 2013). These disturbances can further lead to the system reporting incorrect locations for the sensors. This system was also assessed on a pasture-based dairy system under static conditions. Researchers observed a mean distance error of 0.67 m with 95% of technology-reported data points falling within 2.75 m of reference points and 99% of points falling within 4.93 m (Byrne et al., 2019). The variation in their results may be explained due to signal loss on open pasture. The results from our research suggest system improvements leading to improved static positioning accuracy in freestall-housed dairy cows. In addition to accuracy, the RTLS technology was also evaluated for precision.

We found a median distance error of less than 1.0 m for the precision of this system; which is below the threshold of 1.0 m reported by shows that the RTLS on this particular dairy facility igher precision when compared to precision values reported by others for the same system. Will et al. (2017) reported a median precision value of 1.40 m which was then improved to 0.40 m when filtering methods were applied to the location data. The results from our experiment also support results from other groups working with similar UWB technologies.

Another common UWB RTLS is commercially advertised as having high accuracy and precision, with 95% of technology-reported data points falling within 2.0 m of reference points and precision within 1.0 m. When evaluated by Barker et al. (2018) under research settings with static positions, mean accuracy was 2.70 m (SE= 0.24 m) and mean precision of 1.10 m (SE= 0.08 m) slightly lower than the advertised values. Other groups (Porto et al., 2014) found higher accuracy values with same RTLS with a mean accuracy of 0.11 m (SD= 0.05 m). Our results suggest our system was somewhat less accurate in comparison to Porto et al. (2014); however, data filtering techniques may have led to the high accuracy seen. Both technologies operate on the same frequency bandwidth and signals were sent at the same ISM band frequencies. Further development should investigate impacts of signal transmission regarding different RTLS technologies and their impacts on system performance.

In addition to UWB positioning technologies, indoor localization technologies using wireless local area networks (WLAN) have seen similar accuracy measures. Huhtala et al. (2007) reported that a WLAN tracking system had a mean accuracy of 1.00 m and 30% of technology-reported data points fell within 0.65 m of reference points and 90%

were within 2.00 m; however, accuracy decreased when sensors were in close proximity to each other. These results suggest that although accuracy may be higher using a WLAN system, there is evidence of interference between sensors that may impact performance. The discrepancies in reported accuracy and precision values between studies may be attributed to different conditions and settings under which the system was evaluated. Additionally, indoor RTLS technologies experience signal disturbances from metal structures that result in reduced positioning accuracy (Maalek and Sadeghpour, 2013). Our study evaluated the technology in sand-bedded freestall barns with metal stall dividers which may have contributed to lower performance values. There is also variation in how researchers define the different levels of accuracy and precision (i.e., poor, moderate, high) and what the desired accuracy is for the RTLS being evaluated. For the RTLS assessed in this research, the manufacturer reports accuracy within 1.2 m to 1.8 m which is very close to the average dimensions of a cow-length (1.2 m to 1.6 m). Based on our results, the accuracy is sufficient to locate dairy cows in a commercial barn environment; however, higher accuracy may be required to make predictions on cow behaviors and social interactions.

Future work investigating the integration of location data with weather data may improve our ability to detect animals experiencing heat stress and improve intervention rates. Thus, animal health, welfare and productivity could benefit from the integration of animal monitoring technologies with highly accurate RTLS technologies.

2.6 Conclusions

The results from this study support the use of this ear-attached RTLS technology to determine locations of dairy cows in an indoor barn environment. With adequate understanding of the information reported by the system, producers can effectively use the RTLS technology to locate individual animals in an efficient and accurate manner. The influence of barn framework (metal structures) and farm conditions on system performance requires further investigation. This software possesses the potential to incorporate herd health data with the information gathered by RTLS technology to further improve management applications.

Table 2.1 Precision Measures for Static Location Validation

Precision measures for static location performance for real time location system for its static positioning performance in determining the real-time locations of ear tag sensors attached to dairy cattle. There were a total of six functioning sensors used on three wooden stakes and 138 random locations. Precision was reported for circular error probable with distance error (m) for all technology-reported data points (n= 6,091) and summarized by reference point (n= 138). Mean technology-reported X and Y coordinates were calculated for each reference point and distance differences were calculated for each technology-reported data point.

Distance Error (m)					
n	Mean	Median	SD	Q1	Q3
6,091	1.04	0.87	0.51	1.51	1.40
138	1.99	1.81	0.88	1.33	2.61

Table 2.2 Accuracy Measures for Static Location Validation
Accuracy measures for static location performance of a ultra-wideband (UWB) localization system (Smartbow, Smartbow GmbH, Weiburn, Austria) for its static positioning performance in determining the real-time locations of ear tag sensors attached to dairy cattle. Reference point locations were measured using a laser measuring device and reported as *x, y* coordinates. The accuracy was reported by distance error (m) within the 95% probability distribution for all data points (n= 6,091) and summarized by reference point (n= 138). This value is the square root of the sum squared distance differences between technology-reported X and Y coordinates and their respective reference point coordinates.

Distance Error (m)					
n	Mean	Median	SD	Q1	Q3
6,091	1.99	1.74	1.24	1.07	2.60
138	3.98	3.48	2.48	2.15	5.20

Table 2.3 Percentage of Points Within Radius

Percentage of points within radius of an ultra-wideband (UWB) localization system (Smartbow, Smartbow GmbH, Weiburn, Austria) for technology-reported data points (n= 6,091) that fall within circles of specified distance. Two technology sensors were mounted onto freestanding, wooden stakes (n= 3) at heights of 0.76 m and 1.52 m; each stake was brought to 46 random locations throughout the barn and remained undisturbed for a total of 10 min. There were a total of six functioning sensors used on three wooden stakes and 138 random locations. Distance error is the square root of the sum squared distance differences between technology-reported X and Y coordinates and their respective reference point coordinates. These values were used to calculate the mean percentages of technology-reported data points that fell within circles of specified radii. Circles were centered about reference point locations (0, 0) and radii were set at 0.25, 0.50, 1.0, 2.5, 5.0, and 10.0 m.

	Actual Distance from Reference Point (m)					
	0.25	0.50	1.0	2.5	5.0	10.0
Data Within Radius (%)	1.64	5.96	22.18	72.53	97.14	100.0

Figure 2.1 Barn Layout of Commercial Dairy Farm
Barn layout of the commercial dairy farm used for this research in assessing the static positioning performance of a precision dairy technology. The two on-site barn dimensions were 100 m × 33 m and 117 m × 32 m for barn 1 (left) and barn 2 (right), respectively. Wallpoints or receivers are denoted as red squares.

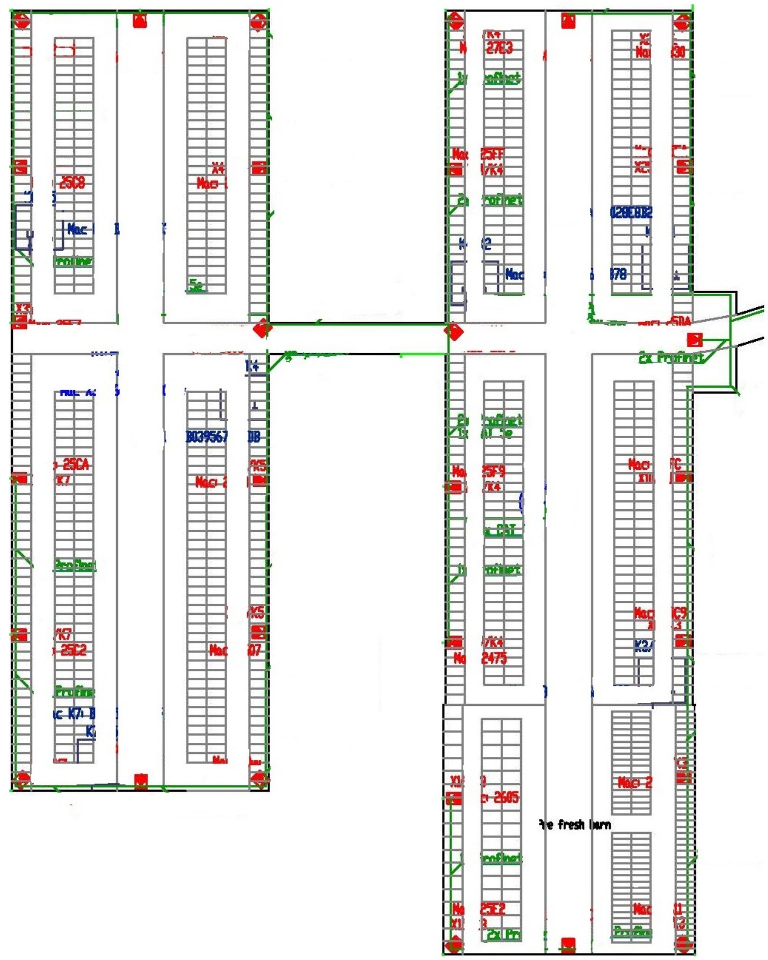


Figure 2.2 Wooden Stake with Two Mounted Sensors
Depiction of one of three wooden stakes constructed. Two sensors were fixed to the stake via rubber bands at heights of 0.76 m and 1.52 m from the ground to mimic the heights of a Holstein dairy cow when lying and standing, respectively.

Figure 2.3 Reference Points Plotted Through AutoCAD
Coordinate data of each reference point (n= 138) were plotted onto AutoCAD-generated barn layout. Red, purple, and blue circles represent one of the three wooden stakes used for the respective reference point.

