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QUESTION 6: STATEMENT OF RESEARCH PROJECT

a. A description of the nature of the research project being conducted.

This research project is designed to quantify physiological and behavioral changes in cattle associated with mastitis, lameness, estrus, and metabolic diseases, using multiple precision dairy farming technologies.

One of the pieces of technology to be used in the study is the reason for the FCC Experimental radio service application. This project will use Kahne's sensor-based monitoring systems that allow continuous monitoring of individual health status of livestock. Kahne's monitoring systems measure pH and temperature inside the rumen of each animal and transmit the data to a receiver.

Consumer pressure for control of zoonotic diseases, pathogens, and medical treatments, coupled with increased awareness of animal welfare issues has altered decision-making processes on farms (Schukken et al., 2008, Bewley, 2010a). Precision Dairy Farming (PDF) is the use of technologies to measure physiological, behavioral, and production indicators on individual animals to improve management strategies and farm performance (Bewley, 2010a).

Precision Dairy Farming technologies have the potential to detect disease early, maximizing individual animal potential. Disease detection in the past has relied on producers observing clinical signs, but once clinical signs are displayed, it is often too late to act effectively. Clinical signs are often preceded by physiological changes that are undetectable with human senses, creating great potential to take preventive action (Bewley, 2010a).

<u>Further information on this project can be seen in the attached study protocol</u> *(Quantification of Physiological and Behavioral Indicators of Mastitis, Lameness, Estrus, and Metabolic Diseases in Dairy Cattle Using Precision Dairy Farming Technologies).*

b. A showing that the communications facilities requested are necessary for the research project.

Kahne's technology is the only rumen monitoring system available with automatic data and information system capabilities. Kahne's rumen monitors detect and reliably record pH and temperature on an ongoing basis. Examining the association between rumen pH and/or temperature to the onset of diseases and to productivity metrics in feedlot cattle is critical to understanding the corresponding conditions and solutions which can deliver significant value to the industry.

c. A showing that existing communications facilities are inadequate.

In vivo rumen monitoring systems available in the marketplace today are cumbersome with limited and poor data collection and information system capabilities, expensive pricing (average pricing is about NZD$1,000 per bolus), limited utility (pH life ranges from 40 to max 150 days), and poor bolus retention. Due to these factors and others, they are still mostly used in limited research environments and require tedious human intervention and fistulated animals.

In addition to this, other devices available are heavier, so they sink and reside in the reticulum. As a result, they suffer from a high incidence of regurgitation. Kahne’s Sentinel bolus is lighter in weight so it bypasses the reticulum and rests in the dorsal sac of the rumen, where the fermentation process occurs.

Kahne’s flexible wing structure is designed to retain the bolus within the rumen. These factors mean that Kahne’s rumen monitoring sensors will provide the most reliable, accurate information for this research study, with very little intrusion on the animals regular activity.

Quantification of Physiological and Behavioral Indicators of Mastitis, Lameness, Estrus, and Metabolic Diseases in Dairy Cattle Using Precision Dairy Farming Technologies

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Primary Research Objective:

To quantify physiological and behavioral changes, using multiple Precision Dairy Farming technologies, associated with mastitis, lameness, estrus, and metabolic diseases.

Background

Consumer pressure for control of zoonotic diseases, pathogens, and medical treatments, coupled with increased awareness of animal welfare issues has altered decision-making processes on farms (Schukken et al., 2008, Bewley, 2010a). Precision Dairy Farming (**PDF**) is the use of technologies to measure physiological, behavioral, and production indicators on individual animals to improve management strategies and farm performance (Bewley, 2010a). Precision Dairy Farming technologies have the potential to detect disease early, maximizing individual animal potential. Disease detection in the past has relied on producers observing clinical signs, but once clinical signs are displayed, it is often too late to act effectively. Clinical signs are often preceded by physiological changes that are undetectable with human senses, creating great potential to take preventive action (Bewley, 2010a).

Mastitis

Mastitis is an inflammatory reaction of udder tissue, usually caused by a bacterial infection in the mammary gland (Harmon, 1994, Sordillo et al., 1997, Oliver and Murinda, 2012). This disease alters udder secretory processes, lowers milk yield, and changes milk composition (Beck et al., 1992, Harmon, 1994). Mastitis destroys some to all secretory cells and epithelial tissue (Harmon and Heald, 1982, Beck et al., 1992). Additionally, mastitis may compromise animal welfare because of the resulting discomfort and pain (Medrano-Galarza et al., 2012, Fitzpatrick et al., 2013).

Mastitis is an important topic in the dairy industry, partly because milk cannot be sold from cows treated with antibiotics, which often occurs with mastitis infections. Milk from treated cows is usually discarded or fed to calves (Blosser, 1979, USDA, 2007). In addition to milk losses from antibiotics, milk fat (Beck et al., 1992, Harmon, 1994) and milk yield decrease, further reducing revenue (Beck et al., 1992).

