THE USE OF IMPLANTABLE MICROCHIPS FOR BODY TEMPERATURE COLLECTION IN CATTLE

BY

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DISSERTATION

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ABSTRACT

Thermal regulation via physiological processes has allowed mammals to survive and thrive in a number of diverse climates. The process of regulating body temperature is complicated and tied to many homeostatic systems in the body. That connection to different systems in the body allows for the use of body temperature to identify deviations from baseline. Rectal temperature (RT) has been used in cattle to identify immune system challenges (febrile response), heat stress, ovulation, and calving, but the ability to monitor body temperature in a production setting is labor intensive. Recently, implantable devices have been developed that have the ability to give real time temperature readings via radio frequency data transfer. The objective of these studies is to determine how well the temperature readings of these implantable devices correlate to RT in cattle exposed to thermoneutral and elevated environmental temperatures, with and without a lipopolysaccharide challenge, and in mature cattle during the periparturient phase (parturition and early lactation disease detection) and around the time of estrus.

In the first experiment it was hypothesized that in response to lipopolysaccharide (LPS) challenge, temperature patterns from three radio frequency implants (RFI) at three peripheral implantation sites {s.c. at the ear (ET), poll (PT), and umbilical fold (UT)} would be similar to RT patterns in weaned steers. Rectal temperature rose rapidly to 39.9 ± 0.30°C after LPS injection, but ET, PT and UT declined in similar fashion. These data do not support the hypothesis that core and peripheral temperature move in synchrony after LPS challenge. The ET implant site had the most robust differences from RT and showed promise as suitable implant location for future studies.

In the second experiment it was hypothesized that the temperature readings from RFIs implanted in the ear of pregnant cows would be positively correlated with baseline RT and negatively correlated with RT during a known health event, and that calving time could be predicted from temperatures measured by using RFIs. Rectal and ET temperatures were positively correlated during both the dry and post-calving periods, and were both higher during periods of high ambient (AMB; > 31 °C) when compared with periods of lower AMB (< 21 °C). A Multiple Local Property Correlation analysis correctly identified all animals experiencing a health event, but had a high false positive rate (flagged 75% of all animals). A negative correlation between RT and ET around parturition or during diagnosed health events (n=12) was not observed therefore this approach has limited value in prediction of parturition or early disease detection when ET is measured at 6 h intervals.

The hypothesis of the third experiment was that temperature measured by a RFI, implanted in the ear of cows (n = 32), would be positively correlated to RT during estrus. Rectal temperature and ET were positively correlated when temperatures were measured every 6 h (6H) as well as every 1 h (1H). Rectal temperature increased by roughly 0.6 °C around estrus using 6H, but ET did not increase significantly during that time, but the patterns of RT and ET were similar. The results of this study indicate that ear temperature monitoring via a RFI is not adequate to detect cattle experiencing estrus.

The hypothesis of the fourth experiment was that temperatures obtained from RFI in steers would be positively correlated with RT under high AMB conditions (20 °C vs. 34 °C) and negatively correlated with RT during a LPS challenge under high AMB. Pearson correlation coefficients for RFI and RT were 0.3 during heat stress, 0.20 during

heat stress with LPS challenge, 0.34 during the thermoneutral period, and -0.42 during the thermoneutral period with LPS challenge. Individual response varied; some animals exhibited negative correlation while others exhibited positive correlation. These data do not support the hypothesis and suggest that individual response be considered when identifying models for use of RFI in temperature monitoring.

The results of these studies indicate that the BioThermo microchips correlate well with RT under thermoneutral conditions. The positive correlation was similar to that in both dry and lactating cattle not experiencing disease events and continued in those cows before and after estrus in the third experiment. The positive correlation was again observed in a second cohort of young steers experiencing thermoneutral ambient temperature in the fourth experiment. There is a repeatable positive correlation between the microchip and RT during periods of thermoneutral ambient temperature and when animals are not experiencing a disease event. The results of these studies indicate that the BioThermo microchip system has potential for use in identifying animals experiencing a range of biological changes through the use of peripheral body temperature. In order for the system to be utilized in common commercial applications will require the development of longer read distances, more frequent data collection, and better data collection and analysis techniques. More research is needed to develop more robust models that take into account varying ambient conditions and individual animal variation.

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CHAPTER 1

REVIEW OF THE LITERATURE

Thermal Regulation

The improvement of survival mechanisms (the production of milk in mammals for example) resulted in vastly complex physiological systems, and in turn, the need to manage those systems within certain physiological ranges. Over time, the need to maintain those complex systems at a steady state resulted in systems of checks and balances which we now describe simply as homeostasis. Through evolution, some animal species have developed advanced systems of body temperature control. A key element in the complex system of body temperature control is the endocrine system, which serves to communicate inputs from an array of physiological variables to central integrators of homeostasis, that results in a steady internal temperature despite variable external conditions. Between the two main classes of body temperature control, ectothermic animals derive most of their body heat from external sources such as solar radiation. In contrast, endothermic animals also utilize external sources of heat, but rely mainly on internal metabolism to maintain core body temperature. Whereas the terms "cold-blooded" and "warm-blooded" are commonly used to describe ectotherms and endotherms, respectively, the terms are misleading. Much variation exists in core temperature for both classifications. Animals that maintain a relatively constant body temperature are described as homeotherms (Cannon, 1929) and this classification includes all traditional livestock. There are two main theories on the evolution of endothermy. The first theory on the evolution of endothermy is based on the fact that

selection for endothermy is based on resting metabolic rates and movements into environmental niches, subsequent efficiencies in metabolism due to homeothermy, and the possibility that regulation of maternal body temperature increases offspring survivability (Bakken and Gates, 1975; Heinrich, 1977; Farmer, 2000.) The second theory on endothermic selection focuses on the fact that heat production is not efficient enough to be the sole basis of selection. This second theory suggests that the ability to regulate body temperature increases the ability to change posture, increase brain size, and allow for higher aerobic capacity (Heath, 1968; Hulbert, 1980; Bennett and Ruben, 1979). Regardless of why, homeotherms have evolved to maintain core temperature through various physiological, morphological, and behavioral mechanisms and balance the heat gained from metabolism and that gained from or lost to the environment.

The maintenance of a relatively stable body temperature in homeotherms revolves around the theory of a "set point." While the thought of a defined point at which body temperature fluctuates has been in discussion since the 1960's (Hammel et al., 1963), subsequent work suggests that the set point is not a specific point, but rather one that fluctuates within a threshold zone (Benzinger, 1969). Active debate on the topic of set point continue with formidable arguments for the existence of a set point as well as the theory that the body regulates heat production and loss, not core body temperature (Cabanac, 2006).

Independent of the set point debate, domestic livestock exhibit circadian rhythms of body temperature that fluctuate around well-defined means, especially when exposed to ambient temperatures within the thermoneatural zone. For example, cattle have up to a 1.4 °C range in temperature throughout the day under ambient temperatures ranging from

22 to 28 °C (Piccione et al., 2003). The circadian rhythm of body temperature in cattle is, however, not present until 9 d of age (Piccione, 2003). In that study, there was little day-night variation in rectal temperature (RT) in the calves until day 9 when morning RT dropped 0.9 °C relative to dusk RT. The immediate shift from constant body temperature to a rhythmic pattern (Piccione et al., 2003), implies that there is a set point in the fluctuation of daily body temperature rhythm. This immediate start of rhythmic rectal temperature may be a function of shifting hormone receptor expression in the hypothalamus or pituitary gland as the neonate shifts heat production from metabolizing the small store of brown adipose present at birth to using feed nutrients, but there is little work to prove this theory.

The neonate has little need to regulate body temperature *in utero* as the healthy dam maintains a relatively constant body temperature. Once parturition occurs, however, the neonate is challenged by the need to regulate its own body temperature. There are two main environmental stimuli that could potentially influence circadian body temperature in homeotherms: ambient temperature and photoperiod. A biological clock based on temperature would result in faster speeds at high temperature and lower speeds at lower temperatures and would not be reliable for time measurement (Barrett and Takahashi, 1995). From an evolutionary standpoint, annual photoperiod changes are more consistent than daily ambient temperature and that consistency is likely critical in correctly modifying long term physiological events. Indeed, photoperiod has been identified to influence reproduction, growth, and immune function, as well as mammary development and output in cattle (Collier et al., 2006). In addition, a hormone generally associated with changes in photoperiod (prolactin; PRL) is also associated with

acclimation to increasing ambient temperature (Collier et al., 2006). Prolactin has also been linked to wool growth during short days in sheep (Allain et al., 1986). Decreasing the length of daily light exposure has also been shown to increase hair growth in cattle (Yeates, 1955). This allows for a thick cover of hair that provides insulation in the winter and the shedding of such hair as days lengthen in the summer and the need to dissipate heat becomes more important. In addition, mammals have acquired the ability to regulate body temperature physiologically through vasoconstriction and vasodilation. Under cold ambient temperatures capillaries constrict and peripheral blood flow is restricted to conserve heat. Mammals also use piloerection to create a buffer zone that traps air and improves insulating action of the pelage. During periods of high ambient temperatures capillaries are relaxed and blood flow to the periphery increases to move heat from the core. Thus, photoperiodic cues and the resulting hormone signals interact with temperature sensing systems and the corresponding hormone systems interact to provide flexibility in regulating body temperature during periods of extreme hot or cold weather.

Thermal regulation is a complex process that has led to the evolution of anatomical and behavioral adaptations among and within species. For example, African elephants (*Loxodonta Africana*), that live where ambient temperature is high year-round, have large ears that increase surface area for heat dissipation around the brain as well as provide the ability to fan air over the top of the body to aid in convective cooling (Buss and Estes, 1971). In addition African, elephants have a sponge-like skull structure with sinuses that act as insulation and keep the brain from becoming hyperthermic (van der Merwe et al., 1995). In contrast to other mammals, male elephants carry their testicles inside the body cavity, as ambient temperatures in Africa would render spermatozoa

ineffective (Short et al., 1967). By carrying the testicles inside of the body cavity elephants can maintain the spermatozoa at body temperature, or roughly 36 °C. Thus, specific adaptations have evolved to cope with variation in ambient temperature.

Although anatomical adaptations are apparent when comparing different species, there are also intraspecies differences. For example, there are differences in anatomical adaptations for thermal regulation among cattle breeds. These anatomical differences are readily apparent when comparing two subspecies of cattle, *Bos taurus* and *Bos indicus*. *Bos taurus* cattle (for example the Holstein-Friesian and Angus breeds) were developed in the northern parts of Europe, have thick strands of hair, denser hair strands, and readily deposit subcutaneous fat. In contrast, *Bos indicus* cattle (for example Zebu breeds) originate from warmer climates, have less dense hair coats, are lighter colored to reflect radiant heat, have lower metabolic rates, a specific fat storage area that also increases skin surface area and increased capacity for heat abatement (Hansen, 2004).

When ambient temperatures are outside of thermoneutral, cattle exhibit behaviors that help them maintain body temperature. During periods of high ambient temperature cattle orient their side into the wind to maximize surface area exposed and increase heat exchange (Keren and Olson, 2006) and stand to increase circulation to the limbs which effectively increases surface area for heat exchange. Cattle seek shade when daily temperatures peak, which reduces radiant heat accumulation by up to 30% (Blackshaw and Blackshaw, 1994). In addition, during periods of high ambient temperature, cattle reduce the number of meal events per day and ultimately limit intake to reduce heat production (Eigenberg et al., 1994, 1998). Grooming also increases in high ambient temperatures, which increases evaporative cooling from deposited saliva and also may

help electrolyte balance as animals ingest salts from the skin (Sly and Bell, 1979). When ambient temperature is below thermoneutral, animals shift behaviors to conserve heat. These include seeking shelter from the wind, huddling together, and turning into the wind to reduce the surface area exposed to the wind (Cymbaluk, 1994; Keren and Olson, 2006). Thus, cattle acutely modify behaviors to increase heat retention or loss depending on climactic conditions.

The Immune System and Body Temperature

A core body temperature fluctuation outside of the normal range indicates a disruption of one or more homeostatic pathway. Thus, body temperature is used as a way to monitor the general physiological state of homeotherms. The most common use of body temperature as a diagnostic tool is during an immune system challenge. The immune system releases pyrogenic factors that stimulate a febrile (fever) response usually resulting in hyperthermia. Because temperature is fast and easy to monitor and does not require time-consuming and costly assays, it is typically the first evaluation performed when trying to diagnose departures from normal homeostasis.

It is the innate immune system that responds first to non-self insults in the body. Phagocytic macrophages are the primary defense and those cells release protein molecules called cytokines and chemokines that act as chemo-stimulators of other immune system cells. The release of cytokines and chemokines results in a general inflammatory response characterized by heat, pain, redness, and swelling of the affected tissue. This response worsens with time and leads to lethargy, a reduction in feed and water intake, and without resolution, death. Fever, as associated with a contracted

disease, is not completely negative. Current theory suggests that individuals experiencing an immune response undergo physiological changes that allow them to regulate body temperature at a higher set point than non-challenged individuals to increase survival (Kozak et al., 2000). For example, an elevated temperature kills pathogenic organisms such as those causing syphilis and gonorrhea before other components of the host immune system can do so (Nelson, 1944). Behavioral modifications such as lethargy and lower water intake during illness, are thought to be survival mechanisms that facilitate recovery and decrease the risk of disease transmission to other animals (Hart, 1988). Most pathogens cannot cross the blood brain barrier and neurons do not have receptors that identify pathogens directly, so chemical signaling from immune cells provides communication with the brain during illness (Johnson, 2002). Cytokines secreted by mononuclear phagocytic cells, like interleukin-1β, reduce feed intake in animals (Plata-Salaman et al., 1988 and Finck and Johnson, 1997). A study comparing mice infected with *Listeria monocytogenes* allowed to eat *ad libitum* and infected mice force fed to normal feed intake resulted in 100% of the force fed mice dying compared with only 50% mortality of the mice fed *ad libitum* which suggests that a reduction in feed intake is beneficial during illness (Murray and Murray, 1979). The benefits of lethargy and reduction of feed intake for an individual animal center around energy availability and heat conservation needed to elicit a robust febrile response. During an immune response interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis facto-alpha (TNF-α) are released, and these cytokines are linked to the febrile response and a reduction in secretion and activity of hormones associated with growth and production such as IGF-1 (De Benedetti et al., 1997; Lazarus et al., 1993). The fact that

IL-1, IL-6 and TNF-α are released nonspecifically and are part of the fastest response to an immune system insult indicates that changes in body temperature are robustly activated before commonly observable responses in livestock occur (e.g. decreased feed intake, lethargy, etc.).