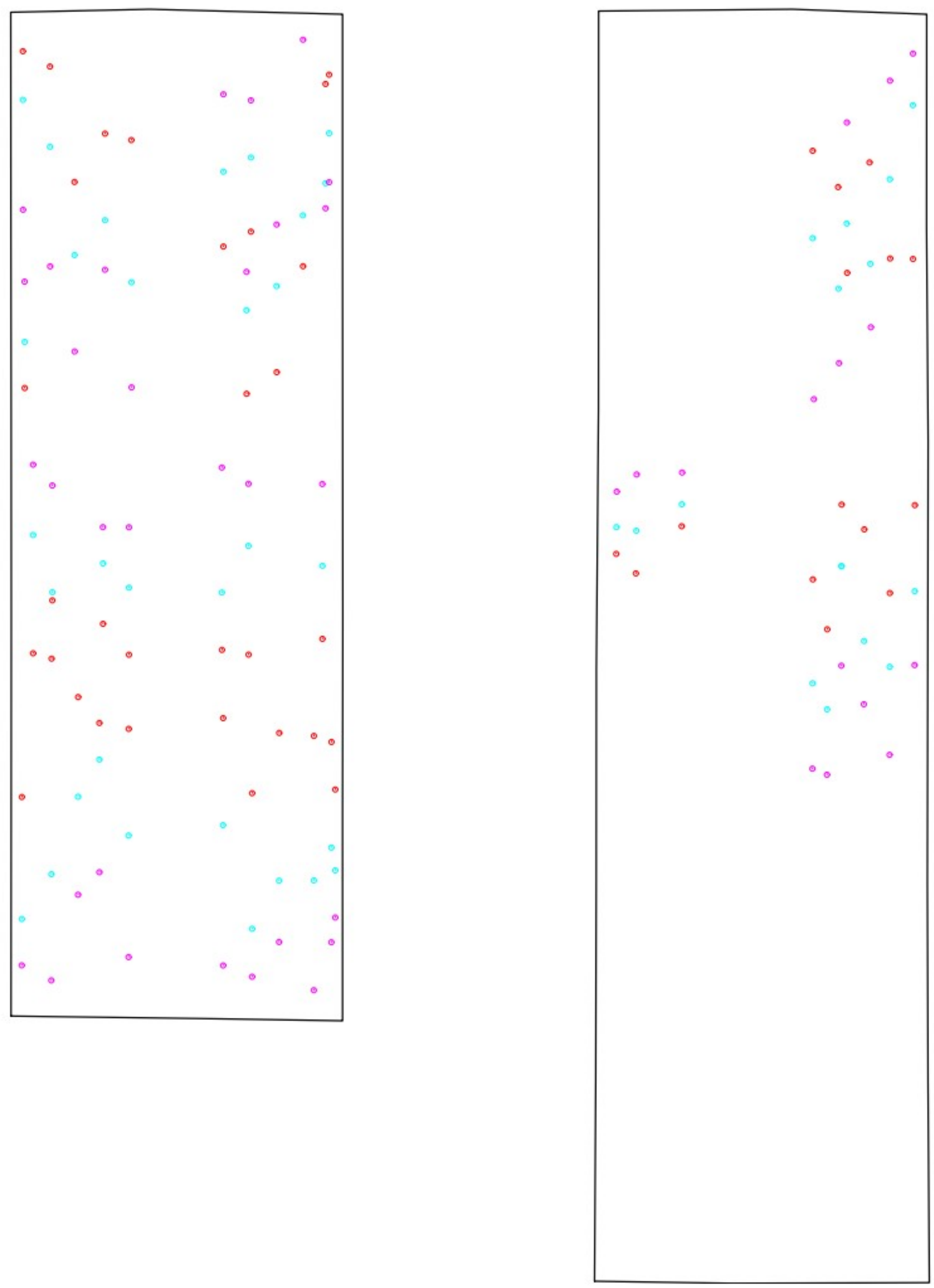


Figure 2.4 Technology-reported Data Points and Reference Points Plotted Through AutoCAD

Technology-reported data points (n= 6,091) were plotted in AutoCAD using their provided *x, y* coordinate values, depicted in green. Reference points (n= 138) are depicted in red, purple, and blue. There were an average of 44.14 (SD= 1.43) technology-reported points for each reference point.

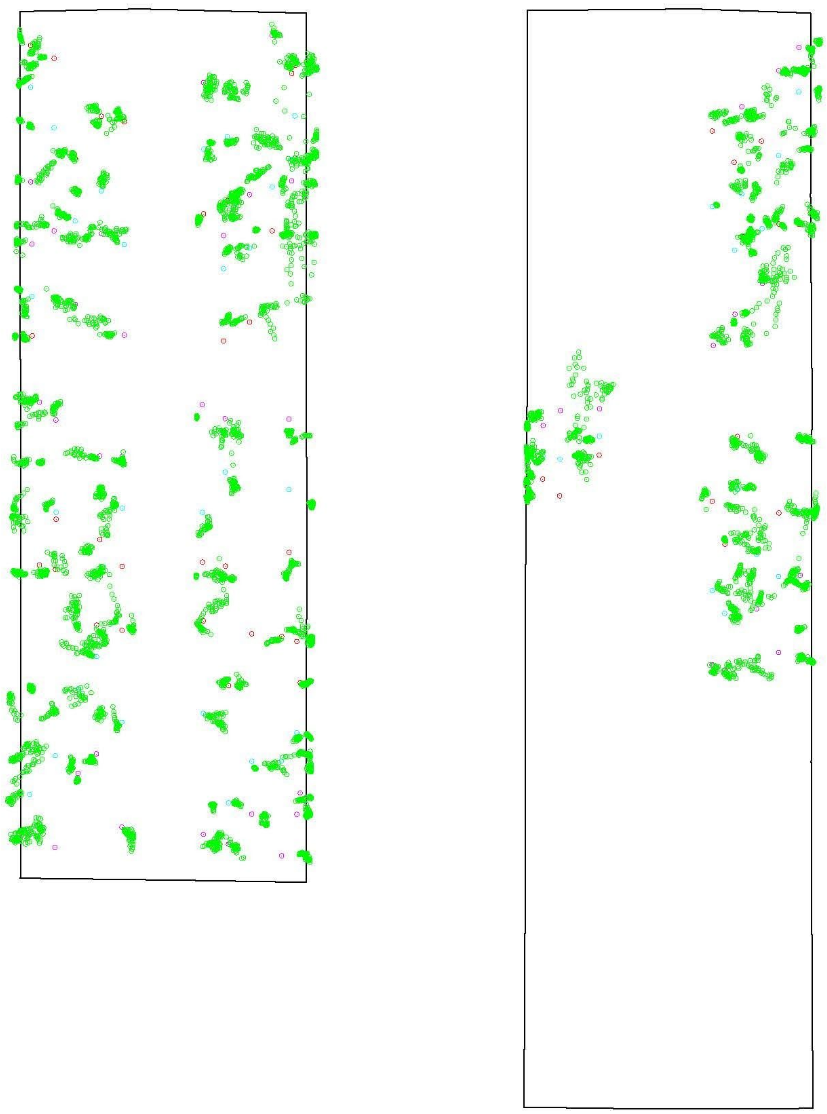
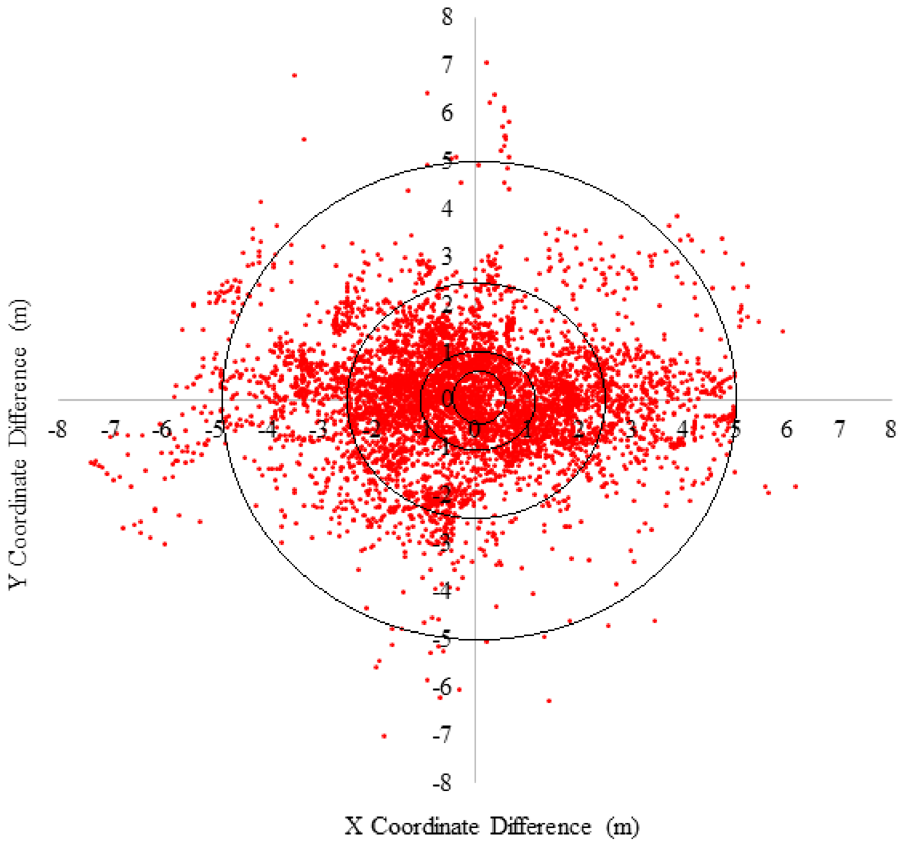


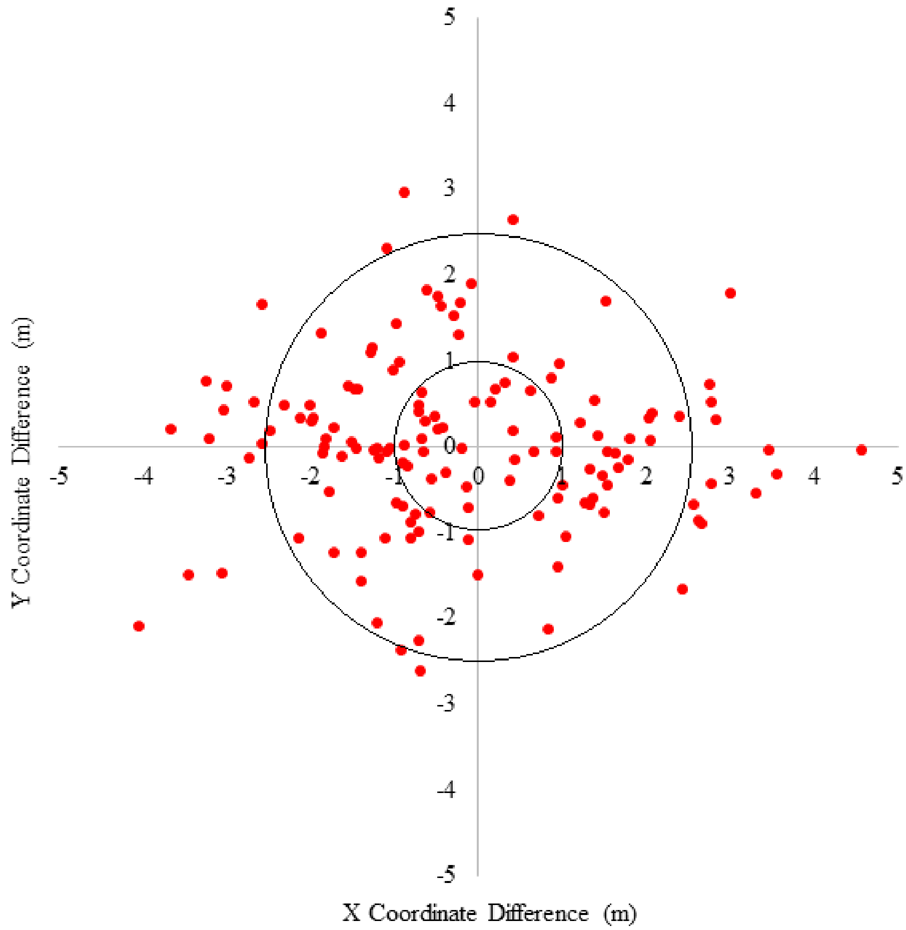
Figure 2.5 a-b Distance Difference Plots for Static Location

Distance difference plots for static location accuracy of an ultra-wideband (UWB) localization system (Smartbow, Smartbow GmbH, Weiburn, Austria) in determining the real-time locations of ear tag sensors. Two technology sensors were mounted onto freestanding, wooden stakes (n= 3) at heights of 0.76 m and 1.52 m. Each stake was brought to 46 random locations throughout the barn and remained undisturbed for a total of 10 min. There were a total of six functioning sensors used on three wooden stakes and 138 random locations. Distance error is the square root of the sum squared distance differences between technology-reported X and Y coordinates and their respective reference point coordinates. Mean distance differences were calculated for X and Y coordinates between technology-reported data points and true reference points. They were calculated for all technology-reported data points (2.5a; n= 6,091) and summarized by reference point (2.5b; n= 138).

a)