Mastitis is a concern even for well-managed herds throughout the world (Schukken et al., 2008). Dairy industry personnel generally accept that economic losses from mastitis are sizable (Blosser, 1979, Beck et al., 1992, Hogeveen, 2011) and are a limiting factor for profitability (Oliver and Murinda, 2012). Optimizing economic results is important for farm survival (Hansson et al., 2011). Bar et al. (2008) claimed mastitis was "undoubtedly the disease with the highest economic importance" to the dairy industry. Economic losses from subclinical and clinical mastitis include: decreased milk production, treatment costs, increased labor, discarded milk, veterinary fees, reduced milk price because of high somatic cell count (SCC), increased risk of subsequent mastitis, increased risk of culling or death (Nielen et al., 1992, Hansson et al., 2011), increased risk of other diseases, and preventive care costs.

Making sound economic decisions, particularly related to disease, allows for financial benefit to producers (Bar et al., 2008, Bewley, 2010b). Therefore, reducing mastitis incidence would be advantageous (Hansson et al., 2011). However, the economic benefits of preventive measures are determined by the implementation cost and the value in reducing the mastitis incidence; Not all preventive measures are economical or provide the same benefits (Hansson et al., 2011, Hogeveen et al., 2011).

Today's non-automated clinical mastitis detection approach involves observing inflammation through visualization and palpation of the udder or milk. Producers can also monitor declines in milk yield because they can indicate a health problem (Lukas et al., 2009, Leslie and Petersson-Wolfe, 2012). However, these changes are not immediate, making “aggressive and early intervention” difficult. This type of intervention may set a cow up to produce more milk and remain healthier throughout her lactation (Aalseth, 2005).

Early diagnosis of clinical mastitis may reduce production losses and enhance prospects of recovery (Milner et al., 1996), making it very important to the dairy industry (Viguier et al., 2009). Proactive action may also decrease antibiotic use, which may decrease the chance of antibiotic residue in the bulk tank (Oliver and Murinda, 2012). In herds with a low frequency of clinical mastitis incidence, herd management and cow immunity is not fully understood (Erskine et al., 1988). While many mastitis management techniques exist, the labor and capital needed for these preventive measures compete with other possible farm improvements (Hogeveen, 2011), making the decision on whether to implement suggested improvements difficult for producers. Unfortunately, subclinical mastitis detection is even more difficult for producers to detect in the absence of clinical signs.

Monitoring subclinical mastitis is important to producers because it can increase bulk tank somatic cell count (Hillerton and Walton, 1991, Nielen et al., 1995) and milk from cows with subclinical mastitis contains greater amounts of bacteria than milk from a cow without mastitis (Harmon, 1994). Eventually, many subclinical infections become clinical (Beck et al., 1992). Therefore, effective mastitis management should include monitoring of both clinical and subclinical mastitis events (Schukken et al., 2008).

Lameness

Lameness is a painful, debilitating disease (Whay et al., 1997). Lameness is recognized as one of the most important health (Dippel et al., 2011) and welfare concerns for dairy cattle (Olmos et al., 2009, Ito et al., 2010) because of the pain, decreased performance, and high prevalence it causes (Cutler et al., 2013).

Cook (2003) discovered that the lameness prevalence for 30 herds across Wisconsin ranged from 7.9% to 51.9%. Researchers evaluating health problems on 90 farms in England found the average lameness incidence rate was 24%, ranging from 4.7% to 47.4% (Esslemont and Kossaibati, 1996). In a Dutch study, lameness rates among 13 commercial herds ranged from 9.3 to 49.2% (Barkema et al., 1994).

The majority of lameness cases occur from hoof lesions (Murray et al., 1996). One of these lesions is digital dermatitis, a widespread, contagious, multifactorial disease of the hoof. In a Canadian study describing farm lesion prevalence, digital dermatitis was the most common lesion found. Digital dermatitis affected 69.7% of tie-stall herds, and 92.1% of freestall herds (Cramer et al., 2008). Early detection of digital dermatitis is key for infection control (Relun et al., 2011). Traditionally, digital dermatitis is evaluated when cows are secured in a trimming chute. However, this is laborious and stressful for the cows. Relun et al. (2011) discovered that digital dermatitis evaluation in the milking parlor, using a headlamp and swiveling mirror, is a reliable method to detect and score digital dermatitis.

Lameness is the third most expensive disorder in cattle after mastitis and reproductive problems. The total cost of lameness per cow per year was estimated at $154.80 (Enting et al., 1997). Cha et al. (2010) reported that the average cost per case of a sole ulcer, foot rot, and digital dermatitis was $216.07, $120.70, and $132.96, respectively.

Estrus Detection

The ability to get dairy cattle bred in a timely and cost effective manner is crucial for maximizing milk production. The economic impact of dairy cattle reproductive efficiency can be determined using ovulation rate, estrus detection rate, and conception rate (Inchaisri et al., 2010; Spielman and Jones, 1939).