In addition to cytokine hormones, the hypothalamic-pituitary-adrenal (HPA) axis is also involved in immune function. Stimulation of the immune system causes release of cortisol through stimulation of the hypothalamus and subsequent release of adrenocorticotropic hormone (ACTH; Chrousos, 1995). Glucocorticoids have generally been thought to have negative effects on immune cells, and have been shown to stimulate apoptosis in thymocytes and cytotoxic T cells and reduce the production of cytokines by lymphocytes (Ashwell, 2000). Current theory places glucocorticoids as immune modulators that exert control in the immune system through negative-feedback effects to ensure relative homeostasis is maintained. Glucocorticoids also have beneficial effects on the immune system, including glucocorticoid-induced apoptosis that results in restructuring of T-cell populations (Ashwell, 2000).

Other hormones, such as PRL, growth hormone (GH), insulin-like growth factor 1 (IGF-1), vasopressin (AVP), and thyrotropin releasing hormone (TRH) have also been implicated in immune system function, but generally are thought to be minor modifiers that offset the effects of the glucocorticoids. In the mouse, PRL does not function as a direct immunolregulatory hormone because PRL receptor knockout mice exhibit normal immune system development and function (Horseman et al., 1997; Bouchard et al., 1999). Yet PRL reduces the suppression of mitogen-induced lymphocyte proliferation by corticosterone in mice (Bernton et al., 1992). Concentrations of GH and IGF-1 increase

during stimulation of the immune system and have been described as antagonists to glucocorticoids (Kelley et al., 1991). Administration of exogenous GH partially reverses the acute, cortisol-induced inhibition of T-cell proliferation in rats (Chatterton et al., 1973). Vasopressin has an effect on the immune response, and more specifically the regulation of fever. Administration of AVP into the brain reduces experimentally induced fever (Kasting, 1989) and administration of an AVP receptor antagonist elevates fever in rats (Cooper et al., 1987). Thyroid hormone receptor-deficient mice exhibit reduced proliferation of immature B-cells in bone marrow (Arpin et al., 2000), but there are no other examples of thyroid hormones directly affecting the immune system. Thyroid hormones are, however, important for regulating metabolic processes and are released during an immune response, resulting in increased body heat production which aids in the febrile response.

While a complicated hormone feedback system is involved in an immune response, it is the quick responding innate immune system, and the cytokines released by those immune cells, that stimulate the first rise in body temperature (Bligh, 1982) before the rest of the hormone systems react. That fact makes core body temperature an excellent diagnostic tool for early detection of disease in livestock.

Heat and Cold Stress

The Economic Impact of Heat Stress on Livestock

A meta-analysis of over 250 journal articles (Pollack and St. Pierre, 2002) estimated that heat stress costs the livestock industry approximately $2.5 billion a year, an amount that represents as much as 50 percent of total net farm income. With regard to

dairy cattle, reports from producers in New York claim an acute 9 kg/d decline in milk production during a particular heat wave (Gooch, 2000). That 9 kg/d loss in milk production translates into a loss of $1,300/d for a farm milking 500 cows at $13/45.4 kg. Further, that estimate does not take into account depressed reproductive efficiency, long term milk lost due to mammary cell loss and subsequent depression of the lactation curve, challenges to the immune system, and effects on young stock. Another analysis of nearly 35 years worth of temperature and milk production data from Australia estimates a milk production loss of six percent due to heat stress in cows (Mayer et al., 1999). Thus, temperature measurement is of interest not only to monitor animal health but performance as well.

Physiological Changes in Cattle During Heat Stress

As homeotherms, cattle can maintain a relatively constant body temperature despite dramatic environmental temperature fluctuation. There is an optimal temperature zone in which cattle can maintain normal homeostasis without needing any additional energy or adaptive behaviors, defined as their thermoneutral zone. For lactating dairy cattle, the thermoneutral zone is reported to be in the –0.5 to 25 °C range (Johnson, 1987 and Berman et al., 1985). As temperature rises beyond the upper limits of the thermoneutral zone, the animal begins to shift energy to heat loss, which alters normal homeostasis. As animals are exposed to increasingly higher ambient temperature, a heat induced stress occurs, resulting in a reduction in health, production and eventually life if the animal cannot control heat accumulation. Identifying when animals are experiencing

heat stress allows producers to implement heat abatement strategies that minimize the negative effects of heat stress.

The difficulty in determining when an animal is experiencing heat stress lies in the large variation among individuals (with different surface areas, coat color and adipose stores) as well as a large difference between sub-species (*Bos indicus* versus *Bos taurus*). As heat abatement in cattle depends on many facets of thermal exchange, it is important to report accurate information with regard to environmental conditions. The environmental condition that an animal perceives is an intricate combination of temperature, humidity, radiant heat, air movement, ionization and barometric pressure. The balance of those elements results in a large range of environmental conditions to which researchers are expected to describe animal perception. It is common practice to use only a few variables in an index to simplify the description of environmental conditions. The most popular combination index is that of temperature and humidity. In order to consistently describe environmental conditions concerning heat stress, it is necessary to have a common way of measuring and relating the various different combinations of dry bulb temperature and humidity. An easy way to do that is to incorporate both into a single index (THI, temperature-humidity index (Mayer et al., 1999)). The common calculation of the THI is:

THI = temperature (°C) + 0.36 dew-point temperature (°C) + 41.2

When THI is used, it is easier to describe average thresholds where animals become stressed. For example, Hahn and Mader (1997) described a THI of greater than

84 during the day without a reduction to a THI below 74 during the night causing mortality in vulnerable animals. Increasing THI to a peak daily THI of 77 also depresses dry matter intake (DMI) in cattle (Johnson et al., 1963) which can have a negative impact on growth and production. The determination of maximum THI critical value to be 76 (Igono et al., 1992) and the confirmation of a negative correlation between THI and DMI (Holter et al., 1997) solidify the relationship between higher THI and a state of anorexia. The relationship between milk yield and THI has been reported as 0.2 kg loss in milk per unit increase in THI over 72 (Ravagnolo et al., 2000). Other research suggests that there is a 0.41 kg milk/d loss for every THI unit increase over 69 (Bouraoui et al., 2002). Heat stress appears to affect cows at a higher yield more than cows producing at a lower rate. Tapki and Sahin (2006) found that high producing cows (>25 kg milk/d) reduced production by 16.1% and low producing cows (<20 kg milk/d) decreased production by 11.6% as cows entered periods of ambient temperature exceeding 25 °C. In addition, RT has been identified as a key determinant of heat stress in cattle. A THI of 93 (+/- 3.1) increased the RT of Holstein cattle by 0.47 °C (Srikandakumar and Johnson, 2004). The digestible nutrient intake also dropped by an average of 1.6 kg per 0.55 °C increase in RT (Srikandakumar and Johnson, 2004). The established state of decreased nutrient uptake and the obvious reduction in milk production during periods with a high THI are the end product of a complex physiological accommodation in the animal.

In order to survive short bouts of heat stress animals have developed behavioral and physiological adaptations. These adaptations include the previously mentioned increase in body temperature and reduction in DMI, but also include increased sweating, respiration rates, urination, and blood flow to the skin surface via vasodilation

(Blackshaw and Blackshaw, 1994). The complexity of the response and the plethora of available research describing such responses lend themselves to an in-depth review of the physiology behind each of the responses.

The most evident explanation for the decrease in DMI with heat stress is the heat increment associated with the digestion of feed and with natural foraging behavior that requires a large amount of movement and muscle use. The increased digestion and energy expenditure are associated with an increase in metabolic rate, and therefore, heat production. By decreasing movement and DMI, an animal can effectively reduce its heat load. In addition, Alvarez and Johnson (1973) reported an increase in plasma catecholamine concentrations during heat stress in cattle. Catecholamines have been known to induce vasoconstriction and reduce the rate of passage through the gastrointestinal tract (Felig and Frohman, 2001). A reduction in rate of passage decreases the need for the animal to expend energy foraging for more feed. A lower rate of passage increases the digestibility of the feed (Warren et al., 1974) allowing the animal to get a similar quantity of nutrients while eating less feed and expending less energy foraging. It is this reduction in DMI that causes the lasting affects of heat stress in lactating cattle, as there is a 2-day lag time between high THI, a reduction of DMI, and the drop in milk production (West et al., 2003).

Whereas RT and feed intake are observed at the whole animal level, there are many physiological changes that occur in cattle during heat stress. These changes affect acid-base chemistry as well as numerous endocrine pathways. Unlike a febrile response, increased body temperature results from high ambient temperature and radiant heat, coupled with metabolic heat and the heat of fermentation in ruminants. Sensory neurons

throughout the body that sense temperature convey messages to the hypothalamus. In response, the hypothalamus coordinates the anterior and posterior pituitary gland responses to changes in environmental temperature. The pituitary gland also controls a large number of the hormonal pathways that are affected by the thermoregulatory system via the hypothalamus. During periods of heat stress, blood cortisol concentrations increase in cattle (Wise et al., 1988), similar to other forms of stress such as an immune challenge (Chrousos, 1995). While the increase in cortisol is similar to that accompanying a fever response, other hormone changes during heat stress are rather different. During heat stress, GH secretion is reduced (McGuire et al., 1991) resulting in a decrease in metabolic activity and a negative effect on milk production, whereas in an immune response GH secretion is increased (Daniel et al., 2002). This signifies the need for a reduction in metabolic heat during heat stress and conversely the need to increase metabolic heat to induce a fever response in a disease situation. The administration of recombinant bovine somatotropin increases RT in cows under heat stress conditions (Elvinger et al., 1992). Circulating PRL concentration increases during periods of high ambient temperature (Tucker and Wetteman, 1976) which may mitigate some of the negative effects of glucocorticoids on the immune system as previously mentioned. Both GH and PRL have direct effects on mammary gland biology, cell metabolism, and ultimately milk production. In addition, during heat stress, both insulin and thyroxine concentrations in the blood decrease (Itoh et al., 1998; Collier et al., 1982), which reduces the metabolic activity of the animal, resulting in lower metabolic heat production. During heat stress the hypothalamus alters the release of hormones to reduce the negative impact high environmental temperature. In a heat stressed animal, hypothalamic signals

down regulate processes that produce heat and up regulate those that dissipate heat. Hormones that stimulate metabolic rate and thus heat production, including GH and T_4, decrease in concentration (Collier et al., 1982).

With regard to blood chemistry, many changes occur as cattle shift cooling mechanisms from conduction and convection to evaporation. As THI increases, cows sweat more and respiration rate rises to increase heat loss via evaporative cooling in the lungs (Hahn, 1999). The increase in respiratory rate results in excessive loss of carbon dioxide, thereby reducing circulating concentrations of carbonic acid, leading ultimately to respiratory alkalosis (Benjamin, 1981). Respiratory alkalosis results in the need for cattle to eliminate bicarbonate via urinary excretion (Benjamin, 1981). The need to balance blood pH during homeostasis results in large shifts in alkalosis and compensatory acidosis during periods of heat stress (Schnieder et al., 1988). With respect to increased sweating, cattle lose significant quantities of sodium and potassium during periods of heat stress (Jenkinson and Mabon, 1973). The changes in acid-base balance and mineral balance, as well as a reduction in DMI due to heat stress, are key factors involved with determining strategies to mitigate the effects of heat stress.

Strategies to Reduce Heat Stress in Dairy Cattle

Because of the aforementioned negative effects of heat stress, it is important to consider ways to mitigate the effects of high ambient temperatures in dairy cattle. Beede and Collier (1986) identified three main management strategies to alleviate heat stress in dairy cattle: 1) environmental modification (blocking radiant heat), 2) genetic selection for animals that are less likely to exhibit the negative effects of heat stress, and 3) altering

nutrition to decrease heat production and maximize DMI. Genetic selection, being a slower approach to solving heat stress issues, has received relatively less research emphasis. Environmental modification often requires considerable investment to incorporate into current management schemes, but is becoming more commonplace in modern facilities, especially those in the southern part of the U.S. where THI tends to be higher throughout the year. Nutritional strategies are integrated much more easily and quickly, with less investment. The use of body temperature as a diagnostic tool means that current and future strategies may require producers to be aware of changes in ambient temperature and humidity and account for them in schemes that use body temperature to identify animals experiencing disease events outside of heat stress.

There are many possible approaches when considering environmental alternatives that reduce heat stress. In some production units, the installation of a shade cloth or a roof can reduce heat stress by reducing radiant heat sources. In grazing situations, the addition of trees in fields, or the use of fields with trees already in them, offers shade to animals during hours of peak daily temperature. Indeed, cows provided with shade had lower RT, reduced respiratory rates, and produced more milk than cows with no shade (Roman-Ponce et al., 1977; Collier et al., 1981). Common barn design incorporates high-pitched roofs (4 in 12 pitch or greater) to raise heat exchange away from cattle and take advantage of thermal flows rising to the top of the building and away from the animals. The simplicity of offering shade is typically cost effective in all production settings and is generally used as the foundation for other cooling strategies.

Whereas shade offers relief from radiant heat exchange, other strategies take advantage of altering THI. Air-conditioning to reduce the temperature of air entering

barns increases milk production nearly 20 percent (Thatcher, 1974; Hahn et al., 1969; Roussel and Beatty, 1970). The limitation to use of tempered-air systems is the cost to run the equipment, which is considered uneconomical (Canton et al., 1982). As technology advances, air-conditioning may become an economical way to cool cows.