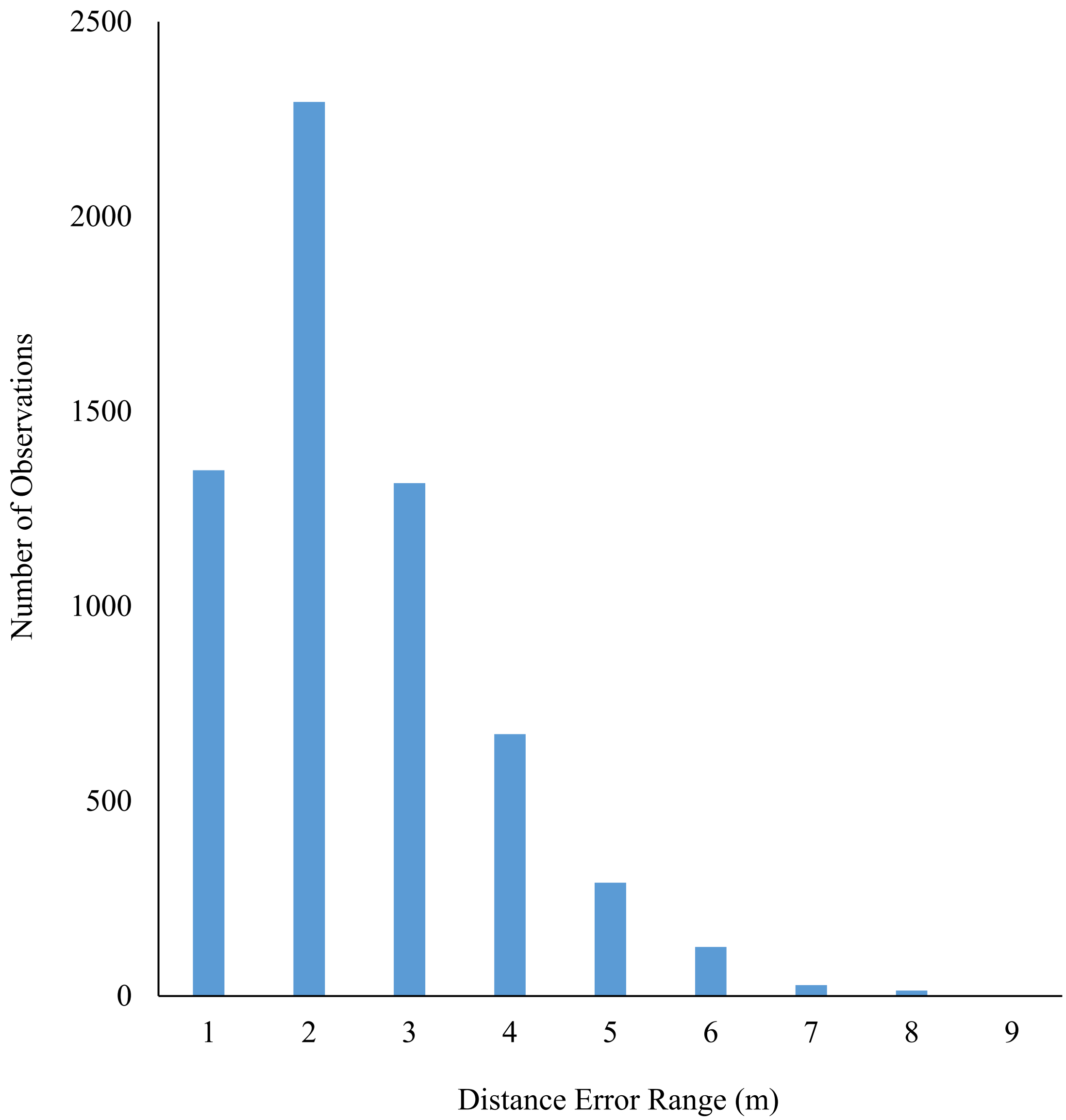


b)



Y Coordinate Difference (m)
X Coordinate Difference (m)
5
4
3
2
1
0
-1
-2
-3
-4
-5
-5
-4
-3
-2
-1
0
1
2
3
4
5

Figure 2.6 Frequency Distribution of Observations by Distance Error Range
Frequency distribution of total observations (n= 6,091) by distance error range (m) from an ultra-wideband (UWB) localization system (Smartbow, Smartbow GmbH, Weiburn, Austria) for assessing its static positioning performance. Two technology sensors were mounted onto freestanding, wooden stakes (n= 3) at heights of 0.76 m and 1.52 m. Each stake was brought to 46 random locations throughout the barn and remained undisturbed for a total of 10 min. There were a total of six functioning sensors used on three wooden stakes and 138 reference point locations. Distance error is the square root of the sum squared distance differences between technology-reported X and Y coordinates and their respective reference point coordinates.



CHAPTER 3. REAL-TIME LOCATION SYSTEM FOR DAIRY CATTLE: EVALUATION OF DYNAMIC POSITIONING IN A COMMERCIAL FACILITY

3.1 Introduction

Real-time location systems (RTLS) present producers with the opportunity to pinpoint the real-time locations, track movement patterns, and obtain area usage summaries of individual dairy cows or the herd. By ascertaining the location of specific animals on-demand, the amount of time spent manually searching for the animal is reduced and thus time and labor efficiency is improved on the farm (Frost et al., 1997). Additionally, information gathered by RTLSs can provide insight into potential health events or concerns with management or barn design. Alterations in movement patterns and area usage within the barn can alert to possible lameness or disease events (Traulsen et al., 2016). Evaluating the performance of RTLSs is essential in establishing the ability of such systems to provide accurate and reliable location information to the producer.

Validating RTLS technologies prior to implementation on-farm is critical. Many positioning systems have been assessed for positioning performance and have reported promising accuracy and precision. In a study conducted by Huhtala et al. (2007), a wireless local-area network (WLAN) technology was able to detect the static positions of sensors within a 1.0 m accuracy, which the authors considered to be acceptable as it was within their desired threshold of 1.0 m. Similarly, Barker et al. (2018) investigated the ability of an ultra-wideband (UWB) technology in determining the static locations of sensors while on a fixed object and while attached to cows while feeding, standing, and lying. They reported the technology, while attached to a fixed object, had an accuracy of 2.7 m and a precision of 1.1 m. However, the technology behaved variably when assessed under dynamic conditions (i.e., while worn by cows participating in certain behaviors). Mean accuracy values were 4.4, 5.6, and 2.8 m for cows while feeding, lying, and standing, respectively. Similar discrepancies between the static and dynamic accuracies of RTLS technologies have been found by other research groups. A similar UWB technology was evaluated for static and dynamic positioning performance for use with dairy cattle. They reported median distance errors of 1.4 m and 1.7 m for static and dynamic accuracies, respectively.

Real-time location systems offer a vast array of potential benefits; however, the efficacy of implementing RTLS technologies is dependent on the performance of the system, specifically how accurate and precise the data are reported to the producer. Accuracies within one cow-length (1.2-1.6 m) would be sufficient in locating cows within the barn or characterize occupancy levels of certain functional areas in the barn. Conversely, higher accuracies (within 1.0 m) would be more suitable to describe cow behaviors or social interactions. To make full use of RTLS's potential, smaller distance errors between technology-reported locations and true locations is desired; accuracies that do not meet the desired level may result in less powerful insights and reduced predictive ability, which subsequently lead to poor labor and time efficiency and erroneous data-reporting (Berckmans, 2006). Therefore, the aim of our research was to evaluate the dynamic positioning capabilities of an ear-attached RTLS (Smartbow, Smartbow GmbH, Weibern, Austria) for use in dairy cattle in a commercial, freestall environment.

3.2 Materials and Methods

This study was conducted on a commercial dairy farm in New York, USA during May 2019. Cows were housed in two on-site freestall barns (barn 1: 100 m × 33 m; barn 2: 117 m × 32 m) that were deep-bedded with sand. For this study, only one barn was utilized. Milking occurred 2-3 times daily, during which alleys were cleaned of manure and stalls were groomed. There were 950 lactating cows of various breeds (Holstein, Jersey, Brown Swiss), grouped in pens based on lactation stage and breed (Holstein or non-Holstein). Twice daily a total mixed ration (TMR) was fed, and clean water was accessible ad libitum. Research was conducted following the Institutional Animal Care and Use Committee approval number 2018-3105.

3.2.1 Real-time Location System

An ultra-wideband (UWB) localization system (Smartbow, Smartbow GmbH, Weiburn, Austria) functions using three main components: sensors (ear tags), receivers (wallpoints), and the system server. Installation of system hardware and software were carried out based on the user's manual directions. Following manufacturer's instructions, ear tags were attached to the animal on the proximal region of the ear. Ear tags (dimensions: 52 mm × 36 mm × 17 mm; weight: 34.0 g) consist of an internal battery with a 2 year lifetime and a 3D accelerometer and integrated microchip. These sensors automatically and continuously collect 2D positions (*x, y* coordinates) and 3D acceleration data. The ear tag sensors relay information to receivers by radio frequency on the 2.4 GHz ISM band. Position data were transmitted to receivers at a frequency of 1 Hz (1/s). Fourteen receivers were evenly distributed and anchored at fixed locations throughout the barn with an average distance between receivers of 19.07 m, which met manufacturer's recommendation of within 25.0 m. Receiver locations (*x, y* coordinates) were determined based on the Cartesian coordinate system established during initial installation of system hardware within the barn. To determine sensor position, ear tag data must be received by at least three receivers nearest the sensor. After obtaining position information from sensors, receivers then exchange these data with the system server that analyzes the data with software and proprietary algorithms. The RTLS server trilaterates the sensor positions using Time Difference of Arrival (TDoA) technique, which requires receivers to generate and record timestamps when signals are transmitted by sensors and when they reach the receivers. The time difference between signal generation and signal arrival is used to calculate the distance each signal traveled from sensor to relevant receivers. These calculated distances are used as the radii of circles centered about the established locations (*x, y* coordinates). For each respective receiver, the ear tag sensor position is reported as the point at which these circles intersect. All sensor positions are reported by the system as *x, y* coordinates in relation to the unique coordinate system established during system installation.

3.2.2 Camera Installation and Measurements

This study sought to assess the dynamic positioning abilities of the RTLS while attached to individual animals (i.e., the system's intended purpose). Due to the possibility of human interference disrupting the cows' normal behaviors, trail cameras (Moultrie M-50i, Moultrie Feeders, LLC, Alabaster, AL, USA) were utilized to capture images of cows at specific locations. This study utilized two adjacent pens in one of the sand-bedded freestall barns on-site. Each pen housed 120 lactating cows in various stages of lactation. One pen was comprised of Holsteins while the second had a variety of Jersey, Brown

Swiss, and cross-bred animals. Eighteen trail cameras were installed on barn fixtures and positioned such that they captured images of cows found in a freestall (n= 10), waterer (n= 4), or feedbunk (n= 4) locations. Specified functional areas in the pens of the barn are shown in Figure 3.1. Using time-lapse mode, 2D images were taken continuously at 1 min intervals for 85 hours. All captured images had a unique identification number and time stamp. At the end of data collection, trail cameras were uninstalled, and SD card data were downloaded onto external hard drives to be organized prior to data analysis.