Estrus in dairy cattle is defined as the period of sexual receptivity (Senger, 2005). Dairy farmers rely on detecting the behavior associated with estrus to determine the best time to breed their animals. However, dairy cows only show estrus for 7 hours with varying intensity (Dransfield et al., 1998). Previous methods used in conjunction with visual observation include tail painting or chalking, androgynous females, Kamar® Heatmount® detectors, and creating sexually active groups with synchronization programs to name a few (Nebel, 2002 and 2011).

Reproductive management programs include more than just when to artificially inseminate dairy cattle. The efficiency of estrus detection can be affected by factors including efficiency, level of estrogen, strength of estrus behavior, time given per day to detect cows in heat, and more. The ability to use records from estrus detection to calculate estrus detection efficiency is vital to improving dairy cattle reproductive management (Heersche and Nebel, 1994).

Precision Dairy Farming technologies are commonly used for estrus detection (Nebel, 2000). The quantification and efficiency of estrus detection can increase with PDF technologies (Firk, 2003). Researchers have explored estrus detection using walking activity (Lopez-Gautius, 2005; Van Eerdenberg, 2008), lying behavior (Bewley, 2010), mounting activity (Dohi et al., 1993), milk temperature (McArthur et al., 1992), vaginal temperature, ear skin temperature (Redden, 1993), tympanic temperature (Scott et al., 1983), rumination time (Reith et al., 2012), and progesterone levels in blood and milk (Delwiche, 2001). The use of synchronization programs in conjunction with automated estrus detection has also been explored (Neves et al., 2012). Quantifying these measurements with automated estrus detection is a movement towards improving the ability to increase estrus detection rates (At-Taras and Spahr, 2001) and overall reproductive management programs.

The previous research completed exemplifies the potential for these systems in the dairy industry. Areas of improvement are evident, like net present value evaluations, and comparisons of the specificity and sensitivity of PDF technologies used for automated estrus detection. The quantification of physiological and behavioral differences during estrus allows dairy farmers to pay closer attention to individual animals instead of masses of dairy cattle at a time.

Metabolic Diseases

Energy metabolism is particularly important in transition cows and early detection of metabolic disease will help to treat the cows. Thus, closely monitoring cows during this critical period is imperative. These diseases include milk fever, ketosis, retained placenta, and left displaced abomasum. Milk fever is a complex metabolic disorder that occurs at the onset of lactation related to increase demand for calcium. Ketosis occurs when the cow is in negative energy balance in early lactation. Retained placenta (RP) occurs when the villi of the foetal cotyledons was not separate from the crypts of the maternal caruncle. Left displaced abomasum (LDA) is a common and economically important problem for cows. When the abomasum becomes enlarged with the gas and fluid, which make the displacement from the normal position to the left side, between the rumen and the left lateral abdominal wall, will be considered as LDA (Coppock, 1974).

Study Design

Proposed start date: January 1, 2014
Proposed end date: July 1, 2015
Study duration: 18 months

General Information

Cows will be housed in two free-stall barns, equal in stall numbers and space. One barn is equipped with 54 dual chamber waterbeds (Advanced Comfort technology, Inc., Reedsburg, WI) and the other is equipped with 54 rubber-filled mattresses. Both stall bases are covered with sawdust. Cow groups will be balanced between barns by days in milk and parity. Cows have access to fresh water in their pens from automatic fill, Rubbermaid 150 gallon tanks and dumping, automatic fill water tanks. Cows have access to an exercise lot for about one hour per day at 10:00 am, weather permitting.

Cows will share a feed bunk, positioned between the free stall barns. The ration consists of corn silage, alfalfa hay, concentrate mix, cottonseed whole, and alfalfa silage. Cows will be fed at 06:00 and 13:30. In order to monitor the diet in relation to the events of interest, feed samples will be component-tested for the lactating herd TMR, the dry cow silage, and the dry cow hay on a quarterly basis and anytime a feed changes (i.e. new silage is delivered). Evaluated components include: percent moisture, ADF, NDF, percent CP, NEL, NEM, NEG, relative feed value, and percent calcium, phosphorus, magnesium, potassium, percent sodium, iron, zinc, copper, manganese, molybdenum, sulfur, and chloride ion. Feed samples of the lactating herd TMR and the dry cow TMR will be obtained twice daily and analyzed for percent moisture using Q-Dry® (Q-Dry®, Germany) and AgriNIR Analyzer (Dinamica Generale®, Italy). The AgriNIR Analyzer will also measure acid detergent fiber, neutral detergent fiber, percent crude protein, percent ash, and percent crude fat.

Every cow in the herd, 100 cows at any point in time, will remain on the study for its entirety. All PDF technologies will remain on every cow in the herd throughout the study, except for those used only for estrus detection purposes in which case will be applied only around a timed estrus event. Technologies will be removed from cull cows before loading the trailer to leave the farm.