The use of fans to move air through buildings improves heat exchange by removing warm humid air. In addition, higher speeds of air across animals improves the exchange of moisture (i.e., convective cooling) from the skin as the animal sweats. Mechanized air movement in modern barns is accomplished using fans (either in the barn or located at one end of the barn pulling the air across the barn as in tunnel or cross ventilated barns). The addition of water to the skin of cattle and use of fans increases evaporative cooling of the cow's micro-environment. The use of sprinkler and fan combinations increases milk production up to 3.6 kg/cow/d during high ambient temperature (Turner et al., 1992; Her et al., 1988). Another way to alter air temperature is through evaporative cooling, where warm outside air is passed through pads saturated with water. Although evaporative pads can reduce inside air temperature, the effectiveness of such systems for cooling cows has been questioned, particular in the southeastern U.S. where humidity is high for extensive periods of the year. For example, evaporative cooling reduces respiration rate and body temperature, but has no effect on milk yield (Taylor et al., 1986; Brown et al., 1974). Further, these strategies are important in the design of new facilities, but are harder to retrofit to older production facilities.

Altering the diet of cattle is rapid, simple, and often a cost effective way to alleviate some of the negative effects of heat stress. Nutritional modulation of the heat

stress response is the subject of many reviews (Fuquay, 1981; Collier et al., 1982; Beede and Collier, 1986; Huber et al., 1994; Sanchez et al., 1994; West, 1994; West, 1998). In summary, these studies suggest that there are six main approaches to dietary manipulation to address heat stress. These focus on the availability of water, feeding time and frequency, formulation of diet based on reduced DMI, increased need for certain nutrients, heat increment due to feed, and the avoidance of excess nutrients.

Water is often the most limiting nutrient during periods of high THI. Murphy et al., (1983) reported that lactating cows increased water intake by 1.2 kg/°C increase in minimum ambient temperature. In addition, offering chilled water reduced body temperature and increased milk production in lactating cows (Milam et al., 1986). The amount of feed offered, time of feeding, and frequency of pushing up feed can influence DMI. During warm periods as DMI decreases, feed often spoils faster making many rations unpalatable, exacerbating the drop in DMI. Therefore, it is important to offer smaller batches of feed, push feed up more frequently, and/or feed more volume at night when it will stay fresher and cows will eat more of it during the cool hours.

After water and feed access and delivery are addressed, the remaining nutritional considerations can be managed together as a special formulation of the ration. As DMI decreases, the need to increase energy density of the ration becomes important to ensure that the cows receive an adequate nutrient supply. An easy way to increase the energy content of the ration is by adding oilseeds, for example, whole cottonseed. In addition, the replacement of calories from forages that increase the heat increment of a ration also helps to alleviate heat stress. As respiration rate increases and blood metabolite concentrations change, additional potassium and sodium chloride can be added to the

ration or offered free choice to meet increasing demand. However, decreasing the forage content and increasing the concentrate proportion of the diet increases the risk of rumen acidosis, necessitating the addition of a buffer product in the ration or as a free choice option. Excess nitrogen in the form of protein in the diet can also occur as more grain is added to the ration. The energetic cost of protein metabolism required to convert excess nitrogen is associated with increased RT (Hassan and Roussel, 1975). Feeding more rumen undegradable protein to shift the protein out of the rumen and into the lower gastrointestinal tract increased milk production 2.4 kg/d in heat stressed animals (Belibasakis et al., 1995). Thus, simple ration formulation changes during months of high THI could alleviate some of the negative effects associated with heat stress.

There is no doubt that heat stress is an economic burden to dairy producers in the U.S., especially in the southern portion of the country. As THI increases outside of the thermoneutral zone, dairy cattle become stressed and physiological processes shift from milk production and reproduction to heat dissipation. It is also important to recognize that high producing animals are more susceptible to reductions in milk yield in association with heat stress. Core body temperature is elevated during periods of high ambient temperature and can be used to identify animals experiencing heat stress. In addition, management strategies can be employed to reduce the effects of heat stress. However, management practices may increase variation in body temperature; for example, when a cow takes a drink of cool water, and body temperature declines. Therefore, it is important to be cognizant of changes in management and stage of lactation when using body temperature as an end point to diagnose animals experiencing disease events during periods of heat stress.

Cold stress

Compared with heat stress, far less is known about the effects of low ambient temperature on adult cattle. Much of this is due to the fact that the lower limit of the thermoneutral zone extends below freezing and common housing facilities usually keep the ambient temperature above that set point. Therefore, mature cows rarely experience temperatures at the lower extreme of their thermoneutral zone. Young cattle, however, more frequently die from cold stress due to a lack of fat cover and a functional rumen (i.e. to provide heat increment of fermentation), and a higher surface area to bodyweight ratio that increases body heat loss. In addition, neonatal calves lose heat through the evaporation of amniotic fluid from their body surface shortly after birth (Carstens, 1994). Indeed, Azzam et al. (1993) observed an increase in mortality as ambient temperature decreased in *Bos taurus* calves.

There are two mechanisms that newborn calves use to maintain short term homeothermy; shivering thermogenesis, via muscle tissue metabolism, and non-shivering thermogenesis via brown adipose tissue metabolism (Alexander, 1979; Alexander et al., 1975). Neonatal Brahman calves showed a 1.8 and 1.2°C decrease in RT in ambient temperatures of 4 and 5°C respectively when compared to ambient temperatures > 25 °C (Godfrey et al., 1991; Stanko et al., 1992). Yet no change in RT was reported during the 4°C experiment for Simmental x Brahman x Hereford calves, suggesting breed differences in cold stress tolerance occur (Godfrey et al., 1991). Further, higher morbidity and mortality rates occur in newborn Bos indicus calves compared with Bos taurus calves under cold stress (Josey et al., 1987). Brown adipose tissue weight is higher

in Bos taurus calves relative to Bos indicus calves (Martin et al., 1999), suggesting that brown adipose tissue contributes to increased survival due to non-shivering thermogenesis during periods of cold stress. In addition, fall born Bos taurus calves had less brown adipose mass compared with spring born Bos taurus calves (Martin et al., 1999), which suggests a seasonal effect on brown adipose tissue deposition. This is likely due to environmental cues (i.e. ambient temperature and photoperiod) in the ability of calves to survive postpartum.

Shortly after calves begin to nurse, the source of energy used to generate heat shifts from brown adipose tissue present at birth to nutrients derived from the diet. The lower critical temperature for calves increases by 0.89 °C/d from d 6 to 11, so an ambient temperature above 14 °C is recommended (Schrama et al., 1993). The calf requires higher energy when ambient temperature drops below 14 °C. Relative to normal energy density, increasing fat supplementation (113 g additional fat/d) in milk replacer during periods of cold ambient temperatures increased fecal scores and BW gain of calves in the first month of life (Jaster et al., 1992). The introduction of solid feed induces rumen development and allows the calf to start producing heat from fermentation in the rumen. Thus, as the calf's digestive tract develops, so does the abilty to cope with lower ambient temperatures.

In adult ruminants that have free access to a balanced ration and proper body condition and hair coat, direct cold stress is rare in dry, cold regions (Webster et al., 1970; Webster, 1974; Young and Christopherson, 1974). The presence of wind and moisture, however, reduce the effectiveness of the animal's coat to provide thermal insulation (Joyce and Blaxter, 1964; Webster and Park, 1967; Ames and Insley, 1975).

The induction of winter hair coat production occurs as photoperiod shifts to shorter days (Yeates and Southcott, 1958) and the hair coat is maintained during cooler ambient temperature (Webster, 1976). In addition to increases in coat length the potential summit metabolism is also increased in animals that have adapted to chronic cold weather (Jansky, 1971). Chronic cold ambient temperature increases appetite (Baile and Forbes, 1974) rumination activity, and rate of passage through the gastrointestinal tract (Westra and Christopherson, 1976).

A decrease in nutrient intake or body temperature could be a sign that an animal is not producing enough heat to maintain homeostasis and could be used as a diagnostic tool to assess cold stress. Decreases in body temperature during periods of cold stress could be used to determine times when altered feeding or housing strategies could be employed to maintain production in mature animals and reduce death loss in neonatal calves.

Body Temperature in Reproduction

Body Temperature and Parturition

In production settings where efficiency is optimized, much of the labor is focused on tending to the majority of animals in a group. Thus, many animals are unattended during times of individual stress such as parturition. Nearly 64% of calf deaths that occur within the first few days of life are caused by difficulty during parturition (Patterson et al., 1987). In addition, Bellows et al. (1987) estimates that providing manual assistance to the cow and calf during parturition could prevent approximately 50% of dystocia-related mortality. The ability to determine when livestock are nearing parturition individually could yield greater opportunity for individual attention and potentially

reduce the number of calves lost due to dystocia. There is also evidence that moving animals in and out of group pens around calving increases stress. Unpublished field data from Wisconsin (Oetzel, personal communication) suggest that moving cows into a calving pen for a week or more increases circulating non-esterified fatty acid concentrations in blood, increases the risk for displaced abomasum, and ultimately decreases milk production. The ability to know when the cow is within 24 hours of parturition could help producers reduce the time spent in the calving area and potentially reduce the incidence of fresh cow disease.

There is evidence that body temperature is useful to identify cows that are nearing parturition. For example, Weber (1910) observed a decrease in body temperature in cattle 28 h before calving. The preparturient decline in temperature in cattle was described as 1°C by Vollmann and Vollmann (1942). More recently, Lammoglia et al. (1997) reported a decrease in body temperature between 8 and 48 h before calving in beef cows. Further, cows gestating female fetuses had a significantly lower body temperature than those gestating male fetuses 3 to 6 d prior to parturition. Specifically, a nadir in body temperature occurred 16 h before parturition and was followed by a steady increase through parturition and into lactation (Lammoglia et al., 1997). Birgel et al. (1994) used temperature as an indicator to predict the occurrence of 43.5 % of parturitions within 22 h in cows. In this study the authors observed that progesterone (P_4) was an indicator of the onset of parturition with 91.3% accuracy within 22 h of parturition. In contrast, body temperature in periparturient mares exhibits a nadir at parturition (Cross et al., 1992), with a mean decline in body temperature of 0.76 °C. One explanation for the decline in body temperature is that as blood flow shifts to the reproductive tract to support an

increase in metabolic activity in that tissue, respiration increases, and the end result of the removal of fetus, amniotic sac, and amniotic fluid from the core of the mare reduces thermal mass. Although species differences are apparent, the relative shift in body temperature could be used to predict parturition,

Hormonal fluctuations potentially drive body temperature changes around parturition. Parturition in sheep is initiated by the fetus, and more specifically through fluctuations in fetal hypothalmo-pituitary axis (First, 1979). Indeed, failure of parturition occurs when fetal congenital pituitary defects are present (First, 1979). The primary pituitary hormone responsible for the initiation of parturition in sheep is adrenocorticotropic hormone (ACTH). The administration of ACTH causes premature birth, and fetal hypophysectomy followed by ACTH replacement induces parturition in sheep (Jones et al., 1978). As fetal ACTH concentration increases, fetal adrenal glands exhibit growth, followed by an increase in fetal adrenal steroid production, including cortisol. The administration of synthetic analogues of cortisol initiate parturition in domestic species (First, 1979). In cattle, where the corpus luteum is needed to maintain pregnancy, concentrations of fetal cortisol appear to increase two weeks prior to parturition and the concentration of maternal prostaglandin F2-alpha ($PGF_{2\alpha}$) increases as well (First, 1979). The increase in $PGF_{2\alpha}$ causes the regression of the corpus luteum, and the subsequent decrease of P_4 and an increase in estradiol (E_2) starting about 1 week prior to parturition (First, 1979). Within 2 days of parturition, coincident with the body temperature decline, E_2 and $PGF_{2\alpha}$ are rising and P_4 is dropping. Maternal glucocorticoids and PRL also increase sharply within 24 h of parturition (Hudson et al., 1976 and Auchtung et al., 2005). Thus, the cascade of hormonal events initiated by the

fetal adrenal steroid system, likely drive body temperature shifts in the periparturient period.

In the study by Lammoglia et al. (1997) ambient temperature, time of day, and birth weight of the calf were positively correlated with body temperature before the onset of the decline in body temperature. Circulating concentrations of progesterone, E_2, thyroxine (T_4), and triiodothyronine (T_3) were not correlated with body temperature before the drop in body temperature, but cortisol and $PGF_{2\alpha}$ were positively correlated with body temperature before the onset of the drop in body temperature. During the drop in body temperature, P_4 became positively correlated with body temperature and $PGF_{2\alpha}$ became negatively correlated with body temperature. After the drop in body temperature none of the hormones examined in the experiment were correlated with body temperature, which suggests that the hormonal-temperature relationship was very time specific and could be used to predict time of parturition.

A drop in body temperature of 1 °C roughly 16 h before parturition provides an opportunity for its use as a management tool to identify animals as they near calving. This decrease in body temperature could coincide with the timing of movement into short-term calving facilities so that stress could be minimized. The identification of animals nearing parturition by at least 8 h would also allow flexibility in moving animals into short-term housing in a production setting. But, these approaches are dependent on a reliable method of frequent body temperature measurement in individual animals.

Body Temperature and Estrus

Reproductive performance is important in all areas of animal agriculture. The ability to get animals pregnant, and keep them pregnant, results in a saleable product. In the dairy industry, getting cows pregnant in a timely manner means that they spend more time at the beginning of the lactation curve where milk yield is highest. This increases the efficiency and overall profitability of the cow. Failure of conception results in premature culling and the need to purchase replacement animals, and increases costs associated with semen, veterinary pregnancy checks, and hormone treatments needed for estrus resynchronization. As number of services increases, producers are more likely to use lower quality semen or an unproven bull to reduce costs (Fricke, 1997). While these practices may result in pregnancies, they reduce the overall genetic merit of the herd, causing long-term losses in profitability. In contrast, a reduced calving interval maximizes the number of available replacement animals that can either be sold or used in the herd as involuntary culling is decreased. The estimated loss to U.S. dairy producers each year associated with failure to detect estrus is roughly $300 million (Senger, 1994). The cost of not getting cows pregnant varies and increases as time passes. Plazier et al. (1997) found that the cost of pregnancy failure varied from -$0.29 to $2.60/d. Marginal increases in heat detection and conception rate are also valued at $1.15 to $1.66 and $1.92 to $2.61 respectively, for a 1 percent increase in each. Thus, there are two primary factors that influence the proportion of cows that a producer can get pregnant 1) the ability to identify animals in estrus and 2) the ability to inseminate animals in estrus using viable semen and proper techniques.