Reference points were determined at the central point of the image capture range; as this is where the expected location of the sensor would be if the cow was positioned at the specific location. To determine the locations, four active sensors were attached to a board (Figure 3.2) facing down following the company's recommendation. The board was brought to each camera location and placed in the center of the image capture range; which was determined through visually assessing the photographs captured of the board and changing the board's position until it was centered. The board then remained undisturbed at the reference point locations for 10 min. During this time the technoly-reported locations were generated and obtained for future comparison. After the 10 min period, a laser measuring device was used to measure the distance from each reference point to X and Y axes of the barn and assigned corresponding *x, y* coordinates. This process is further detailed in the following section.One researcher was selected to be responsible for conducting the measurements utilizing a laser measuring device (Bosch GLM 50C; Robert Bosch LLC, Farmington Hills, MI, USA) in conjunction with a self-leveling 90° laser device (Bosch GPL 5; Robert Bosch LLC, Farmington Hills, MI, USA). These measurements were doneto determine barn dimensions, receiver positions, and reference point positions; all reported as *x, y* coordinates relative to the farm's unique coordinate system. The leveling device was utilized to ensure all measurements were obtained parallel with the floor and at 90° angle with respect to X and Y axes. All measurements were repeated, and the average was reported. Distance from each receiver to nearest neighbor receivers were measured with laser measuring device and barn dimensions were measured from corner to corner. To visually assess receiver layout within the barn, receiver positions and barn dimensions were plotted in AutoCAD.

3.2.3 Image Inclusion Criteria and Data Cleaning

One trained researcher filtered through all images and compiled the final dataset. Only images that clearly depicted a dairy cow with a visible farm-assigned ID number on the ear tag were retained for analyses. Of these remaining images, the image ID number, farm-assigned cow ID, time, date, and reference point location were recorded. Lastly, observation numbers were assigned by reference point location, date, time (hh:mm), and cow ID number. Examples of trail camera images used in this study are shown in Figures 3.3 and 3.4. Observations that did not have technology-reported location data were removed from the data set, resulting in a total of 13,620 individual observations that served as reference points.

3.3 Statistical Analysis

To assess system performance, accuracy and precision were calculated from distance error values. Distance error, sometimes referred to as absolute error, was used to report the accuracy of the system which reflects the magnitude of distance difference between reference point and technology-reported locations. Distance errors were calculated by taking the square root of the squared distance difference between reference points and technology-reported locations. These values were calculated for x and y coordinate differences and combined values for total distance error. Subsequently, average distance error or distance root mean squared (DRMS) values were calculated for each data point by taking the square root of the averages of the sum squared distance differences between reference point and technology-reported positions (*x, y* coordinates). Equations for distance error and DRMS calculations are found below:

$$distance\ error_x = \sqrt{(x_{actual} - x_i)^2} \tag{1}$$

$$distance\ error_y = \sqrt{(y_{actual} - y_i)^2} \tag{2}$$

$$distance\ error_{total} = \sqrt{(x_{actual} - x_i)^2 + (y_{actual} - y_i)^2} \tag{3}$$

$$DRMS = \sqrt{\frac{\sum_{i=1}^{n}(x_{actual} - x_i)^2}{n} + \frac{\sum_{i=1}^{n}(y_{actual} - y_i)^2}{n}} \tag{4}$$

In the equations above (derived from Maalek and Sadeghpour (2013)), x_{actual} and y_{actual} refer to the x or y coordinates of the reference point and x_i and y_i refer to the x or y coordinates of the *i*th technology-reported position respective to the associated reference point. For DRMS, n refers to the number of technology-reported data points for each reference point. Furthermore, distance error values were used to calculate mean percentages of data points that fell within specified radii of circles centered about reference point locations. Specified radii were 0.25, 0.50, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 30.0, and 35.0 m.

Technology-reported data were obtained directly from the commercial company responsible for the technology. Data output included: time stamp (year, month, day, and hour, minute, second, millisecond), farm-assigned cow ID, technology-assigned ID (ear tag number), and technology-reported x, y coordinate information relative to respective ear tag and time point. Data were imported into the statistical software SAS (SAS 9.4, SAS Institute Inc., Cary, NC, USA) which researchers used to carry out the calculations described above to assess system performance.

3.4 Results

Performance parameters were first calculated for all data points (n= 113,487) then summarized by observation (n= 13,620) and further summarized by camera (n= 18) and barn location (n= 3). Prior to removal of outliers, there were a total of 114,633 technology-reported data points with a median distance error of 2.44 m (SD= 6.73 m; Q1= 1.41 m; Q3= 5.13 m). Using results from distance error calculations, outliers detected at the 99th percentile were removed from the final data set used in analyses. Of the 13,620 reference points, there were a total of 113,487 technology-reported data points with an average of 8.33 (SD= 3.95) technology-reported points for each reference point.

For all data points (n= 113,487), we found an average accuracy of 3.82 m with a median distance error of 2.41 m (SD= 2.78 m; Q1= 1.40 m; Q3= 4.95 m). When averaged by reference point (n= 13,620) we found similar results to those described above with mean distance error of 3.10 m and median distance error of 2.50 m (SD= 2.56 m; Q1= 1.51 m; Q3= 4.13 m).

Furthermore, distance error statistics for all data points were summarized by barn location (feedbunk, waterer, freestall) and accuracy was assessed (Table 3.1). For feedbunk locations (n= 42,427), there was a mean distance error of 3.98 m with a median value of

3.89 m (SD= 2.55 m; Q1= 1.61 m; Q3= 5.79 m). For waterer locations (n= 6,013), there was a mean distance error of 4.95 m with a median value of 3.29 m (SD= 5.39 m; Q1= 1.97 m; Q3= 5.13 m). For freestall locations (n= 65,047), there was a mean distance error of 2.58 m with a median value of 2.08 m (SD= 2.35 m; Q1= 1.25 m; Q3= 3.28 m).

Lastly, the percentage of technology-reported data points (n= 113,487) that fell within circles centered about reference point locations with specified radii of 0.25, 0.50, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 30.0 and 35.0 m were calculated; the resulting mean percentages were 0.83, 3.68, 13.25, 51.82, 75.25, 98.54, 99.16, 99.81, 99.88, 100.0%, respectively (Table 3.2). The frequency distribution of total observations (n= 113,487) by distance error (m) range is illustrated in Figure 3.5.

3.5 Discussion

The aim of this research was to assess the dynamic positioning abilities of a UWB localization system (Smartbow, Smartbow GmbH, Weiburn, Austria) in freestall-housed cattle on a commercial dairy farm. In this trial, the RTLS demonstrated a mean distance error of 3.82 m (SD= 2.78 m; Q1= 1.40 m; Q3= 4.95 m)for estimating the locations of ear tag sensors attached to dairy cows. For simply determining which pen a cow may be located in within the barn these distance errors may suffice; however, better accuracy is needed to determine social interactions or behaviors (Gygax et al., 2007, Ipema et al., 2013). These findings support results found by others that evaluated this RTLS for use in dairy cattle and swine facilities. Byrne et al. (2019) assessed the system in grazing dairy cattle only under static conditions (sensors undisturbed at fixed positions) and found accuracies within 5.0 m; reporting that 95% of data points were found within 2.75 m of true locations and 99%

were found within 4.97 m. Similar results were also found in group-housed sows under static conditions, reporting a median distance error of 2.7 m and 35% of data points within 2.0 m of true locations (Will et al., 2017). The system was also evaluated under static and dynamic conditions with dairy cattle housed indoors (Wolfger et al., 2017). They reported accuracies within 3.0 m for both static and dynamic conditions. Under dynamic conditions, 95% of data points fell within 2.93 m of true locations and a mean distance error of 1.22 m (n= 334; SD= 1.32 m). In comparison, the current research found variable accuracy with a median distance error of 2.41 m (n= 113,487; SD= 2.78 m) and 52% of points falling within 2.5 m of true locations. Additionally, we found that 95% of all technology-reported data points fell within 6.84 m of the true locations. There are limitations when conducting research on commercial facilities; such as, time constraints for data collection period, restrictions on camera installation locations, and disruptions caused by employee handling and movement of animals. Due to these limitations, there was a level of expected disturbances that may have lead to the large distance error values we found.

Another UWB RTLS was evaluated by Ipema et al. (2013) under static conditions on a research facility and found very high (within 1.0 m researcher-established threshold) accuracy with a mean distance error of 0.31 m (SD= 0.25 m). The distances between the receivers in their RTLS were within 10.0 m of one another; while receivers in our study were within 19.0 m of one another. These discrepancies suggest that the proximity of RTLS receivers may play a role in system accuracy., Porto et al. (2014) found similar resultswith mean distance errors within 1.0 m for both static and dynamic accuracy of a UWB system in a freestall dairy cow barn. The same system showed varied results when assessed by others in a freestall barn environment with sensors attached to cows moving freely in the barn (Barker et al., 2018). Results showed higher distance errors (compared to previously mentioned research) for dynamic accuracy with mean values greater than 2.8 m. Research conducted by Huhtala et al. (2007) noted that, in undisturbed conditions, the system's accuracy was within 2.0 m for 90% of data points and when attached to moving animals the distance errors increased to over 3.0 m. Other researchers reported differing results when the UWB technology was assessed for determining location and detecting social behaviors of dairy cattle. Ren et al. (2021) used computer vision techniques in conjunction with the RTLS, resulting with a mean distance error of 0.39 m (SD= 0.62 m) under static conditions. The variable accuracies reported by researchers on livestock positioning systems may be due to the diverse settings and conditions under which they were assessed. However, a common issue encountered with many of these indoor positioning technologies is signal disruption (Oppermann et al., 2004, Yavari, 2015). In livestock systems, these signal disturbances are typically caused by metal barn fixtures and bodies of water (waterers or animal bodies). Some technologies automatically adjust for this "noise" by utilizing specific filtering methods which improve the reported system accuracy (Yavari and Nickerson, 2014).

3.6 Conclusions

This study sought to investigate the applicability of a RTLS for use on dairy cattle in a commercial setting. These results suggest that the RTLS technology can determine the dynamic positions of dairy cows within 4.0 m when moving freely in a barn environment. However, distance errors were higher when cows were located in feedbunk and waterer locations compared to those found in the freestalls. These differences may be due to signal disruption from the metal structures at the feedbunks and metal waterers that would result in miscalculating the positions. Additionally, there were fewer number of observations from waterer and feedbunk locations, leading to a smaller set of data to make inferences from. Our findings suggest that this RTLS technology may be useful in locating individual cows in pens; however, improved accuracy would be needed to assess cow time-budgets, social networks, and behaviors. Further development of this technology through incorporating location data with accelerometer and weather data offers the potential to provide producers with more robust health alerts and insight into behaviors during times of environmental stress.

Table 3.1 Distance Error Values by Location in Barn for Dynamic Validation
Distance error (m) values by barn location for a precision dairy technology for assessing its dynamic positioning performance in determining real-time locations of ear tag sensors. Distance error is calculated from the square root of the sum squared distance differences between technology-reported X and Y coordinates and their respective reference point coordinates. These calculations were performed on data after removal of outliers at the 99th percentile, reported for feedbunk (n= 42,427), freestall (n= 65,047), and waterer (n= 6,013) locations within the barn.

	Distance Error (m) by Location in Barn				
	Mean	Median	SD	Q1	Q3
Feedbunk (n=42,427)	3.98	3.89	2.55	1.61	5.79
Freestall (n=65,047)	2.58	2.08	2.35	1.25	3.28
Waterer (n=6,013)	4.95	3.29	5.39	1.97	5.13

Table 3.2 Percentage of Points Within Radius
The percentage of technology-reported data points (n= 113,487) that fell within circles of specified radii were measured for a precision dairy technology to assess its dynamic positioning performance in determining real-time locations. Distance differences (m) between technology-reported X and Y coordinates and their respective reference point coordinates were calculated for each data point. These values were then used to calculate the percentages of technology-reported data points that fell within circles of specified radii. Circles were centered about the true reference point locations (0, 0) and specified radii were 0.25, 0.50, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, and 30.0 m.