All clinical disease events (including, but not limited to, milk fever, metritis, displaced abomasum, ketosis, mastitis, and digital dermatitis) will be recorded, including date of detection, observable signs, test results, and administered treatment.

All technologies included in this project are listed in Table 1. All technologies will be placed on heifers and new cows at least two weeks before first calving. Leg tags (IceQube, Afi, Track-a-Cow, and CowScout) will be attached one at a time from back legs to front legs. DVM boluses will be inserted into the reticulorumen orally, using a bolus gun. Ear tags (Smartbow, SensOor, and FeverTags) will be placed on the ear using an ear tagger, provided by each technology company to fit their respective tag. Ears will be monitored for infection and treated if a problem does arise. The HR Tag will be placed loosely around the cows' neck.

This study will use multiple PDFs (Table 1) to evaluate each one's usefulness in estrus, lameness, mastitis, and metabolic disease detection. Because each focus of detection will evaluate many of the same PDFs, the technology and data management of all PDFs will be split equally amongst the four graduate students involved in the project. To minimize stress on the cows, the graduate students will work together to ensure that the cows receive tags at the same time wherever possible so that they are only handled once. A head chute will be used for tag placement to ensure safety for both the cow and the handler. Each cow will be checked daily upon entrance to the parlor to ensure that any physical damage the tags could cause (i.e. rubbing of skin or hair) will be treated upon notice. If any tag severely rubs or injures a cow, the tag will be moved to the adjacent leg and ointment will be applied to the leg until it is healed. Once healed, the tag will be placed back onto the original leg.

Two weather stations (HOBO U23 Pro v2 External Temperature/Relative Humidity Data Logger - U23-002, Onset, Bourne, MA) will be located in the two freestall barns that will measure daily relative humidity and temperature. Data will be downloaded bi-weekly. Drinking water cools the rumen and may offset a temperature increase around a mastitis, lameness, estrus, or metabolic disease event. Therefore, water temperature will be monitored daily by HOBO Pendant® Temperature/Light Data Logger 8K - UA-002-08 (Onset Computer Corporation, Bourne, MA) placed into each water tank in the two freestall barns. Water intake will then be taken into account when analyzing reticulorumen temperatures obtained from the DVM boluses. Additionally, cows may decrease water intake around a mastitis, lameness, estrus, or metabolic disease event. Monitoring water temperature and the subsequent reticulorumen temperature decrease will allow the researchers to calculate water intake (Cornett et al., 2013).

The following information will be recorded at least once daily for all cows throughout the study:

- Milk yield
- Milking order
- Milk conductivity, fat, protein, somatic cell count
- Physical activity (neck and leg)
- Reticulorumen temperatures
- Tympanic temperature
- Surface ear temperature
- Physical location
- Rumination time
- Lying and standing time
- Resting time
- Somatic cell count
- Number of steps
- Milk temperature
- Body weight

Body condition scores (Table 2) and hygiene scores (Table 3) of each cow in the herd will be recorded once weekly by the same observer. Hock scoring will be visually assessed weekly in each barn. Hocks will be scored according to the Dairy Science Cluster initiative (Table 4).

Because each estrus, lameness, mastitis, and metabolic diseases may produce different physiological and behavioral changes within the cows during different stages of their lactations, the scoring systems and evaluations of each focus differ. These systems are outlined in the following sections.

Lameness Monitoring

Currently no gold standard exists to assess lameness in cattle. However, the GAITRite® system (CIR Systems Inc., Sparta, NJ) may be able to measure gait more definitely. All cows will walk over the GAITRite® system upon exiting the parlor. The GAITRite® system is a portable electronic walkway that measures temporal (timing) and spatial (dimensions) parameters of pressure activated sensors. The system measures cow gait events automatically. The 20-foot-long GAITRite® mat will be placed on the concrete exit alley and measurements will be obtained as cows walk over the mat (Maertens et al., 2011).

The most common method of assessing cow locomotion is visually assessing their gait. Therefore, gait scores (Table 5; adapted from Olmos et al., 2008) will be visually assessed weekly for the extent of the project. One cow at a time will be released to walk past an observer on the way to pasture, so that the observer may watch the cow walk from a front, side, and hind view. Scores for each category will be averaged and an average gait score will be obtained.

Digital dermatitis (DD) lesions will be visually scored using the Dopfer 5 M point scale (Table 6) during milking weekly by the same observer. The cow's feet will be washed off with a hose to be able to see the lesion. A mirror will be used to help give a better view of the DD if needed. The observer will also wear a headlamp to shine better light onto the lesion.