Whereas adequate training, standard operating procedures and quality control ensure that effective insemination can be accomplished, it is difficult to achieve a

successful pregnancy without identifying the correct time to deposit semen. Up to 80% of dairy farms in the U.S. use artificial insemination to impregnate cows (Pursley et al., 1997). Traditionally, heat is detected by visual observation of animals exhibiting estrus behaviors. Of these behaviors, standing to be mounted is currently the most reliable behavior to determine when cows are receptive to breeding (Glencross et al., 1980). The use of visual observation is, however, labor intensive and cannot be performed 24 h/d. Cows tend to exhibit estrus behavior for a short period of time that ranges from 5.7 h to 7.1 h (At-Taras and Spahr, 2001; Dransfield et al., 1998). Further, high producing cows tend to exhibit estrus behavior for less time than low producing cows (Harrison et al., 1990) and cows exhibit roughly twice as much mounting behavior in cold weather than in hot weather (Pennington et al., 1985), which makes visual observation of estrus activity less effective in the summer. There are various methods, besides visual observation, in use today that increase a producer's ability to identify animals that are at the correct point in the estrous cycle to optimize breeding success.

One method to achieve successful pregnancies is complete elimination of visual observations and use of a timed artificial insemination program. Synthetic hormones are used to "reset" the natural estrous cycle and synchronize the cycle so that the time of ovulation is known. The most common protocol involves the administration of gonadotropin releasing hormone (GnRH), followed by $PGF_{2\alpha}$ one week later, followed by GnRH 48 h later, and ending with insemination the next day. This protocol is commonly referred to as Ovsync and has been shown to result in up to 100% of cows exhibiting a new follicular wave (Pursley et al., 1995). In two studies (Fricke et al., 1998 and Fricke et al., 1999) 85% of cows enrolled in an Ovsync program ovulated a follicle with 38%

and 41% pregnancy rates respectively. Other reproductive protocols have been developed that utilize an additional $PGF_{2\alpha}$ injection and/or the addition of an intravaginal P_4 insert (Brusveen et al., 2009; Stevenson et al., 2006). These programs have resulted in similar pregnancy rates when compared with traditional visual observation of estrus behavior (Jobst et al., 2000), but still require precise scheduling and labor to administer hormones.

There has been some movement toward developing systems that can monitor animals passively. The use of pedometers can identify 74% of animals in heat compared to 58% utilizing visual observations of estrus behavior (Heat Seeker; Boumatic, Madison, WI; Liu and Spahr, 1993). Devices mounted near the tail head of the cow (HeatWatch; DDx, Inc., Boulder, CO), are able to identify 87% of animals exhibiting standing behavior versus 54% for visual observation (At-Taras and Spahr, 2001). Another tail head-mounted device (TattleTale; Microdyne Company, Inc., St. Joseph, MO) detected 35 of 38 (92%) synchronized Angus cattle expected to be exhibiting estrus behavior (VanEtten et al., 2006) and 19 of 23 (82.6%) synchronized Holstein cows expected to exhibit estrus behavior (VanEtten, 2007). These systems reduce the need for hormone administration and the labor involved with visual observation, but still identify animals exhibiting standing estrus.

Estrus is another physiological event during which noticeable changes in body temperature occur. Indeed, Kyle et al. (1997) reported an 89% estrus detection rate using temperature sensing vaginal implants. The increase in vaginal temperature peaked at 0.9°C above a 3 d baseline period and the temperature rise lasted for an average of 6.5 h. Further, Redden et al. (1993) observed an elevation in vaginal temperature of 0.6 °C

lasting from 2 to 10 hours around estrus in dairy cows. Using a rectal probe to measure temperature twice daily, Piccione et al. (2003) reported an increase in body temperature during estrus. That increase in RT averaged 1.3°C during both the winter and the summer seasons. The benefit of a system that utilizes body temperature as an indicator of ovulation, rather than behavior, is highlighted by a study by Nieuwenhuizen et al. (1979), wherein 8 of 20 cows did not show estrus behavior, but exhibited an increase in body temperature and did ovulate.

Infrared technology has been employed to identify animals in estrus with a 93% success rate (Hurnik et al., 1985). In that study, Hurnik et al. (1985) determined that infrared technology was useful, but a 33% false positive rate led to the conclusion that the technology is limited for use as the sole detection method for determining time of estrus. The temperature of milk has been explored as a method to detect estrus. Fordham and colleagues (1987) found a strong positive correlation between vaginal temperature and milk temperature measured at four locations in the milking unit when measured at peak milk flow rate. Comparing the previous 3 d milk temperature average with the current day milk temperature, an increase of greater than 0.2 °C resulted in the highest rate of estrus detection (73.3 %) and the lowest rate of false positives (10.8%) (Fordham et al., 1987b). These studies suggest that body temperature measurement holds promise in estrus detection, especially if technologies can be combined to utilize body temperature and milk temperature to increase detection and reduce the number of false positives.

Individual Identification in Animal Management

The Past and Current Use of Animal Identification

Individual animal identification (ID) was being used as far back as 3,800 years ago in Mesopotamia (Blancou, 2001). At that time, simple markings on the hides of animals were used to signify ownership. As time progressed, hot-iron branding evolved and animal registries were formed. Alexander the Great's (356-323 BC) horse was branded with the head of an ox and it appears that all horses owned by the Athenian cavalry were registered by color, brand, purchase price, and owner (Blancou, 2001). For the past 20 or so centuries not much has changed in the identification of animals. Breed registries today resemble very closely those originally developed, except that more information is recorded and at a faster rate.

The greatest accomplishments resulting from improvements in animal ID in the past few centuries center on two areas: 1) increasing the ability to monitor and prevent disease and 2) the use of individual records to improve production efficiency. In the past, effective control of disease was easier, as most animals moved within small geographic areas and controlling animal flow was simple. In the 20th century, advancements in transportation and food preservation have turned animal agriculture, and thus animal movement, into a global market. While this lowers food costs for consumers, it increases the risk of transmitting disease rapidly, thereby putting all of animal agriculture at risk. Even so, great advances have been made toward the eradication of diseases like scrapie and brucellosis, and those involved in the programs have credited the use of individual animal ID as an important contributor to success (O'Rourke, 2001; Houston, 2001).

The past two decades have brought about the widespread use of personal computers, animal databases, genetic tracking, and new ways to use ID to manage individual animals on the farm. The ability to monitor production traits in individual animals allows owners to breed more efficient animals and cull those that are less so. In intensely managed herds, animal movement and breeding programs, as well as many other day to day duties, are much easier with individual ID, which leads to a more efficiently run farm. Consider managing a 3,000 animal unit without some form of individual ID. Despite the trend for production units to increase in size to capture efficiencies of scale, the need to provide individual attention to animals remains the same. Individual animal ID allows for efficient movement of animals at each stage of production in and out of contemporary groups or facilities as needed. Further, individual ID allows producers to tailor programs to ensure each animal receives optimal management. In addition, current data can be linked to past data and then be used cow-side to provide decision support to manage animals more efficiently in real time. New technologies, such as radio frequency ID (RFID), allow for rapid, efficient data collection and a reduction in the error associated with manual data collection.

There are two main classes of animal ID being used in production units today: management ID and animal ID. Management ID is always temporary and does not serve to identify the individual animal, it is used to identify animals that require specific attention. Examples of management ID include leg bands, zip ties, duct tape, neck chains, and weatherproof paint or chalk. All of those tools utilize differing colors to identify animals that need different management. An example on a dairy farm would be the use of blue leg bands to identify

animals that are dry, and the use of chalk to identify a series of health observations postpartum (i.e. a new line drawn for each check-up the cow receives). In contrast to management ID, the goal of animal ID is permanent identification of an individual animal, although due to some performance limitations, this generally results in a semi-permanent ID. Examples of animal ID include brands, tattoos, ear notches, ear tags (i.e. metal, plastic, RFID), and microchip implants. Animal ID allows producers to record individual animal performance and identify animals that need to enter different management groups or protocols. Yet animal ID generally stops at the farm gate with only minimal integration into the movement of animals in commerce.

The U.S. National Animal Identification System

The U.S. National Animal Identification System (NAIS) is still under development, but includes three phases to its implementation. Phase one involves identifying premises where animals are housed, even temporarily. Phase two is identification of individual animals or groups of animals. The third and final phase utilizes the first two phases to track animal movements among premises. The ultimate goal of the system is the ability to trace all animal movements from a specific site, for example an area of a disease outbreak, within 48 h. Australia and Canada already have similar systems in place. The NAIS is not part of a Country Of Origin Labeling (COOL) program, but it could be used in marketing U.S. grown animal products. Establishment of state premises registration systems represents significant progress toward meeting Phase 1 goals. All states currently accept premises registrations and the U.S. total is approaching 500,000 registered premises. A few states have passed legislation for

mandatory premises registration. There is currently no estimated date that the U.S. will begin mandating individual animal ID and there are still many factors that will affect the implementation of Phase 2 and 3 of the NAIS. Specific goals of the NAIS are to be able to track 45% of beef and dairy cattle to premises of origin within 48 hours by March 2010 (USDA, 2008). An additional goal is to be able to identify 98% of poultry and swine operations within a disease outbreak radius within 48 hours by October 2009 (USDA, 2008a)

Those involved with animal health tend to see the value of a tracking system clearly. For example, although bovine spongiform encephalopathy is on the minds of today's consumers, animal health officials realize that a foot and mouth outbreak poses a greater risk and would quickly destroy much of animal agriculture in the U.S. The focus of the NAIS is to be able to track the source of a disease event within 48 hours, contain the spread of exposure in the event of a disease outbreak, and improve consumer confidence here and abroad. Lost markets translate into hundreds of millions of dollars in lost income per year. For example a reduction in U.S. beef exports from $2.5 bil. in 2003 to $631 mil. in 2004 (USDA, 2008b) occurred after bovine spongiform encephalopathy was found in the U.S. The implementation of the NAIS will most likely be focused on mitigating losses in export markets.

Despite the underlying economic reasons to support implementation, the willingness of consumers to pay for the NAIS is not a given. Thus, unless there is a large increase in of public concern and subsequent government funding, the cost of implementing the NAIS will largely fall on the producers who will be responsible for identifying the animals before the animals move in commerce. The need for technologies

aimed at adding value to the producer in addition to meeting the needs of the NAIS has become apparent. Technologies like RFID and implantable microchips that were initially investigated over two decades ago (Holm, 1981), are now resurfacing due to lower costs of production and the growing need for individual animal ID for management purposes and inclusion in the proposed NAIS.

Radio-frequency ID devices have been identified as the leading technology to track animals through production systems. The development of International Organization for Standardization (ISO) protocols (ISO 11784, ISO 11785) for RFID streamlines the vertical integration of the system and allows producers to select from multiple companies making compliant devices. The competition in the market place among companies due to the proposed implementation of the NAIS has decreased the cost of the technology to the producer. Use of RFID on farms has potential to increase handling efficiency and reduce data transcription errors while automatically logging data into microcomputer databases that can easily be used for management, as well as for animal tracking in the proposed NAIS.

The Use of RFID Systems in Temperature Collection

As described in previous sections, body temperature measurement can be used for disease detection, estimation of time of parturition, detection of heat or cold stress, and identification of animals in estrus. The need to collect temperature data with minimal labor has led to the design of many elaborate systems. Primitive systems use thermal probes connected via wires to computer modules that log data, whereas more recent systems employ onboard temperature sensing equipment and radio frequency (RF) data

transmission. The use of wired systems yields useful information, but is obviously not practical in production settings. Success has been achieved with surgically implanted (i.e. subcutaneous behind last rib) RF devices (Lefcourt and Adams, 1996; Telonics, Mesa, AZ), vaginally inserted RF devices (Kyle et al., 1997; Wildlife Materials Inc., Carbondale, IL), and rumen bolus RF devices (Prendiville et al., 2002; Innotek Inc., Garrett, IN). All of those methods provide accurate temperature readings, but have one common design flaw: they all have onboard power supplies resulting in variable length of operation. While perhaps less important in market steers that are slaughtered before 30 months of age, dairy cattle can have productive lives for up to 10 years, and none of the previously described systems have power capacity to last that long. In addition, surgical insertion is rather costly, can increase the risk for local infection, and implantation site damage can affect meat yield and quality. Vaginally inserted RF devices can fall out and are not practical around calving for obvious reasons. Relative to other RF devices, rumen boluses show increased variability due to differences in diet composition and heat of fermentation as well as large drops in temperature associated with water intake (Bewley et al., 2008). Due to the variability in rumen bolus data, the ability for similar systems to become separated from the animal, and the fact that those systems use onboard power systems that can fail, injectable microchip systems have more potential for long-term application.

The advancements of microprocessors and a corresponding decrease in circuitry size has allowed for the design of much smaller implants. In addition, the cost of producing the microchips is generally cheaper than that of the previously mentioned systems and the increased cost of more antennae needed to cover the entire facility could

theoretically be spread out effectively over time. That, coupled with a passive system that has no power limitations at the animal level, has lead to the development of microchips with temperature recording capabilities (Biothermo microchip, Digital Angel Corp., St. Paul, MN). The microchips are 12 x 2 mm passive devices and run at 134.2 kHz (ISO compliant for current animal ID systems). The microchips have a resolution of ±0.1 °C and an accuracy of ±0.5 °C as measured using a National Institute of Standards and Technology ITS-90 certified thermometer. The microchips are easily implantable and lack the complications of failing power supply, increased variation due to nutrient uptake, and possibility of the device coming out of the animal that rumen bolus systems have. However, a relatively short read distance limits the application of the microchip system. The current technology used with the BioThermo microchips is a handheld, battery powered reader that operates in the 125 to 400 kHz range and allows for approximately 5 cm of read distance compared to read distances of up to 60 m for some of the previously described systems using active devices and a large stationary antenna. The ability to maintain a small device size translates into having a much smaller antenna, and antenna size limits the read distance. Some of this can be overcome by using a stationary interrogator with a higher power output, but that requires multiple collection points or the ability of the microchips to log data onboard and upload data when in range of an interrogator. Another solution to the read-distance problem could be an alternate frequency. Some suggestion has been made to move to a higher frequency, in the MHz or GHz range, to gain read-distance. But evidence shows that as frequency increases, the ability to interrogate through material, in this case tissue, decreases. Further, background noise, especially metal in the case of production units, becomes more of a problem with

higher frequencies. For these reasons, maintaining a relatively low frequency system for the use in implantable animal ID is an advantage.