	Distance from Reference Point (m)								
	0.25	0.50	1.0	2.5	5.0	10.0	15.0	25.0	30.0
Data Within Radius (%)	0.83	3.68	13.25	51.82	75.25	98.54	99.16	99.81	99.88

Figure 3.1 Research Barn and Specified Functional Pen Areas

Floor plan and layout of the research barn (dimensions: 100 m × 33 m) used in this dynamic validation. The three functional areas of the pens where cameras were installed are shaded. Feedbunks are shaded in blue, waterers are shaded in green, and freestalls are shaded in pink.

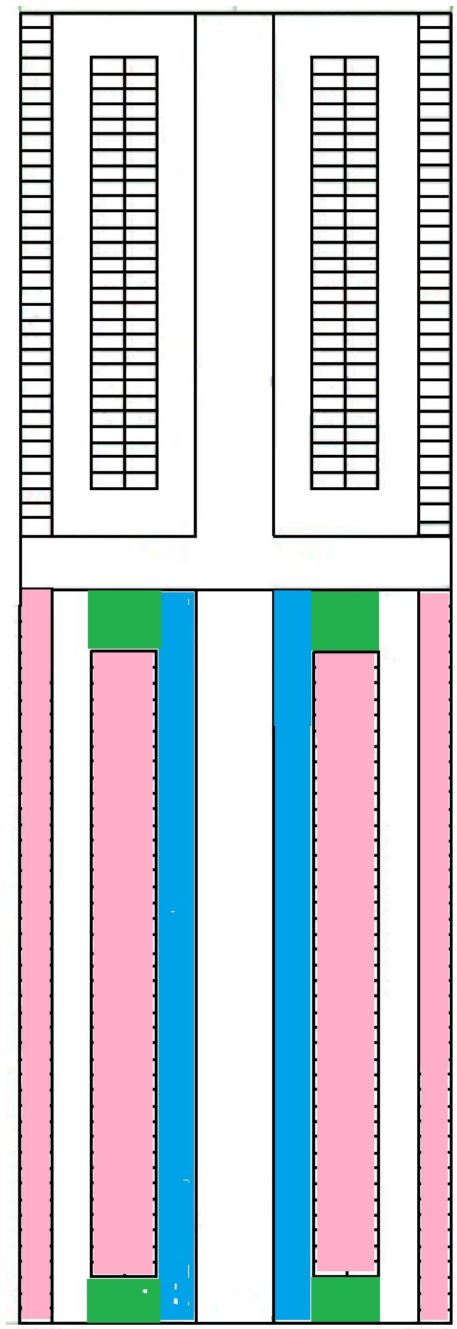
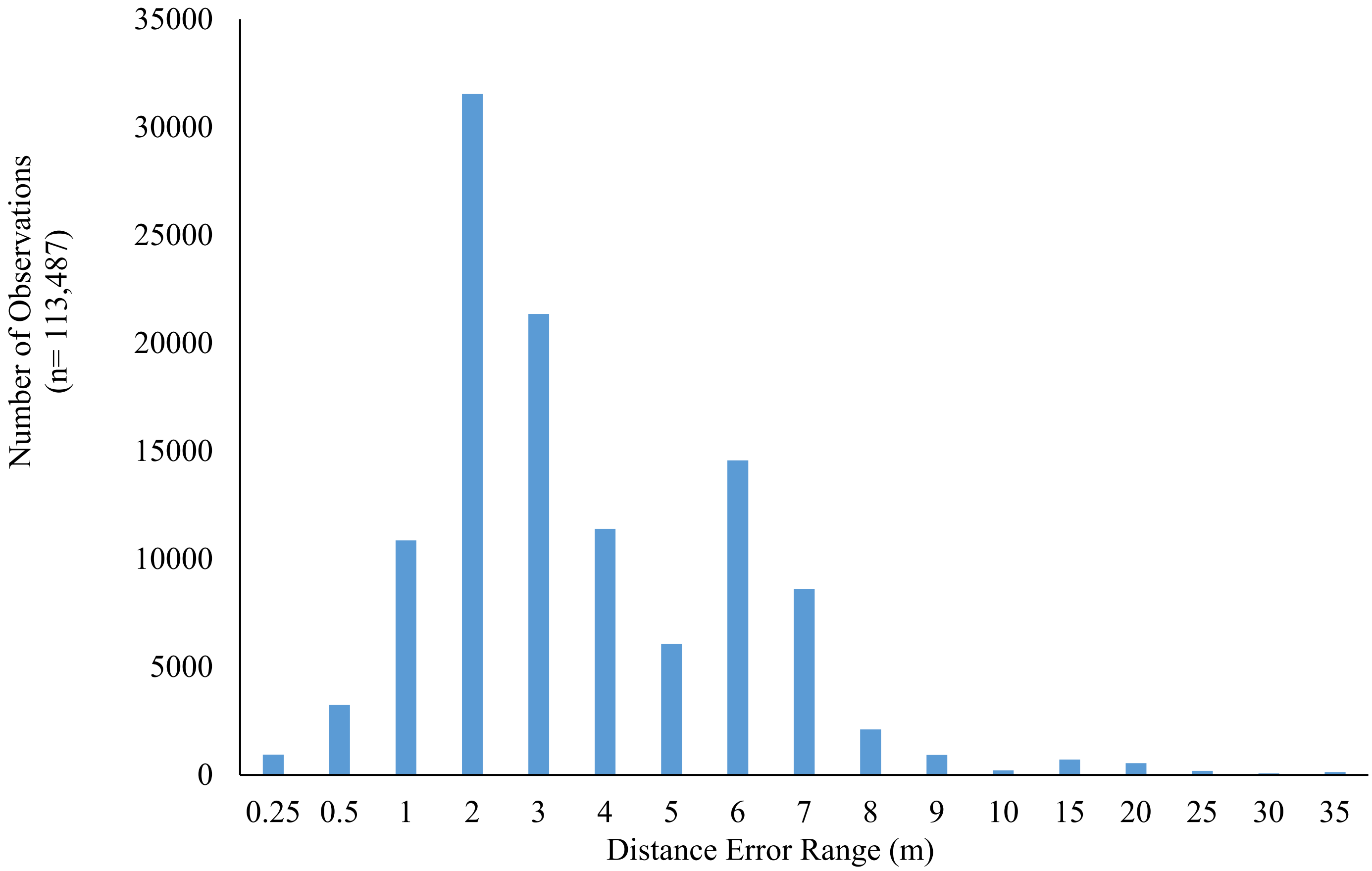


Figure 3.2 Board Used in Reference Point Location Determinations
Trail camera image of the board with attached active technology sensors lplaced within one of the freestall locations. The board was brought to all camera locations and placed in the center of the image capture range which would serve at the reference point locations. The *x*, *y* coordinates of each reference point was determined by measuring the distance from reference point to the X and Y axes of the barn with a laser measuring device.

Figure 3.3 Trail Camera Photograph of Cow in Freestall
This image depicts one cow located in the freestall with a visible farm-assigned identification tag and its respective number. The farm-assigned ID was associated with an active technology sensor and was included in the final data set.

Figure 3.4 Trail Camera Photograph of Cows Located at Feedbunk
This image depicts two cows located at the feedbunk, captured by the trail camera. Only one cow had a visible farm-assigned identification tag and its respective number. The farm-assigned ID was associated with an active technology sensor and was included in the final data set. The cow without a clear farm-assigned ID tag was not included in the data set.

Figure 3.5 Frequency Distribution of Observations by Distance Error Range
The frequency distribution of total observations (n= 113,487) by distance error range (m) from the assessment of the dynamic positioning performance of a precision dairy technology in determining real-time locations. Distance error (m) was calculated from the square root of the sum squared distance differences between technology-reported X and Y coordinates and their respective reference point coordinates.



CHAPTER 4. HEAT STRESS EFFECTS AND DAIRY CALF BEHAVIOR: ASSOCIATION OF AN ELEVATED TEMPERATURE-HUMIDITY INDEX WITH CHANGES IN DAILY BEHAVIORAL PATTERNS DURING THE PREWEANING PERIOD

4.1 Introduction

Heat stress causes a plethora of negative effects on dairy cattle development, performance, and welfare and is an economic loss for producers (Collier et al., 2017). The major environmental factors contributing to heat stress are ambient temperature and relative humidity, which are commonly combined into a temperature-humidity index (THI) (Bernabucci et al., 2010). Under elevated THI or when temperatures exceed the thermoneutral zone (TNZ), dairy cattle exhibit behavioral and physiological changes to maintain core body temperature (CBT) (McDowell, 1972). It is widely accepted that THI of 72 or greater is the threshold of heat stress for mature dairy cattle (West, 2003). Mature dairy cows under heat stress showed reduced dry matter intake (DMI) and decreased milk production (Collier et al., 2006, Garcia et al., 2015). Additionally, when subjected to heat stress, mature dairy cows have shown reduced reproductive performance and impaired fetal development (Dahl et al., 2016, Menta et al., 2022). Evidence also shows that maternal heat stress affects neonatal calves in utero and their performance post-parturition (Tao et al., 2012). Calves from heat-stressed dams had reduced birth weights and compromised immunity compared to calves from cooled dams. Additionally, Dahl et al. (2016) reported impaired reproductive ability and reduced milk yield in the first lactation of calves from heat-stressed dams versus cooled dams. These findings support the need for further investigation into the impacts of heat stress on dairy calves.

Raising high quality replacement heifers not only has economic costs that affect profitability, but also has impacts on the herd's future productivity (St-Pierre et al., 2003). The preweaning period is a pivotal moment in a dairy calf's lifetime that supports growth and development, preparing them for a successful future (Drackley, 2005). Milk and feed intake during this period is essential in maximizing growth and controlling feed costs. Additionally, optimizing immune function is important in managing disease susceptibility in calves (Brown-Brandl et al., 2005). Environmental conditions, such as heat stress, during this time play an important role of growing calves during this time and may have prolonged effects that last into maturity.

Although environmental thresholds for defining heat stress have been heavily investigated in mature cows, there are no clearly defined thresholds which indicate heat stress in calves. For mature cows, many groups define heat stress at THI ≥ 72 (West, 2003, Allen et al., 2015). Heat stress conditions for dairy calves have been described by upper critical temperature (UCT) of the TNZ ranging from 26 - 32°C (Neuwirth et al., 1979). Several groups have sought to develop a THI threshold for heat stress studies with dairy calves; however, no set threshold has been accepted thus far (Peña et al., 2016). Preweaned calves (3 – 4 wk) first showed responses (increased heart rate and skin temperature) to heat stress at ambient temperatures > 32.2°C with 60% RH (Neuwirth et al., 1979). Collier et al. (2018) reported an upper critical temperature for calves up to 200 kg at 26 °C whereby calves expressed increased respiration rates and elevated rectal temperatures. Kovács et al. (2018b) investigated heat stress in dairy calves using ambient temperature and THI to characterize heat stress. They reported that the physiological changes (respiration rate, heart rate, and rectal and skin temperatures) associated with heat stress were more highly correlated with ambient temperature than THI.