An infrared (IR) thermography camera (Flir i5 IR Camera, Flir, Boston, MA) will be used to obtain images of all hooves at the coronary band weekly from the dorsal view in the parlor during milking (Nikkhah et al., 2005). An area of skin above the coronary band will also be imaged on the dorsal side of the hoof to act as a control. Before taking images, hooves will be cleaned with water in the parlor and then dried with a towel to remove dirt and debris that could affect measurements. Plantar aspect measurements (Alsaaod and Büscher, 2012, Stokes et al., 2012) will be taken at the same time weekly in the parlor with the IR thermography camera in the same manner as the dorsal side of the hooves.

In order to evaluate hooves for lesions, every two months a trim table will be used to gently turn cows on their sides and an observer will restrain their feet to the trim table to prevent injury to the cow and human. The observer will spray hooves with water to clean them, and a mild soap will be used if needed. Hooves will then be wiped dry with a towel. The same observer will examine lesions throughout the study and lesions will be photographed to safely obtain lesion measurements. A standardized identification tag will be held next to the cows foot and photographed at the same time of the lesion examination to identify actual lesion size. While hooves are under restraint, all cows with a lesion will have an algometer (Pain Test FPX 50 Algometer Wagner instruments, Greenwich, CT) applied to their lesion to quantify pain. The algometer tip (1 cm flat rubber end) will be pressed against the hoof lesion until the nociceptive threshold is reached. The mechanical nociceptive threshold (kilograms of force causing the hoof to withdraw from the algometer) will be measured while cows are secured on the trim table.

A professional trimmer will routinely trim hooves every 6 months to prevent lameness and to ensure that lesions are properly treated.

Metabolic Disease Monitoring

The following information will be recorded daily for <u>all fresh cows (days 1-21 DIM)</u> at 8 AM ± 2 hours:

After milking, all the fresh cows will be hold in the holding pen, and do the exam below.

- Rectal temperature with a GLA thermometer (GLA Agricultural Electronics, San Luis Obispo, CA)
- Manure score evaluation (Table 7)
- Behavioral score evaluation (Table 8)
- Subcutaneous fluid content (skin tenting to check for dehydration)
  - A small amount of neck skin will be pinched lightly and pulled away from the cow ("tented"). The time it takes for it to return to normal will be recorded. A cow will be considered dehydrated if the skin takes longer than two seconds to snap back to normal.

The following information will be recorded for all fresh cows on approximate day 10 before calving.

- Blood tests
  - One vial of blood will be collected in the 2 ml tube from the tailhead vein via syringe and 20 gauges 1.5 inch needle.
  - The blood will be stored in the tube, and the blood samples will use iCheck to determine the β-Carotene.

The following information will be recorded for <u>all fresh cows on</u> <u>days 3, 5, 7, 9, 11,14,17, and 21 days post-calving</u>:

After milking, all the fresh cows will be hold in the holding pen, and do the exam below.

- Respiration rate by counting the number of times the flank expands and contracts in one minute.
- Metricheck to check uterine health (Table 9).
  - Metricheck device which scoops discharge from the anterior vagina. Depending on the appearance of this discharge, a cow is diagnosed as having endometritis or not. If the cow has endometritis the treatment is to uses Metricure which is deposited through the cervix into the uterus
- Rumen fill score (Table 10).
- Milk fever by using the table clinical sign of the milk fever (Table)

The following test will be performed on <u>3, 7, 14, and 21 days post-calving</u>:

After milking, all the fresh cows will be hold in the holding pen, and do the exam below.

- Blood tests
  - Approximately 20 cc of blood will be collected from the tailhead vein via syringe and 20 gauge 1.5 inch needle.
  - One drop of blood will be removed from the syringe and will be applied to the end of the ketone test strip that fits into the Precision Xtra™ (Abbott Laboratories, Abbott Park, Illinois) handheld machine. The strip will draw the necessary amount of blood (1.5 μL) into a sample well and start analyzing BHBA. The meter takes 10 seconds to check BHBA levels and will then displays the results in mmol/L.
    - Cows that are ≥ 1.4 mmol/L with the Precision Xtra™ test will be considered positive for ketosis.

- http://www.abbottdiabetescare.com/precision-xtra-blood-glucose-and-ketone-monitoring-system.html
- The remaining blood will be evenly-divided between two 10 mL red-top tubes. The first blood sample will be sent to the University of Kentucky Veterinary Diagnostic Lab for evaluation of BUN, calcium, phosphorus, magnesium, sodium, potassium, chloride, total protein, albumin, globulin, AST, CK, GGT, Alkaline Phosphatase, glucose, creatinine, total bilirubin, and AG ratio. The second blood sample will be stored in a -20°C freezer at the University of Kentucky and then sent to the University of Wisconsin to measure NEFAs.
- Only on the day 3 after calving, the blood will collected from the tailhead vein via syringe and 20 gauge 1.5 inch needle. And 5 ml of blood sample will be iCheck to determine the β-Carotene.