Individual RFID devices were originally developed to identify animals on a permanent basis for use in management systems that track individual animal performance, but the individual ID of animals also fits well into programs that track individual animal movements like NAIS. The plan for NAIS to track animal movement and reduce the spread of disease requires that all new technologies synchronize with current recommendations from the NAIS plan. In addition to use in the NAIS, many daily tasks can be expedited with the use of electronic ID. Vaccination schedules, reproductive synchronization protocols, general cow movement between groups, and individual milk production monitoring may be streamlined with the use of electronic ID. This is due to the reduction in transcription error and the absence of the need to visually identify animals using standard ear tags with hard-to-read numbers as well as the ability to store and update information using handheld personal computers linked to wireless electronic ID interrogators during common cow side practices. These advantages reduce labor expenditure, increase treatment accuracy, and ultimately lead to increased animal health and profitability on the modern production facility.

Summary

The fact that body temperature is regulated during homeostasis and is linked to immune function, parturition, and reproduction and the relative ease with which body temperature can be monitored make it a useful tool in the management of livestock. The impending animal ID system, which would require individual ID, hastens the need for

value added ID systems that will allow livestock producers to manage animals more efficiently. The advent of an implantable microchip (Biothermo microchip, Digital Angel Corp., St. Paul, MN) that has the ability to produce real-time temperature readings could provide such a system that will meet animal ID requirements as well as provide useful data to livestock producers. This system could be used to identify animals experiencing physiological changes and allow producers to make management decisions in a timely manner.

During an immune system challenge, immune cells release hormones that elicit a febrile response. The release of hormones in response to an immune challenge is also involved with other biological functions like appetite, motor activity, and metabolism. Feed intake and lethargy can be monitored to identify sick animals, but come after the initial rise in body temperature. The ability to identify animals before lethargy and reduced intake set in can hasten diagnosis and treatment. An initial test of the Biothermo microchips is needed to determine if the temperature readings from the microchips correlate to RT both under homeostatic conditions and during a febrile response.

Parturition is a stressful time for both neonate and dam. Nearly two-thirds of calf mortality that occur in the first few days of life are associated with difficulty during parturition. Many of the hormones involved in parturition are linked to body temperature regulation and there is evidence that body temperature drops by roughly 1 °C between 8 and 48 hours of parturition. The ability to use body temperature to identify animals nearing parturition could reduce pen movements, time in calving areas, and be used to increase animal observation to identify times when calving assistance is needed. There is

also benefit to test these Biothermo microchips in cows nearing parturition and during the postparturient time when there is an increase in the incidence of health events.

Reproductive performance is important to all areas of animal agriculture. In the dairy industry, the cost of not getting cows pregnant can be $2.60/d or more. In addition, an increase of one percentage point in conception rate is valued at up to $2.60 per cow per reproductive cycle. One of the necessities in a successful breeding program is correctly identifying animals that are in heat. This is often done by visual observation for animals exhibiting estrus behaviors like standing to be mounted. Cows tend to exhibit estrus behavior for a relatively short amount of time (5-7 h), and labor constraints do not allow for 24 h per day observation. Body temperature has been shown to rise by as much as 1.3 °C from 2 to 10 h around estrus. The potential use of body temperature to identify animals in heat could increase conception rates and profitability. Therefore, it would be beneficial to test these Biothermo microchips in cows during the estrous cycle.

Extreme environmental temperature can act as a stressor to homeotherms. As ambient temperatures exceed the thermoneutral zone, mammals are unable to utilize physiological, morphological, and behavioral adaptations to regulate body temperature. A stress response is invoked when ambient temperature rises above the thermoneutral zone. Heat stress results in negative hyperthermy, in contrast to a febrile response where a higher set point body temperature is beneficial. Increases in body temperature could identify times where heat abatement strategies could be employed. It is also difficult to determine the difference between heat stress and a febrile response when identifying animals that need heat abatement or medical treatment. Therefore it would also be

beneficial to test the Biothermo microchips during periods of high ambient temperature in conjunction with a lipopolysaccharide challenge to determine if differences are apparent.

It is therefore the goal of this dissertation to test the Biothermal microchips at varying times in the life-cycle of cattle and during different ambient conditions and to determine if correlation exists between temperatures collected from these microchips and temperatures collected via rectal probe.

CHAPTER 2

THE USE OF RADIO-FREQUENCY DEVICES TO MONITOR BODY TEMPERATURE IN STEERS GIVEN LPS

Abstract

Early detection of disease can hasten administration of treatments, alter the health status of animals and on a larger scale, prevent the spread of disease through a herd. Immune stimulation is often manifested as elevated core body temperature, as measured by rectal temperature (RT). Injectable radio frequency implants (RFI) are now produced with the capacity to remotely monitor temperature at the site of implantation, yet the fidelity of peripheral site temperature, determined by RFI, relative to core temperature in cattle is unknown. We hypothesized that in response to lipopolysaccharide (LPS) challenge, patterns at three peripheral implantation sites would be similar to RT patterns in weaned steers (n=4; BW 77 +/- 2 kg). These three sites were 1) under the scutiform cartilage at the base of the left ear (ET) 2) s.c. on the midline, posterior to the poll (PT) and 3) s.c. on the midline beneath the umbilical fold (UT). Animals were housed in controlled temperature rooms (between 18 and 21°C; n=2/room) and fed *ad libitum* cubed alfalfa and water and 0.9 kg/d of pelleted grain. Room temperature and humidity were logged every 15 min. and RT, ET, PT and UT temperatures were collected every 8 h daily. On d 7, 21, 22, 36 and 37, rectal and implant temperatures were taken every 5 min for 6 h, every 15 min for 3 h and every 30 min for 15 h. To test the RFI during a simulated immune system challenge, 0.1 ug/kg of LPS (E. coli 055:B5) was injected i.v. at 1000 h on d 22 and 37. Mean basal temperatures (°C) were RT (38.7 ±0.20), ET (37.1

±0.86), PT (36.7 ±0.57), and UT (36.3 ±0.97). Rectal temperature rose rapidly to 39.9 ±0.30°C after LPS injection, but ET, PT and UT declined in similar fashion. The drop in peripheral temperature was biphasic and consistent among sites. These data do not support the hypothesis that core and peripheral temperature move in synchrony after LPS challenge. However, these RFI have potential for use in the early detection of diseases that alter basal temperature.

Introduction

The identification of sick animals in a timely manner is pertinent to animal agriculture. Production losses due to a variety of illnesses can affect the profitability of production units. Estimates for dairy cattle alone averaged $172/cow/year in 1990 (Miller and Dorn, 1990). As animal production units become larger, it is becoming more important to identify unhealthy animals as quickly as possible to initiate treatment to ensure recovery and limit the spread of disease to reduce production losses. Currently, popular methods of identifying sick animals rely on visual and physical assessments and the use of RT to identify animals outside of steady state (i.e. a fever). The rectum has been used as an easily accessible point to monitor fluctuations in body temperature. Labor input to monitor RT is time consuming and expensive, especially for animals not in group-housing. In addition, the use of RT as a diagnostic tool requires a database to identify animal-specific fluctuations. When using only one temperature reading per day, it is also difficult to adjust for natural diurnal changes in animal body temperature (Araki

et al., 1984) as well as changes attributed to ambient temperatures (e.g. high rectal temperature during periods of heat stress; Legates et al., 1991). Some methods of remote temperature sensing have been proven to detect fever associated with an endotoxin challenge (Davis et al., 2003). While Davis and coworkers determined that those systems were not convenient or practical, the ability to use frequently collected data to define models that could identify animals experiencing fever was an encouraging result.

There is also benefit in the individual identification of animals in systems where group management is possible. This allows producers to create management groups and intensively manage animals during higher risk periods (e.g. periparturient animals). The advent of a National Animal Identification System provides an impetus for value-added products to combine with individual identification and serve as management tools to improve profitability. This development in conjunction with the ability to use body temperature as a diagnostic tool has lead to a new push in the commercial development of radiofrequency identification devices that also provide real-time temperature monitoring. One such system is the injectable Biothermo microchip (Digital Angel Corp., St. Paul, MN). The objectives of this study were to determine if the implantable microchips, implanted at three locations, yielded data that correlated to RT during thermoneutral conditions and during induced fever caused by lipopolysaccharide challenge.

Materials and Methods

Animal Description and Sampling Procedure

The use of animals in this experiment was approved by the University of Illinois Institutional Animal Care and Use Committee. Four weaned Holstein steers (77 ±2 kg)

were used in the study. The steers were moved from outdoor housing to an indoor temperature-controlled facility and housed in two rooms with each room housing two steers. The rooms measured 2.75 x 3.36 meters and the flooring was rubber-coated expanded metal with a manure collection pit under the flooring. Inside each room, the steers were kept in individual stalls measuring 0.92 x 1.75 meters. Air exchange in the room was approximately 15 changes per hour and the rooms were kept between 18 and 21 °C. Steers were fed 0.9 kg of grain/d and had *ad libitum* access to water and alfalfa cubes. At 1000 h daily the grain was fed, fresh alfalfa and water were offered rooms were pressure washed, and the manure collection pit was emptied.

Upon entering the facility the steers were implanted with three Biothermo microchips (Digital Angel Corp., Saint Paul, MN) that were part of a passive full-duplex, ISO 11784 and 11785 compliant system that operates at 134.2 kHz radio-frequency. The microchips were 2x12 mm and delivered via a pre-packaged sterile positive displacement syringe to three implant locations. The locations were 1) subcutaneously under the umbilical fold 2) subcutaneously on the midline posterior to the poll and 3) under the scutiform cartilage of the left ear. The steers were then given one week to acclimate to the facility and observed daily for inflammation around the injection sites. Photoperiod was initially set at 12 h of light and 12 h of dark. The animals were also used in another study examining photoperiodic effects on immune function during LPS challenge. On d 7, steers were placed on either short day photoperiod (8 h light:16 h dark) or long day photoperiod (16 h light: 8 h dark) and photoperiod treatments were reversed on day 22.

Rectal temperature was measured with a digital thermometer with an 8.6 cm long, round end probe connected to a digital readout (model TM99A, Cooper-Atkins,

Middlefield, CT). Rectal temperature was taken by inserting the probe into the rectum and gently applying pressure to ensure that the probe was touching the rectal wall. Rectal temperature was recorded when no change in the reading was observed for 3 seconds. Animals were not restrained during temperature collection. Microchip queries were performed with a handheld scanner that provided chip ID number and current temperature reading (Biothermo Pocket EX Reader, Digital Angel Corp., St. Paul, MN). The microchip queries were performed before taking RT.

An initial set of temperatures was taken on d 7 to serve as a baseline before photoperiod change, however, those data are not presented, as photoperiod did not influence body temperature. The first baseline temperature recording for this study began at 0800 h on day 21. Rectal temperature was recorded and each microchip was scanned and recorded every 5 min from 0800 until 1400 h, every 15 min from 1400 h to 2000 h, and every 30 min from 2000 h to 0800 h the following day. That temperature measurement schedule was again followed on day 22 and LPS was administered into the jugular vein at 1000 at a dose of 0.1 μg/kg BW (*E. coli* 055:B5; reconstituted with 0.9% saline solution, Sigma-Aldrich, St. Louis, MO). Temperature collection ceased at 0800 h on d 23 and steers were given a 2 week recovery period. A second replicate was performed with the same 4 steers following the same protocol (including baseline day and LPS challenge) starting on d 36 and ending on d 38 although photoperiod treatments were reversed in that replicate.

Statistical Analysis

A statistical analysis using PROC MIXED in SAS (version 9.1, SAS Institute Inc., Cary, NC) was performed to compare day 7 baseline temperatures with day 21 temperatures, and photoperiod was not found to significantly affect body temperature. Rectal temperature and microchip data were analyzed using the PROC MEANS and PROC CORR functions in SAS. To determine correlations, data during the baseline measurement periods were analyzed as a complete set and data during the LPS challenge were divided into pre-challenge (0800 h to 1000 h), during challenge (1000 h to 1400 h), and post-challenge (1400 h to 0800 h the following day). Results are expressed as Pearson's Correlation coefficients (*r*). P values less than 0.10 were considered to suggest a likely correlation and P values less than 0.05 were considered to indicate a statistically significant correlation.

Results

Period One

During the first baseline day (Figure 2.1), values for rectal temperature (RT), and microchips at the ear (ET), poll (PT), and the umbilical fold (UT) averaged 38.7, 37.0, 36.5, and 36.1 °C with standard deviations of 0.2, 1.0, 0.6, and 1.2 °C, respectively. During the first baseline period, correlations between RT and ET, RT and PT, and RT and UT were all significantly positive (r = 0.21, 0.16, and 0.16 respectively; $P < 0.01$).

On the first d of LPS challenge (Figure 2.2) the correlation coefficients (r) for RT and ET, RT and PT, and RT and UT were 0.53, 0.61, and 0.43, respectively ($p < 0.01$) for the pre-challenge period, -0.40 ($p < 0.01$), 0.30 ($p < 0.01$), and 0.03 ($p = 0.66$)

respectively, for the 4 h period following the LPS injection, and -0.12 ($p = 0.22$), 0.53 ($p < 0.01$), and 0.21 ($p < 0.03$) respectively, from 1400 h to 0800 h the following day. An inverse biphasic response was observed between the rectal and RFI temperatures during the LPS challenge.

Period Two

Mean baseline temperature values were 38.7, 37.3, 36.7, and 36.5 °C with standard deviations of 0.2, 0.7, 0.5, and 0.7 for RT, ET, PT and UT respectively. Correlations between RT and ET, RT and PT, and RT and UT were all significantly positive ($r = 0.11$, 0.36, and 0.22 respectively; $p < 0.03$).