In addition to establishing environmental indicators of heat stress in dairy calves, there has been growing interest in investigating physiological (respiration rate, heart rate, rectal temperature) and behavioral patterns changes that could effectively detect onset of heat stress. Several groups reported that respiration rate and rectal temperature were strong indicators of heat stress in calves or steers subjected to heat stress in either shaded or non-shaded environments (Brown-Brandl et al., 2005, Kovács et al., 2018b). Under elevated THI (74.22 and 87.72) calves had increased heart rates and rectal temperatures versus calves below a THI of 70.01 (Kim et al., 2018). These researchers also observed that as THI increased, there was an increase in serum cortisol levels. Behavioral responses to heat stress that have been investigated are feeding behaviors, activity levels, and standing and lying behaviors. Dado-Senn et al. (2022) investigated the behavioral responses of preweaned dairy calves exposed to heat stress in utero, postnatal, or both conditions. Calves born to heat-stressed dams had increased standing times compared to calves from cooled dams. Calves subjected to postnatal heat stress had decreased milk intake versus calves that were not heat stressed. Seasonal effects were also observed; calves born in the summer had decreased average daily gain (ADG) and lower starter DMI when compared with calves born in winter (Place et al., 1998, Rauba et al., 2019). Broucek et al. (2009) reported decreased starter DMI for calves raised under high ambient temperatures (greater than 26.5°C) and elevated THI (greater than 74.8) when compared with calves under moderate ambient temperatures (15.7°C and 19.5°C) and moderate THI (59.7 and 65.2). Calves (4 wk) provided shade structures had increased daily lying time and increased frequency of lying bouts compared to calves who did not have access to shade (Kovács et al., 2018a). Kim et al. (2018) observed that calves subjected to elevated THI (74.22 and 87.72) had increased standing times and decreased lying times when compared to calves at THI of 70.01. These findings show that there are environmental, physiological, and behavioral measures that can prove useful indicators of heat stress in dairy calves.

Recording and observing the aforementioned variables could prove labor intensive. Using precision dairy technologies (PDT), dairy producers could monitor for heat stress-related measures in their calves. Environmental data could be obtained on a large-scale by utilizing local meteorological station data or more precise barn conditions could be measured (Shock et al., 2016). There are a variety of PDTs that have been validated for behavioral monitoring of both mature dairy cattle and calves. For preweaned dairy calves, the most widely used PDTs are automated calf feeders and accelerometers (Costa et al., 2021). Automated feeders are PDTs that provide milk and starter grain to dairy calves, and in doing so they continuously monitor and record the feeding behaviors of individuals. Automated milk feeders (AMF) track milk intake, drinking speed, and number of visits (rewarded and unrewarded) to the feeder. While automated starter feeders can record starter grain intake, eating rate, and number of visits (rewarded and unrewarded). These AMF can detect variations in a calf's normal feeding behaviors such as decreased milk intake, slower drinking speed, or decreased unrewarded visits that have been found in calves prior to onset of illness (Sutherland et al., 2018, Cantor and Costa, 2022). In addition to AMF, accelerometers are another PDT that has been validated for behavior monitoring in dairy calves (Bonk et al., 2013, Finney et al., 2018, Costa et al., 2021). Accelerometers (tri-axial accelerometers) are a PDT that rely on gravitational pull and tilt while attached to the calf to determine rate of acceleration. This information is then used to determine behaviors such as activity level, step counts, and lying and standing behaviors (Bonk et al., 2013, Finney et al., 2018). Precision dairy technologies are non-invasive tools that would provide insight into calf health and behavioral patterns without subjecting them to further stressors associated with handling. Therefore, the aim of this retrospective cohort study was to investigate the association of heat stress with precision dairy technology (AMS and pedometers) recorded feeding behaviors and activity levels of preweaned dairy calves.

4.2 Materials and Methods

This study was conducted from June 2018 to May 2019 at the University of Kentucky Coldstream Research Dairy Farm located in Lexington, KY, USA. A total of 96 Holstein dairy calves were enrolled on this trial. All calves enrolled were part of the Institutional Animal Care and Use Committee approval number 2018: 2864. This research was conducted following the quality standards of Strengthening the Reporting of Observational Studies in Epidemiology Veterinary Guidelines (Sargeant et al., 2016).

4.2.1 Management and Feeding

The methods are described by Cantor et al., 2021, briefly calves were removed from dam ≤ 6 h after parturition and placed in individual sawdust-bedded pens (3.0 × 3.0 m). Upon removal, calf birth weights (41.45 ± 6.55 kg) were taken utilizing an electronic scale (Brecknell PS1000, Avery Weigh-Tronix LLC, Fairmont, MN, USA) and calves were fed maternal colostrum. All calves were fitted with an RFID tag attached to the left ear such that the automated milk feeder (AMF; CF1000, Förster-Technik, Engen, Germany) could identify each upon entrance to the feeder. Pedometers (IceQube, IceRobotics, Edinburgh, Scotland) were also secured on the rear left leg with a Velcro band. At 48 h of life calves were tested for passive transfer (serum BRIX of 8.0%), all met transfer threshold. Calves were individually housed and bottle fed 6 L/d of 840.0 g milk replacer (MR; Cow's Match; Land O'Lakes Animal Milk Products Co., Shoreview, MN, USA) divided into two feedings. When calves presented signs of strong vigor and suckle reflex, they were moved into a group pen (4.57 × 10.67 m) with 6 ± 3 other calves present. Each pen housed an AMF, an automated calf starter feeder (Compact Smart, Förster-Technik, Engen, Germany), automated waterer, and hay trough. The automated starter feeder provided ad libitum pelleted calf starter (22% CP (DM); Special Calf Starter and Grower, Baghdad Feeds, Shelby, KY, USA). Chopped alfalfa hay was offered ad libitum in the hay trough (1.83 × 0.33 × 0.16 m). Automated milk and starter feeders were calibrated weekly following manufacturer's instructions. Group pens were sawdust-bedded and complete cleaning and sanitation occurred every two weeks. During cleaning processes, all calves in pen group were moved to adjacent pen with identical dimensions and conditions. Calves remained with their pen group throughout the study to ensure all animals were of similar body weights, body dimensions, and age. Calves were offered up to 10 L/d of MR (140 g/L) at the AMF for 50 d on feeder until undergoing step-down, gradual weaning. At 51 d MR allotment was reduced by 50% for 14 d and then reduced to 20% for 7 d until weaning was completed at 70 d.

4.2.2 Health Exams

Health assessments were completed once daily (0830 h) from birth until 14 d post-weaning for all calves, further described on (Cantor et al., 2021). Briefly, BRD assessment, researchers followed the UW Calf Health Chart by assigning scores for nasal discharge, eye discharge, ear tilt, cough, and body temperature and calculating the sum of all 5 measures. Diarrhea was assessed by scoring the consistency of the fecal sample based on the following: score 1 (soft and does not hold form, piles and slightly spread), score 2 (runny and readily spreads), or score 3 (watery, liquid consistency that splatters). One of two trained researchers (inter-observer agreement $\kappa = 0.90$) evaluated calf lung consolidation twice weekly using a portable linear rectal ultrasound (Ibex Pro, E.I. Medical, Loveland, CO, USA) with a 70% isopropyl alcohol transducing agent. Ultrasound settings were as follows: 9.0 cm depth, 6.2 MHs frequency, and gain of 23 dB (near 13 dB; far 36 dB). Lobar consolidation was considered if any lung lobe was greater than or equal to 3.0 cm^2. Based on methods described by Buczinski et al. (2015), a BRD bout was characterized by a BRD score ≥ 5 and lobar consolidation in at least one lobe ≥ 3.0 cm^2.

If calves met criteria for a BRD bout, they were labeled as "sick positive" on the day of BRD diagnosis and on days following diagnosis until BRD signs and lung consolidation resolved. Following BRD diagnosis, calves were treated according to herd veterinarian protocols with subcutaneous administration of enroflaxacin (Baytril, Bayer, Leverkusen, Germany) at a dosage calculated by BW (100 mg/15 kg BW). Fifteen days after BRD diagnosis calves were treated with 2.5 mg tulathromycin per kg BW (Draxxin, Zoetis Animal Health, Parsippany, NJ, USA) if BRD symptoms had not resolved.

4.2.3 Precision Dairy Technologies

To monitor activity behaviors, calves were equipped with leg-attached tri-axial accelerometers (IceQube, IceRobotics, Edinburgh, Scotland). These sensors automatically measured movements or accelerations at a frequency of 4 Hz and using company-derived algorithms translate these data into 15 min intervals of movement behaviors and posture information. Data files obtained for this research included daily summaries of lying time, number of lying bouts, and number of steps for each individual calf. Furthermore, using proprietary algorithms the IceQube software calculated daily activity index scores as a function of average daily activity duration and daily total number of steps (Gladden et al., 2020).

Feeding behaviors and intakes were recorded by automated milk and starter feeders which were both equipped with software (KalbManagerWIN, Förster-Technik, Engen, Germany) that analyzed the data to report the following measures: daily number of rewarded and unrewarded visits, daily MR and starter intakes, and milk drinking speed (L/min). For this explorative analysis we focused on milk intake and drinking speeds.

4.2.4 Environmental Conditions

Environmental conditions were recorded at 15 min intervals by wireless data loggers (HOBO Pro V2, Onset Computer Corp., Bourne, MA, USA) placed within the calf barn. Ambient temperature and relative humidity were averaged by hour then averaged by day and assigned by date to a specified season: winter (Dec - Feb), spring (Mar - May), summer (Jun - Aug), or fall (Sep - Nov). Because this research focused on elevated temperature-humidity indices, data associated with the winter months were not included in the analyses. Daily THI and seasonal THI were calculated using the equation: temperature-humidity index (THI) = $(1.8 \times T°C + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T°C - 26)$ (NOAA, 1976), where T°C is the ambient temperature (°C) and RH is relative humidity. Environmental conditions by season are reported in Table 4.1 and environmental conditions by heat stress group are found in Table 4.2. A heat stress bout was defined at

THI ≥ 70. Overall, we observed that of the 96 calves on this study, 57 experienced at least one heat stress bout (THI ≥ 70).

4.3 Statistical Analysis

Statistical analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Statistical significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. Before analysis, the univariate procedure was used to assess normality of data and collinearity between variables was assessed with PROC CORR. Linear mixed models were used to further assess normality of data with visual assessment of residuals plots and determination of covariance structure. Data were checked for outliers, and no significant outliers were found and all data were retained for analysis. Linear mixed models were used to determine optimal covariance structures that best fit our models. The covariance structures that were assessed were first order autoregressive (ar1), Toeplitz (toep), and compound symmetry (cs) andthe structure used for each model was selected based on which resulted in the lowest Akaike information criterion (AIC) value. After determining the proper covariance structure for all linear mixed models, stepwise backward elimination was used to reduce multivariable models where effects with a *P*-value < 0.20 were retained.

Mixed linear regression models were used to assess the effect of heat stress and heat stress × day interactions on response variables, which were milk intake, drinking speed, lying bouts, lying time, step count, and activity index. Calf was used as the subject and day was a repeated measure. Any heat stress × day interactions at $P < 0.15$ were further assessed using the Tukey-Kramer method for multiple comparisons to adjust the differences of least squares means. If no interaction effect was found, the interaction was removed from the model. Collinearity was assessed between variables and from these findings it was determined that birth weight would be assessed for model inclusion as a covariate at P-value of < 0.20. Birth weight was significant and remained in the milk intake model (P = 0.01) but was not significant (P >0.20) in all other models and was removed.

Using the Akaike information criterion, autoregressive first order covariance structure provided the best model fit for 3 of the response variables (milk intake, activity index, and step count). For lying bouts and lying times, the Toeplitz covariance structure provided the lowest AIC value and was the best fit for the model.

4.4 Results

For spring, the mean temperature was 13.46 ± 7.36°C; mean relative humidity was 70.28 ± 9.78; mean THI was 56.47 ± 11.18. For summer, the mean temperature was 24.91 ± 2.08°C; mean relative humidity was 76.31 ± 8.77; mean THI was 74.31 ± 3.01. For fall, the mean temperature was 15.66 ± 8.30°C; mean relative humidity was 81.06 ± 9.21; mean THI was 60.01 ± 13.32. Fifty-seven calves experienced at least one heat stress event (THI ≥ 70). Mean daily THI for the heat stressed group was 74.72 (SD= 2.48; min= 70.22; max= 80.67) and mean daily THI for non-heat stressed group was 53.99 (SD= 10.71; min= 26.08; max= 69.91). Environmental conditions averaged by season are reported in Table 4.1, and group environmental conditions are reported in Table 4.2.