- Haptoglobin (Frim Tec, Germany) on 3, and 7 days post-fresh:
  While cows are milking, a vial of 5 ml milk sample will be collected. A composite sample will be obtained by attaching the composite milk sampler to the pipeline in the parlor.
    - 50 µl of milk should be inserted into one well from each sample of milk using the pipette and pipette tip. Haptoglobin concentration was measured by means of enzyme immunoassay on the milk.
    - Haptoglobin is one of several compounds known as the acute phase proteins of inflammation. Certain diseases in cattle have been associated with an increase in serum haptoglobin. Tissue injury and inflammation induce the release of mediators of inflammation known as cytokines, one of which is interleukin-6, or hepatocyte stimulating factor.

The following information will be recorded at calving:

- Calving date, time, approximate length, and ease of calving
    - The DHIA scoring system will be used to record the ease of calving (Table 11)
- Calf sex and weight at birth

Diagnosis:

In order to early detect the left displaced abomasum (LDA), Retained placenta, Ketosis, and milk fever. The testing upon will help to define.

A cow will be considered to have a left displaced abomasum (LDA) if she has a decreased appetite accompanied by an audible, high pitched tympanic resonance (ping) produced by percussion of the left abdominal wall between the 9th and 12th ribs (Kelton et al., 1998). If a cow is suspected of having an LDA, her abdomen will be flicked with the middle finger of the examiner and he/she will listen for the ping with a stethoscope. Right DAs are less common and therefore will not be sought after extensively in this research project, but will be recorded if they occur. Moreover, DA not only costs of treatment, but also reduces the milk production in a short term (Detilleux et al., 1997; Raizman and Santos, 2002),also has a higher culling rate (Geishauser et al., 1998; Gröhn et al., 1998; Raizman and Santos, 2002).

A cow will be considered to have a retained placenta if, after 24 hours after calving, the fetal membrane is visible at the vulva or can be identified by vaginal examination in the uterus or vagina (Kelton et al., 1998).

When the cow deceased appetite and there was evidence of decreasing milk production, urine, and breath ketones will be considerate as a clinical ketosis. Since ketosis is not as easy to diagnose as some of the other illnesses discussed in this project, a Precision Xtra test will be performed on a small amount of the cow's blood if she is even slightly suspected of having this problem.

Mastitis Monitoring

Cows will be evaluated for both clinical and subclinical mastitis cases in this study. In order to determine whether the technologies signaled an alert related to a clinical mastitis event, the researchers need to identify clinical signs as soon as they are detectable. Therefore, the cows will be closely monitored by one of the four graduate students involved in this project during each milking and all observances will be recorded.

Each lactating cow will have their somatic cell count (SCC) measured at each milking during the study period with the Mastiline (Isogen Animal Care, the Netherlands) system. A cow will be considered to have subclinical mastitis if she has a somatic cell count (SCC) > 200,000 cells/mL based on Mastiline results. High SCC milk from the same cow at subsequent milkings any time within 14 days of the most recent episode will be considered the same case.

A cow will be considered to have clinical mastitis if she has a visually abnormal milk secretion (i.e. clots, flakes, or watery milk) from one or more quarters. Milk clots will be detected by examining the Ambic dairy cow mastitis detector (Coburn Company, Whitewater WI), which will be connected to the milk hose and will catch any clots present in the milk. The Ambic dairy cow mastitis detector will be checked for clots after each cow is milked. If the graduate student present at the milking observes clots in the filter, the cow will be sampled for bacteriological, leukocyte differential, and SCC evaluation (explanations below) at the following milking.

The abnormal milk may or may not be accompanied by inflammation (i.e. heat, swelling, or skin discoloration) of the udder. A grade level will be recorded for each clinical mastitis case (Table 13), including which quarter is exhibiting the signs. If there are no signs of clinical mastitis, no information will be recorded. Abnormal milk from the same quarter of the same cow at subsequent milkings or at any time within 14 days of the most recent episode will be considered the same case. A new case of clinical mastitis will be defined as the first recorded case of mastitis for the cow per lactation or if the case is following > 14 days of normal milking from the quarter that was diagnosed as having mastitis (Hogan et al., 1989; Schukken et al. 1990; Barkema et al. 1997; Hertl et al. 2010). Quarters within cows will be considered separate for clinical mastitis detection. For example, if a quarter is detected with clinical mastitis and a different quarter on the same cow is detected three days later, these will be considered different cases and both will be tracked, recorded, and sampled.

Mastitis occurring in fresh cows, or cows within 21 days after calving, will be analyzed separately from cows 22 to 400 days in milk because that time is when the cows are most susceptible to contract other diseases that may also affect the technology data. Additionally, mastitis that presents itself during the fresh period usually results from the dry period and may cause slightly different physiological responses than mastitis contracted during lactation. Cows will enter the study on their third milking, and are then eligible for sampling for subclinical and clinical mastitis evaluation. Fresh cows will be sampled, regardless of their mastitis status, on milking 4 and 14.

Cows will be sampled for individual quarter bacteriological culture, milk leukocyte differential, and SCC analysis at the milking following the one where clinical signs are detected. These samples will be obtained from the quarter(s) showing clinical signs and from all quarters in the case of subclinical mastitis.