On the day of the second LPS challenge (Figure 2.3) the correlation coefficients (r) for RT and ET, RT and PT, and RT and UT were -0.19 ($p = 0.07$), -0.02 ($p = 0.87$), and -0.07 ($p = 0.47$), respectively for the pre-challenge period, -0.48, 0.36, and 0.38 ($p < 0.01$) respectively, for the 4 h period following the LPS injection, and -0.25 ($p < 0.01$), 0.53 ($p < 0.01$), and 0.17 ($p < 0.08$) respectively, from 1400 h to 0800 h the following day. There was a similar inverse biphasic response of rectal and microchip temperatures during the second LPS challenge, when compared with the first LPS challenge.

Discussion

The ability to identify sick animals quickly, provide the needed treatment, and reduce morbidity and mortality is important to animal producers. Systems that aid in that process have received new interest as the size of animal units are growing and producers try to offer individual care in large group settings. The purpose of this study was to

evaluate an available system that utilizes implantable microchips that offer individual identification as well as real-time temperature monitoring.

Identifying an ideal location for radiofrequency devices has been the topic of debate. The first consideration for placement in the animal is the ability to recover the implant to prevent its inclusion in the food chain and also to eliminate the reuse of devices in systems where unique numbers are needed (Sheridan, 1991). The second consideration for placement in the animal is ease of use under production settings. A recommendation by Dorn (1987) was to place the implants subcutaneously on the neck just cranial to the shoulder on the left side. Further evaluation of implantation sites by Merks and Lambooij (1989) included subcutaneously at the front of the head, at the base of the ear, intramuscularly in the neck, and at the lateral side of the neck, cranial to the shoulder. A site under the scutiform cartilage of the ear was described by Fallon and Rogers (1991), and was determined to be a suitable placement site for implants by Hasker et al. (1992) due to the ability to recover them at the abbatoir. In this study we believed, the position under the scutiform cartilage would be the ideal spot based on the recommendations of previous authors and the prospect of having a deeper implantation that would be less affected by external temperature fluctuations than the subcutaneous sites. However, the two other sites were included to test the performance of the implants in areas that were closer to the ambient environment relative to the base of the ear where the implant was insulated by the scutiform cartilage. The umbilical implantation site was also thought to have some benefit to dairy producers where the implants could be read from milking units attaching on the underside of the animal. Microchips implanted at the umbilical fold, subcutaneously behind the poll, and under the scutiform cartilage of the

left ear were all positively correlated to RT in steers in a controlled, thermoneutral environment. A second collection of temperatures in this experiment confirmed the positive correlation between each chip and RT at thermoneutral ambient temperatures. The mean temperatures for the implant sites were lower than RT, but expressed a similar pattern. These observations support the concept that implantable devices that measure temperature provide consistent, reliable indications of body temperature similar to traditional RT responses.

During an experimentally induced fever caused by LPS injection, RT increased in a bimodal fashion as shown in previous research by Davis et al. (2003). In contrast to RT, which increased after LPS challenge, temperature at the three microchip locations was reduced in a biphasic pattern. Whereas a negative correlation was exhibited only between RT and the ear location, the pattern of the temperature change was similar for all three microchip locations. A second LPS challenge resulted in similar results for RT and implant temperatures. The consistency of these results suggests that the patterns have value in a system that monitors animals for fluctuation in body temperature caused by fever, albeit in an inverse manner.

The hypothesis that the temperature recorded by the microchips would be positively correlated with RT during an immune system challenge was proved to be incorrect. The negative correlation between the RT and implant temperatures was unexpected, but robust, especially for the implants located at the base of the ear. Gordon et al. (2002) reported a decrease in tail temperature in rats when LPS was administered. In the study by Gordon et al. (2002) core temperature rose from 37.0 to 38.5 °C and tail temperature dropped from 28 to 25.5 °C after LPS injection indicating a shift in heat loss

mechanisms to conserve heat for a fever response. Our hypothesis was based on the supposition that the microchip implants were located close enough to the core (UT), to the head (PT), and deep enough (ET) that they would all be positively correlated with RT. One possibility is that as blood flow was shunted from the periphery to the body core during the febrile response, a reduction in temperature occurred at subcutaneous sites. Indeed, the results of the present study indicate that the implant locations were located in areas that are susceptible to changes in blood flow and body temperature regulation during an induced fever response, as there was a consistent decrease in temperature recorded at all locations in response to LPS.

It is of interest to consider the physiological basis of the biphasic fluctuation in temperature among the variable sites where temperatures were recorded. Lipopolysaccharide injection results in a decrease in arterial pressure. Transient hypoxia in peripheral tissues is one potential outcome of blood flow shunting to the core as fever develops. Such hypoxia would eventually overcome the vasoconstrictive response to avoid complete anoxia and cell death, causing an increase in blood flow to the periphery and a rise in peripheral temperature. In contrast, it has been hypothesized that the first rise in core temperature is a result of peripheral stimulation of the temperature regulation zones in the brain, followed by a latency period while hypothalamic regulation is stimulated (Morimoto et al., 1987). It is unclear whether hypoxic conditions at the periphery or a latency in immune system signaling are responsible for the changes in peripheral temperature during a LPS challenge.

Although standard deviation was greater at the implant sites when compared with RT during baseline collection, the nadir in temperature at implant sites during LPS

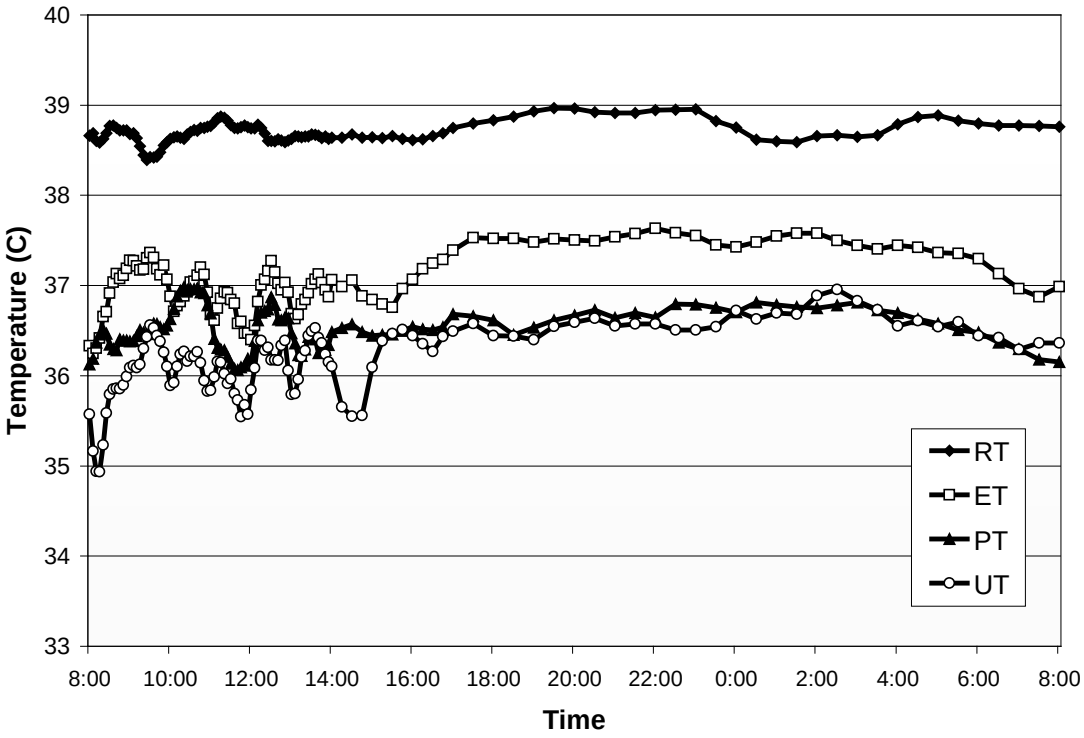
challenge was farther from the mean when compared to the peak in RT. The increase in deviation from the mean allows these data to be tested in different modeling scenarios to help identify animals experiencing a health event that produces fever. The ability to have real-time temperature measurements in a production facility would hasten time to diagnosis and treatment and add value to an identification system used solely for animal tracking. Implanted microchips also add security to an animal identification system compared to traditional identification systems by increasing the difficulty of removing the device. These implantable devices are also passive in nature and require no power to operate, which increases the useful lifetime of the devices. The benefits of using implants in an animal identification system, in addition to the added value of real-time temperature monitoring that has been shown in this study, makes these devices candidates for future animal identification systems. The results of this experiment suggest that further research is warrented to determine how the microchips perform in animals experiencing naturally occurrring disease.

Implications

The biphasic temperature patterns measured from the implants which were synchronized with biphasic responses in RT during LPS challenge, suggest that the microchips may add value to an ID system by detecting fluctuation in body temperature associated with disease. The consistency of results obtained at the base of the ear, along with that location's popularity with other forms of identification indicate that it would be a viable place to further study the performance of the microchips.

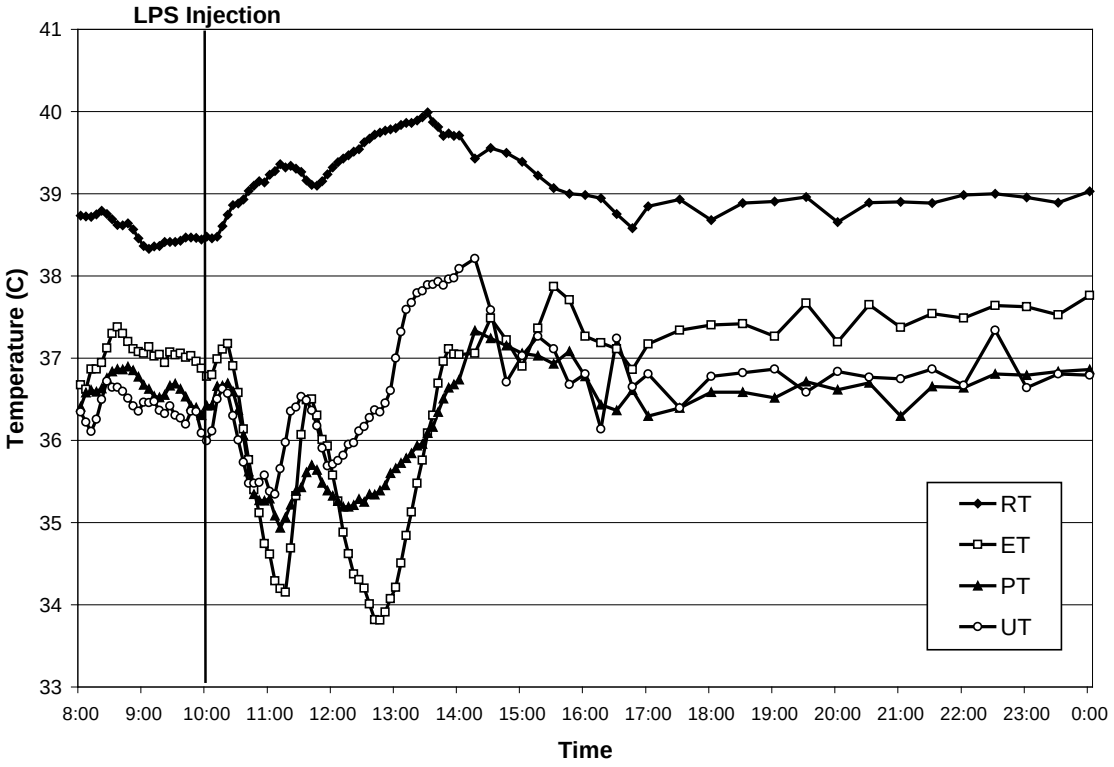
Figures

Figure 2.1. Three point running average of rectal temperature and temperatures from three microchips implanted at the periphery during baseline collection at thermoneutral ambient temperature.



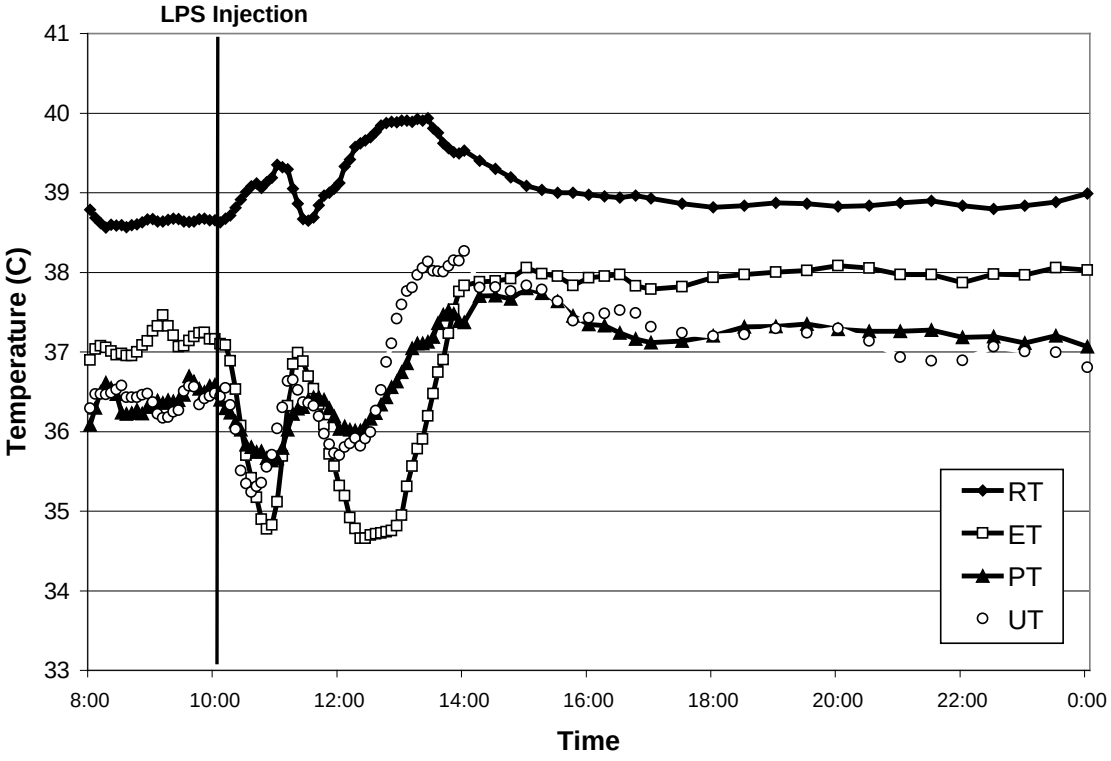
Recorded temperatures from the rectum (RT) and three microchips implanted under the scutiform cartilage of the left ear (ET), s.c. on the midline behind the poll (PT), and under the umbilical fold (UT) during the first 24 h collection period under thermoneutral environmental temperature. Temperatures were recorded every 5 min from 0800 h to 1400 h, every 15 min from 1400 h to 2000 h and every 30 min from 2000 h to 0800 h the following day. Mean temperatures for RT, ET, PT, and UT were 38.7, 37.0, 36.5, and 36.1 °C with standard deviations of 0.2, 1.0, 0.6, and 1.2 respectively. Pearson's Correlation coefficients (*r*) were 0.21, 0.16, and 0.16 for RT and ET, RT and PT, and RT and UT respectively ($p < 0.01$ for all).