Association of heat stress with milk intake and activity behaviors (activity index and step count) are reported in Table 4.3. For average daily milk intake there was no heat stress × day interaction (P = 0.15), so the interaction was removed from the model. Birth weight was set as a covariate (P = 0.01) and remained in the milk intake model. For average daily milk intake, there was a tendency ($P = 0.06$) for heat stress to increase milk intake in calves. For activity index there was no heat stress × day interaction ($P = 0.64$), so the interaction was removed from the model and we found no effects of heat stress on activity index ($P = 0.20$). For total step count, there was no heat stress × day interaction ($P = 0.84$), so the interaction was removed from the model. For total step count, there was a tendency for an effect of heat stress ($P = 0.09$), where heat stressed calves had increased step counts compared to non-heat stressed calves.

Association of heat stress with lying behaviors and heat stress × day interactions are illustrated in Figures 4.1 and 4.2. For daily lying bouts, there was a significant heat stress × day interaction ($P = 0.03$); however, after accounting for Tukey corrections there were no significant daily differences in number of lying bouts between heat stressed and non-heat stressed calves detected ($P > 0.05$; Figure 4.1). For average lying time, there was a significant heat stress × day interaction ($P = 0.01$); accounting for Tukey corrections there were significant differences detected on day 3 ($P = 0.01$; Figure 4.2); heat stressed calves had decreased lying times (mean= 16.5 h/d; SD= 0.50 h/d) compared to non-heat stressed calves (mean= 19.43 h/d; SD= 0.37 h/d). For average daily milk drinking speed, there was a significant heat stress × day interaction ($P < 0.0001$); however, following Tukey adjustments there were no significant daily differences between heat stressed and non-heat stressed calves detected ($P > 0.05$; Figure 4.3).

4.5 Discussion

This study aimed to investigate the relationship between elevated THI and feeding and activity behaviors in Holstein dairy calves during the preweaning period. Our findings demonstrate that calves subjected to heat stress exhibited increased restless behaviors and there may be a tendency for heat stress to increase milk intake. The automated feeder provided useful information on the average daily milk intakes and average milk drinking speeds of calves on this study. We found no interaction of heat stress × day for milk intake, however, after removal of the interaction term we saw a tendency for heat stressed calves to consume a greater average quantity of milk (8.44 ± 0.07 L/d) when compared to non-heat stressed calves (8.25 ± 0.08 L/d). Water is lost via respiration and sweating during times of heat stress which increases the water requirements for the animal. The increased milk intakes observed in heat stressed calves may be driven by the calves' increased water requirements under elevated THI levels. However, for drinking speeds although we found a significant heat stress × day interaction there were no significant daily differences between heat stressed and non-heat stressed calves. Contrastingly, Dado-Senn et al. (2022) reported reduced overall milk intake and decreased intake per visit to the feeder in calves subjected to heat stress conditions compared to normal. The decreased milk intakes seen may be explained due to hypothalamic regulation of satiety signals (Baile and Della-Fera, 1981). When the core body temperature exceeds thermoneutrality, a thermoregulatory response of the hypothalamus is to transmit signals to receptors to suppress satiety and alter feeding behaviors. Discrepancies seen between our findings and those of others may also be attributed to varying milk feeding and weaning strategies across groups. Calves under average THI of 74.8 had decreased starter intake compared to calves under THIs of 65.2 and 59.7 as reported by Broucek et al. (2009). Others found that calves born in spring and summer consumed less starter and metabolizable energy than calves born in winter or fall (Rauba et al., 2019). The reduced feed intake can serve to lessen the metabolic heat load generated through digestion (West, 2003) and may assist in decreasing the core body temperature during times of heat stress.

Activity behaviors were also monitored during this research and found varying results. We did not find any significant heat stress effects or heat stress × day interactions with activity index. For total step counts, there was no heat stress × day interaction, but there was a tendency for heat stress to increase total step counts. We also saw effects of heat stress on lying behaviors in the preweaned calves. For daily lying bouts, there was a significant heat stress × day interaction, but there were no significant daily differences to explore. For daily lying times we found a significant heat stress × day interaction and found significant differences on day 3; where heat stressed calves had decreased lying times compared to non-heat stressed calves. These results suggest heat stressed calves may express more restlessness than calves not experiencing heat stress. These results are similar to those reported by Kovács et al. (2018a) where they investigated behavioral indicators of thermal discomfort in heat stressed bull calves. They reported an increase of at least 71.2% in the frequency of lying bouts for heat stressed bull calves without a shade structure when compared to non-heat stressed calves with the structure. Additionally, calves under heat stress conditions had decreased lying times when compared to non-heat stress calves (Kim et al., 2018, Kovács et al., 2018a). Kim et al. (2018) observed decreased lying times and increased standing times for beef calves at THIs of 74.22 and 87.72 compared to THI of 70.01. Some hypothesize that increased standing times during heat stress may improve the surface area of cows exposed to improve evaporative cooling from the skin (Allen et al., 2015). These results suggest that heat stressed calves are less comfortable in their environment than non-heat stressed calves. The increased restlessness may also indicate the calves’ attempt to find more suitable conditions, such as shade structures or better wind speed and air circulation.

Although heat abatement methods in dairy cows are widely discussed, there has been limited focus on implementing similar strategies with dairy calves. Some strategies that may benefit calves during heat stress are providing shade structures, evaporative cooling through fans and sprinklers, and improved ventilation (Wang et al., 2020). The information gathered by PDT during heat stress can be used to help producers develop heat abatement strategies for their calves or assess preexisting methods. Our findings suggest that these technology-recorded behaviors can characterize heat stress in preweaned dairy calves. Further investigation is required to better understand th behavioral response of calves during heat stress as well as developing methods to assess the effectiveness of heat abatement strategies.

4.6 Conclusions

In summary, changes in activity levels during heat stress suggest that affected calves exhibit increased restless behaviors reflected in decreased lying times and a tendency towards increased step counts when compared to their non-heat stressed counterparts. This increased activity may be because calves are seeking shade or changing posture due to thermal discomfort.The tendency for calves to consume more milk during heat stress suggests that they are attempting to meet their increased water requirements through milk consumption. These results support further investigation into the potential of precision dairy technologies in detection of behavioral indicators of heat stress in preweaned dairy calves. By monitoring the signs of heat stress in dairy calves, precision dairy technologies could alert to producers when heat stress abatement strategies would be necessary. Additionally, they could use the technology-recorded information to assess the effectiveness of their heat abatement strategies and aid them in improving heat stress intervention and management.

Table 4.1 Environmental Conditions by Season

Environmental conditions by season for average daily temperature (°C), relative humidity (%), and THI (mean ± SD) of the experimental calf pens. Data were recorded at 15-min intervals by HOBO data logger (HOBO Pro V2, Onset Computer Corp., Bourne, MA, USA) then averaged by hour and then averaged by day.

	Daily Environmental Conditions		
	Spring	Summer	Fall
Temperature (°C)	13.46 ± 7.36	24.91 ± 2.08	15.66 ± 8.30
Relative humidity (%)	70.28 ± 9.78	76.31 ± 8.77	81.06 ± 9.21
THI	56.47 ± 11.18	74.31 ± 3.01	60.01 ± 13.32

Table 4.2 Environmental Conditions by Heat Stress Group

Environmental conditions by heat stress group for average daily temperature (°C), relative humidity (%), and THI (mean ± SD) of the experimental calf pens. Data were recorded at 15-min intervals by HOBO data logger (HOBO Pro V2, Onset Computer Corp., Bourne, MA, USA) then averaged by hour and then averaged by day.

	Daily Environmental Conditions	
	Heat Stress	No Heat Stress
Temperature (°C)	25.12 ± 1.79	11.91 ± 6.85
Relative humidity (%)	77.08 ± 8.49	74.78 ± 11.88
THI	74.72 ± 2.48	53.99 ± 10.71

Table 4.3 Association of Heat Stress with Milk Intake. Activity Index, and Step Count for Preweaned Dairy Calves

Association of heat stress with milk intake (L/day), activity behaviors index (Index), and step count (steps/day) (LSM ± SEM) for preweaned dairy calves (n=96) while on an automated milk feeder Compact Smart, Förster-Technik, Engen, Germany) and wearing a leg-attached pedometer technology (IceQube, IceRobotics, Edinburgh, Scotland).

Behavior	No Heat Stress	Heat Stress	SEM	F-value (df)	Heat Stress	Treatment × Day
Milk intake (L/d)	8.25	8.44	0.10	$3.72_{1,47}$	0.06	0.15
Activity index	2611.62	2721.25	84.64	$1.68_{1,47}$	0.20	0.64
Step count (steps/day)	470.83	498.39	16.12	$2.92_{1,47}$	0.09	0.84

Figure 4.1 Association of Heat Stress with Lying Bouts for Preweaned Dairy Calves

Association of heat stress1 with average daily lying bouts (bouts/day) (LSM ± SEM) for preweaned dairy calves (n=96) while on an automated milk feeder (Compact Smart, Förster-Technik, Engen, Germany) and wearing a leg-attached pedometer technology (IceQube, IceRobotics, Edinburgh, Scotland).

1Heat stress was characterized as temperature-humidity index (THI) ≥ 70

Significance* ($P \leq 0.05$)

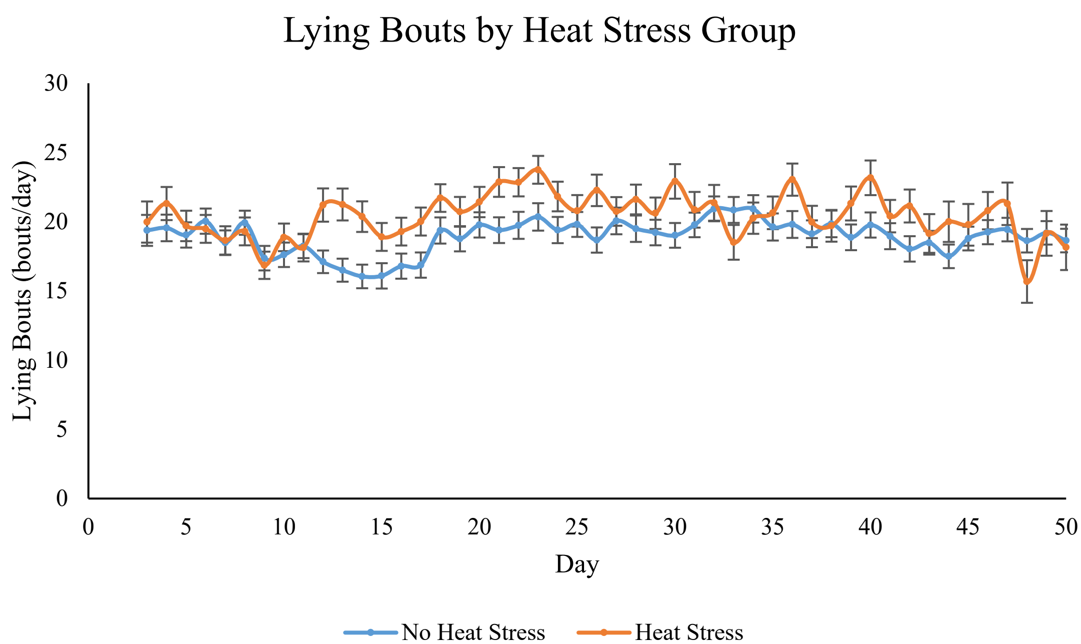


Figure 4.2 Association of Heat Stress with Lying Times for Preweaned Dairy Calves
Association of heat stress[1] with average daily lying times (h/day) (LSM ± SEM) for preweaned dairy calves (n=96) while on an automated milk feeder (Compact Smart, Förster-Technik, Engen, Germany) and wearing a leg-attached pedometer technology (IceQube, IceRobotics, Edinburgh, Scotland).