For bacteriological culture, approximately 5 mL of milk will be collected aseptically from the infected quarter(s) of a cow diagnosed with clinical mastitis and from all four quarters of a cow with subclinical mastitis. Samples will be taken aseptically according to procedures recommended by the National Mastitis Council (Hogan et al., 1999). Teat ends will be washed thoroughly, dried with individual disposable paper towels, and then cleaned with 70% isopropyl alcohol swabs. Milk samples for microbiological analysis will be collected into sterile pop-cap tubes and kept frozen until evaluation. Milk samples will be cultured in a laboratory at the University of Kentucky. Ten microliters from each milk sample will be plated onto one quadrant of a Difco Columbia Blood Esculin agar plate with 5% calf's blood obtained from the University of Kentucky Coldstream Dairy. Plates will be incubated for 48 hours at 37°C. Bacteria on primary culture medium will be identified tentatively according to colony morphologic features, hemolytic characteristics, gram stain, and catalase test. A single colony from each isolate will be grown further in BHI broth (brain-heart infusion) for 24 hours. All isolates will be gram stained and identified under a microscope. Isolates identified presumptively as staphylococci will be tested for coagulase by the tube coagulase method (BBL Coagulase Plasma, rabbit with EDTA). Gram negative rods, yeast, and isolates identified presumptively as streptococci will be evaluated by Vitek 2 Compact (Biomerieux).

For the milk leukocyte differential sample, approximately 4 mL of milk will be taken from each quarter directly after the bacteriological samples are obtained. The Q-Scout (Advanced Animal Diagnostics, Research Triangle Park, NC) system will be used to evaluate the milk leukocyte differential from the sample. Company directions will be followed to run this sample.

Lastly, about 90 mL from each quarter will be obtained in a non-sterile polypropylene flip-top vial for SCC evaluation directly after the milk leukocyte differential sample is obtained. Samples will be preserved and refrigerated until delivery to the University of Kentucky Regulatory Services lab once weekly.

The herd manager will be notified of cows with clinical mastitis and they will be treated as he deems necessary. Cows will be included in the study regardless of their treatment regimen, but this information will be recorded and evaluated at the end of the study.

Reproductive Management

All cows will be visually observed for estrus behavior for 30 minute periods twice a day for the entire study. The planned times will be 3:30 and 14:30. This information will be compared to increased activity (IceQube, Afi, Track-a-Cow, SCR HR Tag, and CowScout), temperature deviations (Smartbow, SensOor, and FeverTags), and rumination deviations (SCR HR Tag).

Approximately 150 cows will enter into a synchronized breeding program at some point during the period of 18 months. Throughout this study, some cows may have multiple calvings resulting in multiple lactations. Therefore, some cows may be entered into the synchronization program more than once over the study period. The Ovsynch-56 protocol (Table 14) will be used in order to visually observe estrus behaviors in cattle in groups of 8 to10 cattle at a time. It is important that cattle are added to the breeding program in groups in order to create sexually active groups to encourage a stronger display of estrus (Sweberg et al., 2013; Van Vliet et al., 1996). The voluntary waiting period for the herd will be 45 to 85 days postpartum.

The following information will be collected for lactating cows monitored for estrus (8 to

10 cows that are 45 to 85 days postpartum) prior to start:

- Body Condition score evaluation (Table 2)
- Gait score evaluation (Table 5)
- Reproductive tract examination with Ultrasonography

Ultrasonography will be used four times for each breeding group. Ultrasonography is a widely used tool by veterinarians and reproductive specialists to determine cyclicity and reproductive health of dairy cattle (Fricke, 2002). This will allow researchers to visually see important reproductive structures that identify different stages in the estrous cycle and succession to estrus. This method is considered to be safe for reproductive management (Lamb, 2001). Ultrasonagraphy at 45 to 85 days postpartum will determine if cows have resumed ovarian cyclicity (confirmed by the presence of a corpus luteum). Eligible cows will be presynchronized using the G7G protocol, starting with an injection of prostaglandin ($PGF_{2\alpha}$), (25 mg, Lutalyse, Pfizer Animal Health, New York, NY). Two days later, cows will receive an injection of GnRH (100 ug, Factrel, Fort Dodge Animal Health, Fort Dodge, IA). Seven days after the GnRH injection, the OVSYNCH protocol, excluding the final shot (Pursley et al., 1995), will be initiated. Cows will receive an injection of GnRH, then 7 days later an injection of $PGF_{2\alpha}$. An additional $PGF_{2\alpha}$ shot will be given on the same day, 6 hours later. An outline of the presynchonization and modified OVSYNCH is explained below.