Figure 2.2. Rectal temperature and temperatures from three microchips implanted at the periphery during the first lipopolysaccharide challenge at thermoneutral ambient temperature.



Recorded temperatures from the rectum (RT), and three microchips implanted under the scutiform cartilage of the left ear (ET), s.c. on the midline behind the poll (PT), and under the umbilical fold (UT) during the first lipopolysaccharide (LPS) challenge. Lipopolysaccharide was administered in the jugular vessel at 1000 h (0.1 μg/kg). Temperatures were recorded every 5 min from 0800 h to 1400 h, every 15 min from 1400 h to 2000 h and every 30 min from 2000 h to 0800 h the following day (data from 0800 h to 0000h are shown). Pearson's Correlation coefficients (*r*) during the 4 h period immediately following the LPS injection were -0.40 ($p < 0.01$), 0.30 ($p < 0.01$), and 0.03 ($p = 0.66$) for RT and ET, RT and PT, and RT and UT respectively.

Figure 2.3. Rectal temperature and temperatures from three microchips implanted at the periphery during the second lipopolysaccharide challenge collection at thermoneutral ambient temperature.



Recorded temperatures from the rectum (RT) and three microchips implanted under the scutiform cartilage of the left ear (ET), s.c. on the midline behind the poll (PT), and under the umbilical fold (UT) during the second lipopolysaccharide (LPS) challenge. Lipopolysaccharide was administered in the jugular vessel at 1000 h (0.1 μg/kg). Temperatures were recorded every 5 min from 0800 h to 1400 h, every 15 min from 1400 h to 2000 h and every 30 min from 2000 h to 0800 h the following day (data from 0800 h to 0000h are shown). Pearson's Correlation coefficients (*r*) during the 4 h period immediately following the LPS injection were -0.48, 0.36, and 0.38 for RT and ET, RT and PT, and RT and UT respectively ($p < 0.01$ for all).

CHAPTER 3

CORRELATION OF RECTAL TEMPERATURE AND PERIPHERAL TEMPERATURE FROM IMPLANTABLE RADIO-FREQUENCY MICROCHIPS IN PERIPARTURIENT COWS

Abstract

The periparturient cow is challenged by a relative change in metabolic state, which is often exaggerated by a decrease in dry matter intake and stress induced by housing change. These challenges leave the animal susceptible to both metabolic and pathogenic insults. The ability to identify animals early in a disease event may allow for earlier intervention and lead to higher treatment success. There is evidence that there is a change in body temperature prior to parturition. Injectable radio frequency implants (RFI) with the ability to remotely monitor temperature at the site of implantation are available. Temperatures recorded from these RFI are positively correlated with baseline rectal temperatures in a thermoneutral environment but negatively correlated to rectal temperature when cattle are challenged with lipopolysaccharide. We hypothesized that the temperature readings from RFIs implanted under the scutiform cartilage of the ear of pregnant cows, would be positively correlated with baseline rectal temperature and negatively correlated with rectal temperature during a known health event. We further hypothesized that calving time could be predicted from temperatures measured by the RFIs. Multiparous dairy cattle (n=40) were implanted with an RFI one wk prior to dry off. After implantation, rectal and RFI temperatures (RT and ET) were recorded every 12 h until 7 d before expected calving date, when sampling frequency increased to every 6 h

until 14 d after calving. Ambient temperature (AMB) was logged at 30 min intervals and included in the statistical model. Mean RT and ET were 38.9 (0.6) and 37.4 (0.8) and 39.2 (0.7) and 37.1 (1.6) for the dry and post-calving periods respectively. Rectal and ET temperatures were positively correlated during both the dry and post-calving periods ($r = 0.55$ and 0.49 respectively; $P < 0.001$ for both). Rectal and ET temperatures were both higher during periods of high AMB (> 31 C) when compared with periods of lower AMB (< 21 C). A Multiple Local Property Correlation analysis correctly identified all animals experiencing a health event, but had a high false positive rate (flagged 75% of all animals). A negative correlation between RT and ET around parturition or during diagnosed health events (n=12) was not consistently observed therefore this approach has limited value in prediction of parturition or early disease detection when ET is measured at 6 h intervals.

Introduction

The three weeks prior to and three weeks after parturition, known as the transition period (Grummer, 1995; Drackley, 1999), are important times in the life cycle of a dairy cow. As pregnancy reaches term, the mammary gland shifts from colostrogenesis to lactogenesis, and the physiological state of the animal at parturition is transitioning from supporting a fetus to meet the demands of milk production. The transition is mediated by a multitude of hormones including prolactin, estradiol, progesterone, and the glucocorticoids, which are summarized by Bazer and First (1983). In addition there is a reduction in DMI around parturition (Marquardt et al., 1977), and a concomitant reduction in nutrient intake and thus energy status of the animal. These endocrine

changes and reduced nutrient intake have implications on the immune function of the animal (reviewed by Goff and Horst, 1997). Altered energy status and immune function can result in the animal being more susceptible to stress and disease. Therefore, the transition period is a time where increased attention must be given to decreasing exposure to high pathogenic loads, ensuring proper access to feed and water, and increased disease monitoring.

Immune challenges during the transition period are inevitable, but early detection and proper management may decrease the severity of transition disease events and hasten recovery of sick animals. At its peak, rectal temperature (RT) increases by 1.1 degrees C during puerperal metritis, yet RT initially increases 72 to 48 h before clinical diagnosis (Benzaquen et al., 2007). In addition, an increase in RT is observed during mastitis (Vangroenweghe et al., 2004). Bacterial infections, like metritis and mastitis, stimulate the innate immune system resulting in the release of cytokines that signal the brain that an immune challenge has occurred (Johnson, 2002). Certain cytokines have been shown to induce a febrile response and cause a reduction in hormones related to growth and production, like insulin-like growth factor-1 (Lazarus et al., 1993; De Benedetti et al., 1997). The fact that these cytokines are released without specificity suggests that temperature monitoring could be used to identify animals in the herd that may not yet be showing clinical signs of disease but have early onset of fever.

The physical act of parturition is also a challenge for the dam and the offspring. Although parturition often occurs without incident, there are instances when assistance is needed. Nearly two of three calf deaths that occur within the first few days of life are associated with difficult parturition (Patterson et al., 1987). In addition, movement into

maternity pens before parturition can cause stress if done between 2 and 10 d before calving (Cook and Nordlund, 2004). The ability of the producer to predict when parturition will occur may improve their ability to provide an adequate area for the cow to give birth as well as be prepared to provide assistance at parturition if needed. Rectal temperature has been shown to decrease around parturition (Weber, 1910; Vollman and Vollman, 1942). The drop in temperature has been shown to be between 8 and 48 h before parturition with an average nadir time of 16 h before parturition (Lammoglia et al., 1997) suggesting that temperature monitoring may be a good predictor of parturition.

Although shifts in body temperature is useful for identifying animals nearing parturition as well as cows experiencing disease events, the process is labor intensive and the variation in body temperature requires frequent sampling to make it an effective approach to monitor individual cows. A system has been developed that uses injectable devices (Digital Angel Corp., St. Paul, MN) that have the ability to monitor temperature in real time via radio frequency (RFI) readings. Temperature estimates from these devices are positively correlated with RT in healthy steers in thermoneutral environments and negatively correlated with RT during a fever caused by a lipopolysacharride challenge (Reid et al., 2005). The first objective of this study was to determine if a microchip, implanted at the base of the left ear, would be positively or negatively correlated with RT during the dry period and early lactation. The second objective of this study was to determine if the data collected could be used to identify animals that were nearing parturition or experiencing a disease event.

Materials and Methods

Animal Description and Sampling Procedure

The use of animals in this experiment was approved by the University of Illinois Institutional Animal Care and Use Committee. Forty-one multiparous cows were used in this study. One cow was removed from the study due to a prolapsed uterus and resulting health complications. The animals were part of another experiment examining photoperiodic effects on feed intake, subsequent milk production, behavior, and immune function during a targeted 42 d dry (Velasco et al., 2008). One week prior to dry off cows were moved into one bay of a mechanically ventilated, free stall barn with 4 bays, with each bay containing 10 stalls and individual feeding gates (American Calan, Northwood, NH). Upon entering the facility, the cows received a Biothermo device (Digital Angel Corp., Saint Paul, MN) at the base of the left ear under the scutiform cartilage. The devices were 2x12 mm and delivered via a pre-packaged sterile positive displacement syringe. The Biothermo devices are part of a passive full-duplex, ISO 11784 and 11785 compliant system that operates at 134.2 kHz radio-frequency. The cows were observed daily for 1 wk for inflammation around injection sites; no inflammation was observed. After 7 d, cows were dried off and provided either short day photoperiod (8 h light:16 h dark) or long day photoperiod (16 h light: 8 h dark) for the remainder of the dry period (dry period averaged 40 days). Groups of cows were dried off weekly from May 26, 2005 to September 1, 2005. One week prior to expected calving date cows were moved into individual calving pens within each photoperiod bay. Upon calving, cows were moved to a mechanically ventilated tie stall barn and exposed to ambient photoperiod. Cows had ad libitum access to feed and water throughout the study. Post-calving

examinations were performed daily by the University of Illinois Dairy Farm staff and included visual observation of feed intake, general attitude of the animal, RT, milk production, and presence or absence of uterine discharge. Diagnosis of clinical disease was performed by the University of Illinois veterinary staff.

Rectal temperature was collected with a digital thermometer with a 10 cm long, round end probe connected to a digital readout (model M500, GLA Agricultural Products, San Luis Obispo, CA). Microchip queries were performed with a handheld scanner that provided chip ID number and current temperature reading (Biothermo Pocket EX Reader, Digital Angel Corp., St. Paul, MN). Temperature measurement was performed at 0900 h and 2100 h each day during the dry period and collection frequency was increased to 4 times per day (0300 h, 0900 h, 1500 h, 2100 h) starting one week prior to calving and continuing through 14 DIM.

Statistical Analysis

A statistical analysis using PROC MIXED in SAS (version 9.1, SAS Institute Inc., Cary, NC) determined that photoperiod did not significantly affect RT or ET ($P > 0.5$). Rectal temperature and microchip data were analyzed using the PROC MIXED and PROC CORR functions in SAS, with ambient temperature (AMB) included as a covariate in all models. Because AMB was a significant covariate, a PROC MEANS analysis was performed to investigate the differences between rectal and peripheral temperature during periods when AMB was higher than 30 °C and lower than 21 °C. Correlation results are expressed as Pearson's correlation coefficients (r). P values less than 0.10 were considered to suggest a likely correlation and p values less than 0.05 were

considered to indicate a statistically significant correlation. In addition, a Multiple Local Property Correlation (MLPC) analysis (Bergquist et al., 2004) was performed on the post calving data set in MATLAB (The MathWorks, Natick, MA). The MLPC analysis allowed for comparison of small subsets of data by observing the distribution of the data, allowing a comparison of data during a health event to be compared to a "normal" temperature profile. Data sets with a normal distribution curve were assumed to be similar, data sets that exhibited skewness greater than one standard deviation from the mean and/or kurtosis 1.25 standard deviations from the mean were assumed to be different.

In addition, a statistical process control analysis was evaluated using the data from one cow that experienced a clinical case of metritis to determine if that method could identify a deviation from the mean before clinical diagnosis of the disease. The analysis was performed using SPC IV software (Quality America Inc., Tuscon, AZ).

Results

Dry period

The mean RT for the dry period was 38.9 ±0.6 °C and the mean radio-frequency microchip temperature (ET) was 37.4 ±0.8 °C. Pearson's Correlation coefficient (r) for the entire dry period was 0.55 ($P < 0.001$). The mean RT and ET for the pre-calving period (-7 d to parturition) are presented in Figure 3.1. The mean RT for the pre-calving period were 39.2 ±0.1, 38.7 ±0.1, 38.7 ±0.1, and 39.3 ±0.1 °C for 0300, 0900, 1500 and 2100 respectively. The mean ET during the pre-calving period was 37.9 ±0.2, 37.1 ±0.2,

36.7 ±0.2, and 37.6 ±0.2 °C for 0300, 0900, 1500 and 2100 respectively. Pearson's Correlation coefficient (r) for the pre-calving period was 0.38 ($P < 0.001$).

Post-calving period

The mean rectal and RFT for DIM 1 through 14 are presented in Figure 3.1. Mean value for RT during the post-calving period was 39.2 ±0.7 °C and the mean value for ET was 37.1 ±1.6 °C. Daily variation in RT and ET is shown in Figure 3.2. Mean RT was 39.6 ±0.2, 38.9 ±0.1, 38.9 ±0.1, and 39.5 ±0.1 °C for 0300, 0900, 1500 and 2100 respectively. Mean ET was 37.1 ±0.2, 36.2 ±0.2, 36.4 ±0.2, and 37.1 ±0.2 °C for 0300, 0900, 1500 and 2100 respectively. Pearson's Correlation coefficient (r) for RT and RFT in the pre-calving period was 0.49 ($P < 0.001$). The use of MPLC analysis and the resulting use of boundaries on skewness and kurtosis to flag animals that had a disease event resulted in a model that was able to identify 100% of the animals experiencing a health event (12 animals: 6 cows with retained placental membrane; 1 cow with ketosis; 1 cow with metritis; 1 cow with toxic mastitis; 1 cow with retained placental membrane, ketosis, and metritis; 1 cow with retained placental membrane, ketosis and a displaced abomasums; and 1 cow with retained placental membrane, metritis and an inverted bladder). However, the resulting model displayed a high false positive rate of approximately 75%. An illustration of the MPLC comparisons (maximum distance, variance, and maximum distance between two points) for a cow experiencing no health events (normal distribution) and for one cow experiencing a health event (non-normal distribution) is depicted in Figure 3.3.