1Heat stress was characterized as temperature-humidity index (THI) ≥ 70

Significance* ($P \leq 0.05$) and tendency^ ($0.05 < P < 0.10$) indicate daily differences between heat stress group

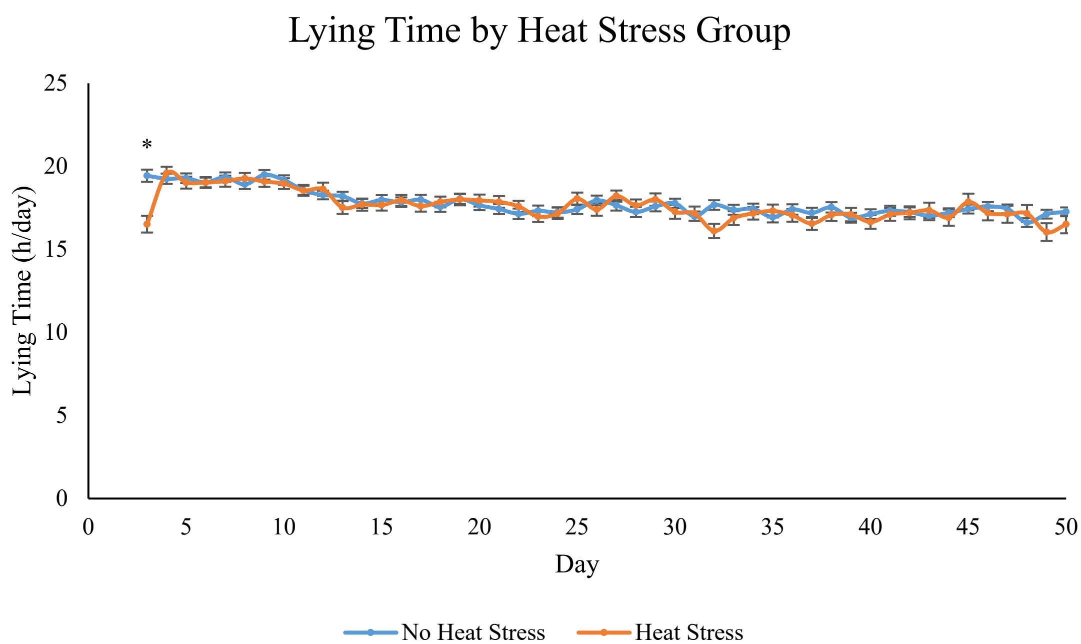
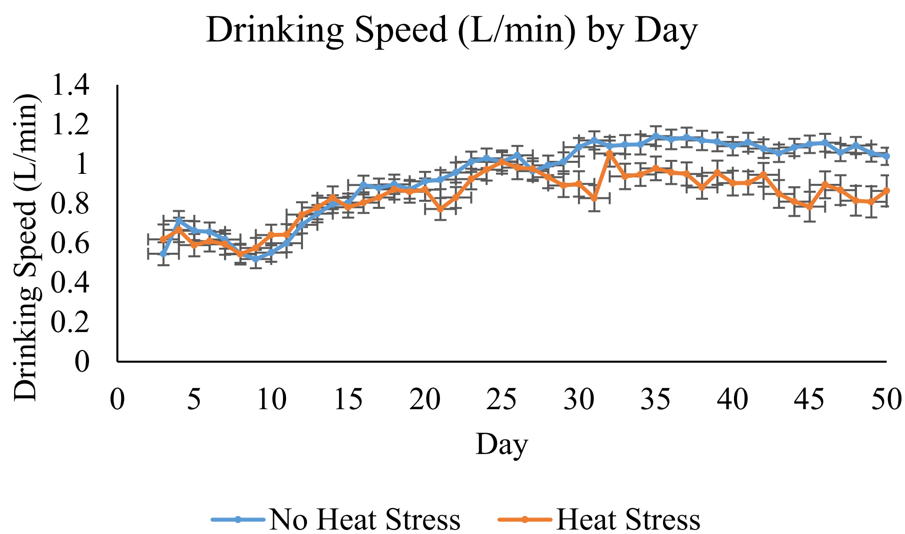


Figure 4.3 Association of Heat Stress with Milk Drinking Speeds of Preweaned Dairy Calves

Association of heat stress[1] with average daily milk drinking speed (L/day) (LSM ± SEM) for preweaned dairy calves (n=96) while on an automated milk feeder Compact Smart, Förster-Technik, Engen, Germany) and wearing a leg-attached pedometer technology (IceQube, IceRobotics, Edinburgh, Scotland).

1Heat stress was characterized as temperature-humidity index (THI) ≥ 70

Significance* ($P \leq 0.05$)



DISSERTATION GENERAL DISCUSSION AND CONCLUSIONS

The first objective of this dissertation was to evaluate the positioning performance of a real-time location system for use in dairy cattle on a commercial dairy farm. The system was assessed under static and dynamic conditions to provide deeper insight into the precision and accuracy of the system. Real-time location systems are an emerging precision technology in the livestock sector that can provide useful information to producers that can aid in managing their herd. The benefits of using precision technology in dairy cattle have been widely reported, with major improvements in time and labor efficiency when compared to farm management styles sans the use of precision technologies. Through pinpointing the location of an individual dairy cow, producers can quickly locate cows to provide medical care, breed those in estrus, and move them to a new group. In addition, this technology can track movement patterns and summarize specific area usage for their herd. Understanding cow flow and area preference/avoidance can signal producers to design flaws or management concerns that may threaten animal health and welfare.

To assess the system performance under static conditions, sensors were attached to wooden stakes and left undisturbed in pens without human or animal interference. During this time the sensors sent signals that the system interpreted into positions using *x, y* coordinates unique to the farm. The reference point *x, y* coordinates were determined using a laser-measuring device. The technology-reported locations were then compared to their respective reference point locations which was used to calculate the distance error. The average accuracy of this system was found to be 1.99 m with all technology-reported data points falling within 9.0 m of the true locations. When assessing the dynamic positioning abilities of this system, trail cameras were used to capture images of cows at feedbunk, freestall, and waterer locations in the barn. The trail cameras served to avoid interference or disruption of normal movement patterns that occur with direct visual observation. Technology-reported locations were compared to the reference point *x, y* coordinates of the central point of the camera photograph angle. After analysis of all images, the system had a mean distance error of 3.82 m with nearly all data points falling within 30.0 m of the reference point locations. We found that distance errors were larger for the dynamic validation when compared to static, which was due to the nature of dynamic testing. When the sensors are attached to the animal, there is an increase in expected signal disruptions as it moves throughout the barn. Dairy barns are typically made up of metal structures which are known to interfere with radio signals. Additionally, cow bodies are largely composed of water which is another source of signal interference. Our findings suggest that the accuracy of the RTLS is sufficient in locating an individual cow in the barn because the distance errors found in static testing were approximately within one cow-length. Distance errors during dynamic testing were between 2 to 3 cow-lengths which is not ideal in making specific inferences. However, this level of accuracy would still provide the producer with information of the cows' relative location in the barn (i.e., pen location). With this knowledge, producers can save time spent searching for cows on their farm. This time can then be distributed across other on-farm tasks, resulting in improvmenets in time and labor efficiency. Additionally, the results from these validations demonstrate the limitations of RTLS when implemented under certain conditions. The technology had variable accuracies in determining cow locations in certain areas. This was reflected in the larger distance errors seen at feedbunk and waterer locations with some values exceeding 10.0 m. This level of accuracy would not provide helpful insight if a producer was intending on making inferences on behaviors like drinking and eating times. With these limitations in mind, further research should investigate the incorporation of data from other precision dairy technologies to provide more robust inferences on cow behavior and social interactions. Understanding the social behaviors of one's herd could be used to detect sources of stress and develop welfare indicators. As animal welfare becomes a more important topic in dairy cattle, social mechanisms and relationships between individual animals may be understood through location and proximity information obtained from RTLS. Future work investigating the use of location tracking and proximity sensing with dairy cattle could serve to improve animal welfare.

Additionally, a final objective of this dissertation was to investigate a relationship between heat stress and technology-recorded dairy calf behaviors during the preweaning period. The negative effects of heat stress on adult dairy cattle are widely understood; however, literature reporting on the impacts on dairy calves has been limited. Therefore, there is validity in evaluating the effects of heat stress on calves. The preweaning period serves as the foundation for healthy and productive calves and supports their success when entering the milking herd. Environmental conditions outside of the thermoneutral zone can cause changes in behaviors, health status, and performance. Precision dairy technologies can be used to monitor specific variables that may be useful in detecting calves that are negatively impacted by heat stress. One of the more common technologies used with dairy calves are automated milk feeders, which can track a multitude of feeding behaviors with the dairy calves. Of particular interest in this dissertation, the variables of focus were drinking speed and milk intake. These were selected due to findings from other groups that calves experiencing disease bouts exhibit deviations in normal milk feeding behaviors.

Additionally, leg-attached accelerometers can record the activity levels of calves which can also indicate health issues if there are deviations from normal behaviors. For this study, accelerometers were used to track total number of steps, number of lying bouts, lying times, and activity indices of dairy calves. This retrospective study followed 96 calves for seven weeks after being trained on the automated milk feeder. The behaviors of interest were investigated for their association with heat stress which was defined as temperature-humidity index greater than or equal to 70. Heat stress × week interactions were discovered between average daily milk intake, drinking speed, number of lying bouts, and total step counts. Heat stressed calves drank less milk at slower drinking speeds when compared to unaffected counterparts. Additionally, there were increased number of lying bouts, activity indices, and step counts for heat stressed calves in comparison to non-heat stressed calves. These results suggest that precision dairy technologies can be successfully used to detect changes in calf feeding behaviors and activity levels that are associated with elevated THI. Integrating technology-recorded behaviors with environmental data could support early detection of heat stress impacts and facilitate the development and implementation of effective heat abatement strategies on-farm. Furthermore, monitoring behavioral changes of individual calves could allow for a more precise and targeted approach towards heat stress abatement methods; thus, improving comfort, health, and welfare of calves on an individual basis. Additionally, these behavioral changes could be used to develop a method to assess the effectiveness of a producer's current heat abatement techniques and make recommendations on management decisions to positively affect their young calves.

In conclusion, this dissertation found that the real-time location system was able to accurately determine the locations of individual dairy cows in an indoor environment.

These results support future research into combined application of real-time location technologies with other available precision technologies. One such example could use rumination data and cow movement patterns to detect cows experiencing lameness issues or other health events. Furthermore, the technology may be useful in alerting to bunching behaviors that cattle exhibit during times of heat stress through detecting high concentrations of animals in certain areas. Lastly, precision dairy technologies can detect the differences in feeding behaviors and activity seen in preweaned dairy calves experiencing heat stress. This knowledge can be used to develop alerts based on behavioral changes that could serve as early indicators of heat stress in dairy calves. With this information, producers could better manage calves during times of heat stress and evaluate the success of their heat abatement strategies.

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

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Extension Articles

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

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