The day of the last $PGF_{2\alpha}$ injection will be designated day 0 of the experimental protocol. On day -7, the remainder of the precision dairy technologies used for this study will be assigned to each cow including the following (See Table 1 for more details on each technology below):

- AccuBreed™ Heat Detector is placed on the top of the rump of each cow in the breeding group and secured with a removable patch. Its purpose is to detect mounting behaviors and length of those behaviors in relation to the onset and duration of estrus. A cow that stands to be mounted by another cow is known to be in standing heat (Walker et al., 1996) which is the golden standard for estrus detection.
- Anemon Vaginal Temperature Sensor is placed into a bucket of disinfectant to ensure sterility before being placed in the vagina, with an insertion tool similar to an artificial insemination gun, after cleaning the vulva with a disinfectant the sensor. Gloves and lubricant will be worn by researcher inserting vaginal sensor.
- Anemon Activity Transmitter will be placed inside of a leather case that slides onto a neck strap that remains on only the group of 7 to 10 cows.
- Medria Heat Phone® will be placed on the same strap on the opposite side similar to the above transmitter.

The Anemon sensors and transmitters and Medria HeatPhones® will be removed on day 7 of the protocol. Cows will be ultrasounded again upon removal of these technologies to check for resumption of estrous cycling and condition of the reproductive tract. Accubreed™ will remain on cows in the synchronization program for 14 days after the end of the Ovsynch 56 protocol to monitor cows that may come back into estrus after being bred. Some dairy cows for various reasons will not conceive or become pregnant which will result in them coming into estrus during the consecutive estrous cycle (Fonseca et al., 1983). Decisions to continue synchronization or visual observation for cows that are detected in estrus during this time period (the consecutive estrus cycle) will be made by the herd manager.

Blood samples (10 ml) will be taken from each cow on day -2, -1, 0, 1, 2, 7, 9, and 11.

Plasma will be separated and stored at -20 C until the concentration of progesterone is determined by radioimmunoassay (Coat-a-Count Inc.). Ultrasonography will be done on day 0 (recording presence and location of corpus luteum and the pre-ovulatory follicle), day 5 (confirming regression of corpus luteum and ovulation of the preovulatory follicle recorded on day 0), and day 11 (recording presence and location of newly formed corpus luteum). Progesterone and ultrasound data will confirm that cows responded as predicted. Any cows that do not display anticipated physiological changes will be excluded from final analysis.

Cows will be left alone from days 2 to 5, except for breeding as determined by the herd manager. This will allow information regarding expression of estrus to be collected from the precision technologies. In addition to data collected from the technologies, visual heat detection information (collected in 30 minute periods, 4 times daily from day 2 to day 5 or until all cows have shown heat) will be used in analysis.

All cows in the herd will be monitored for visible heats using visual observation on a daily basis at 3:30 and 14:30 that will be compared to data collected from technologies that will be on the cows during the entire study. Kamar® Heatmount® detectors will be placed on tail-heads of all cattle in the lactating cow group that are not on the synch protocol. This will allow observers to easily detect cows that have stood to be mounted since the last visual observation period. These will be removed once the Kamar® Heatmount® detectors have been activated by another cow mounting the cow wearing the activated Kamar patch. Visual heat detection for the synchronized cows will occur for a 4 day period 4 times a day (03:30, 10:00, 14:30, and 22:00) for 30 minutes each on days 2 to 5 of the synch protocol or until all cows have shown heat. Observations will be scored based on the Van Eerdenburg et al. (1996) scoring scale for observed estrous signs, including modifications used in Roelofs, 2005 (Table 15). Cows in this group will also be given a heart rate monitor to notice increased activity possibly affecting heart rate during estrus (Janzekovic et al, 2005). Polar Sport Tester-Profi® heart rate monitors will be placed on cows in the synchronized group during days 2, 3, 4, and 5, which are the days of predicted increased activity during estrus. Ultrasound gel will be used in order for the heart rate monitors to detect the heart rate effectively.

Breeding decisions will be made by the herd manager with the priority on cows observed visually in heat. Once cows are visually detected in heat, peaks in data from precision dairy farming technologies will be consulted to finalize the decision. The hypothesized order of priority is: Accubreed (since standing heat is the golden standard for the onset of estrus (Nebel, 2000)), Anemon (vaginal temperature and neck activity deviations as secondary signs of estrus), and then any of the remaining technologies that all cows are equipped with. Each cow will be bred on an individual basis. Reports from each software detailing cows identified in heat will be uploaded to files every AM (8am) and PM (5pm) shift.

Pregnancy check with ultrasonography will be made at 30 and 60 days post-breeding. Pregnancy diagnoses are a common practice in determining the maintenance of a pregnancy by the cow (Lamb, 2001). There is a risk for cows to not have the ability to maintain a pregnancy or to lose the pregnancy between days 38 and 90 (López-Gatius et al, 2002). Therefore, it is necessary to monitor the pregnancy after breeding to reduce days open, increase conception rate, and insure absences of abnormalities in the reproductive tract and cycling (Fonseca et al, 1983).

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