The results of the SPC analysis for cow #7713 are shown in Figure 3.4. Significant deviation outside of 3 sigma levels is observed at two time points; roughly 2 d before parturition and roughly 2 d before clinical diagnosis of metritis. Using SPC, this specific animal would have been identified as experiencing a health event before clinic signs were apparent.

Periods of High Ambient Temperature During the Dry Period

During the dry period, the mean rectal and ET temperatures for the data set with AMB over 31 °C were 39.7 ±0.1 and 38.1 ±0.1 °C respectively (Figure 3.5). The mean RT and ET values for the periods when AMB was below 21 °C were 38.8 ±0.04 and 36.9 ±0.1 °C respectively (Figure 3.3). Rectal temperature during the high AMB was significantly higher than RT during periods of lower AMB ($P < 0.01$) and ET during high AMB was also higher than ET during periods of lower AMB ($P < 0.01$).

Discussion

The daily patterns of RT and ET were similar in the dry period and post-calving, which supports the use of ET as a replacement for the labor-intensive RT approach. Indeed, these data compliment the daily rhythmicity of body temperature in cattle reported by Piccione et al. (2003). The observation that the daily temperature patterns of RT and ET were similar and correlations between RT and ET were high indicates that ET is a potentially useful management tool. The primary limitation, however, is the continued need to collect measurements manually rather than through an automated system. Development of a remote query approach to automatically collect temperature

data from multiple animals will be essential to further application of real-time temperature monitoring to management of production animals.

The positive correlation of ET and RT during periods of high AMB suggests that ET can be used to identify periods when a cow experiences heat stress, but more importantly support the concept that peripheral body temperature measurements move in parallel with core temperature, and thus ET is an appropriate proxy for RT during heat stress. Further, the previously noted circadian fluctuation in body temperature (Piccione et al., 2003) persisted during heat stress. Because body temperature can be influenced by other environmental factors such as feeding or drinking (Bewley et al., 2008), any data collected will require modeling to adjust for external factors before final output is interpreted. Again, the data collection frequency will need to be increased before application to animal management.

Previous research suggests that body temperature decreases prior to parturition (Weber, 1910; Vollman and Vollman, 1942; and Lammoglia, 1997). Prediction of the timing of parturition is of interest to producers, as cow movement could be optimized to minimize stress around calving. However, temperature measurement is labor-intensive using current methods. In the current study, RT and ET, taken at 0900 and 2100, were positively correlated during the dry period. During the pre-calving period (-7 d to parturition) the correlation between rectal temperature and ET was lower, but significant. Thus, ET is a suitable replacement for RT methodology in estimating body temperature at any specific time point. Rectal temperature dropped slightly one day before calving, but the drop in temperature was not significant. In addition, ET was observed to decrease around -3 d and after rebounding, decreased again starting -1 d and continuing through

the first few days of lactation (Figure 3.1). The drops in ET were not found to be significant. Our results show that rectal temperature and ET, taken at 6 h intervals, are not suitable predictors for the detection of the onset of parturition. It should be noted that several cows exhibited temperature patterns that suggested that parturition was imminent (as in cow #7713, Figure 3.4), but the variation among cows, at the current 6 h interval, was too high to use the data as a prediction tool.

Because the transition period represents a time when mature cows are at high risk of pathogen mediated and metabolic disease, an effective means of measuring body temperature in real time offers an opportunity to identify a disease event before clinical signs appear. Post-calving, the MPLC analysis identified all cows experiencing a health event, but had a high false positive rate. That analysis is based on comparing an animal to herdmates in a similar stage of lactation. The use of larger amounts of data increases the power to identify animals experiencing a disease event, but requires that the animal have enough temperature points as the health event occurs that would put her data set out of the normal distribution range. With the current technological limitations of temperature recording this is not an efficient method to identify at risk animals and hasten treatment application. In a case where temperature is expected to rise 72 to 48 h prior to a clinical diagnosis of metritis as reported by Benzaquen et al. (2007), the MPLC does not appear to be robust enough to identify the animal until a time point similar to the normal clinical diagnosis. Of the 3 cows diagnosed with clinical metritis, 1 cow did have a significant difference in ET roughly 2 d before clinical diagnosis of metritis by the veterinary staff (Figure 3.6). It appears, from this study, that using the animal as its own reference may be more effective for early detection of disease, but the frequency of

temperature collection (i.e. every 6 h) did not yield significant differences often enough to be recommended as a management tool. The use of Statistical Process Control (SPC) appears to have promise as a tool to evaluate real-time data generated by the microchips used. The SPC analysis identified a time point outside of 3 sigma levels roughly 2 d before clinical diagnosis of metritis in cow 7713. In addition, SPC analysis also identified a time point roughly 2 d before parturition in that same animal. While data from one animal is not enough to provide statistical significance, further research is warranted to evaluate SPC in identifying animals nearing parturition or experiencing health events related to a febrile response.

Implications

The results of the present study highlight the impact of AMB and fluctuations in body temperature throughout the day when using temperature as a management tool. The results also indicate that ET is a potential technology for animal management as it is positively correlated with RT. However, the limitation of this study lies in the frequency of data collection. Whereas all health events were identified using the MPLC analysis, the interval of data collection was insufficient to identify animals early enough to hasten treatment. Using individual animals as their own reference may be a viable option, but more frequent temperature collection is still necessary to increase the power of statistical inference and accurately identify animals nearing parturition or a sick animal before a clinical diagnosis would occur. More research is needed to identify the optimal temperature collection frequency that would result in a viable model to identify animals nearing parturition or experiencing heath events as well as the opportunity to identify

animals experiencing health events not typically associated with a large increase in body temperature, such as ketosis.

Figures

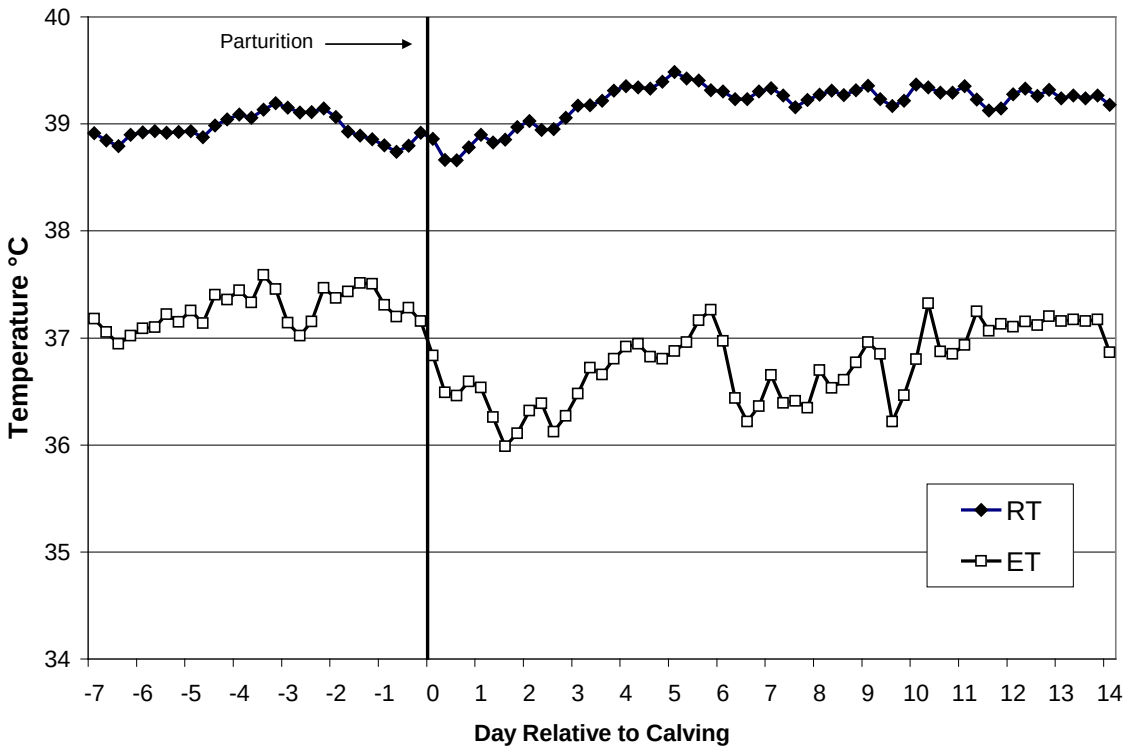


Figure 3.1. Mean rectal (RT) and radio-frequency microchip (ET: located under the scutiform cartilage of the left ear) temperatures from 7 d prior to calving to 14 d post-calving for all animals on the study. Each point represents a temperature collection (performed every 6h). The vertical line depicts time of parturition.

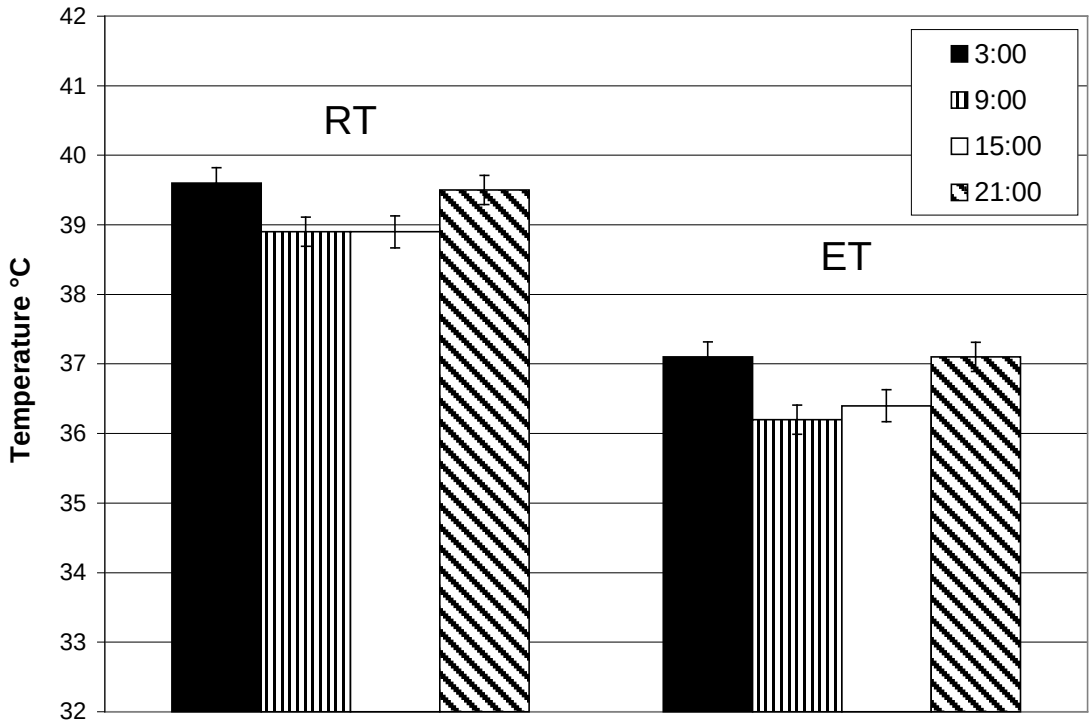


Figure 3.2. Mean daily temperature readings, taken at 03:00, 09:00, 15:00 and 21:00 h daily, for rectal (RT) and radio-frequency microchip (ET: located under the scutiform cartilage of the left ear) temperatures for 14 d post-calving.

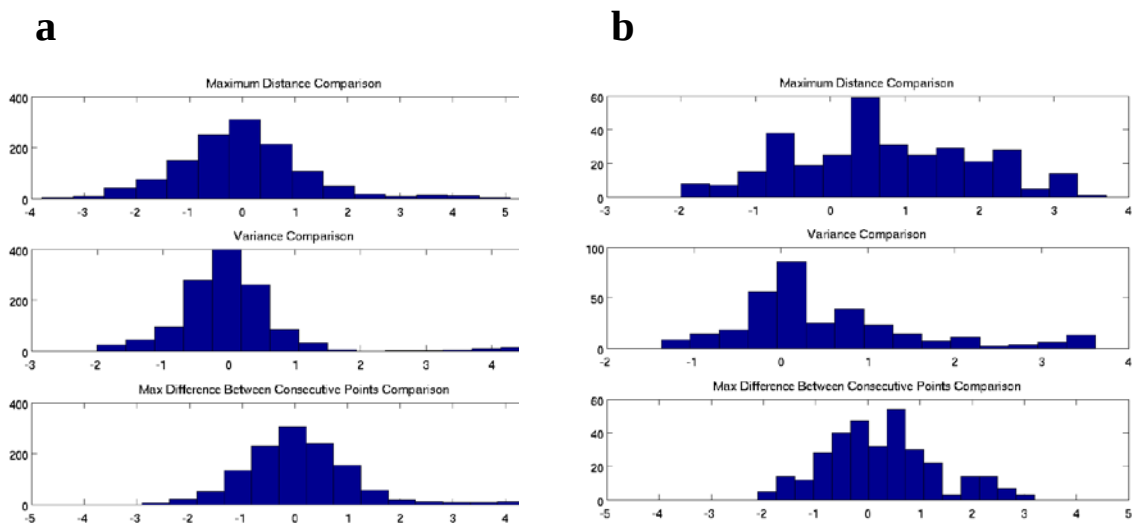


Figure 3.3. Multiple Local Property Correlation analysis results (maximum distance, variance, and maximum distance between two points) for once cow with no health events (a: normal distribution) and for one cow that was diagnosed with metritis (b: non-normal distribution). Cows with data sets exhibiting skewness greater than one standard deviation from the mean and/or kurtosis 1.25 standard deviations from the mean were flagged as animals experiencing disease events. The model correctly identified all animals experiencing health events, but also resulted in a high false positive rate (75% of all animals were flagged).

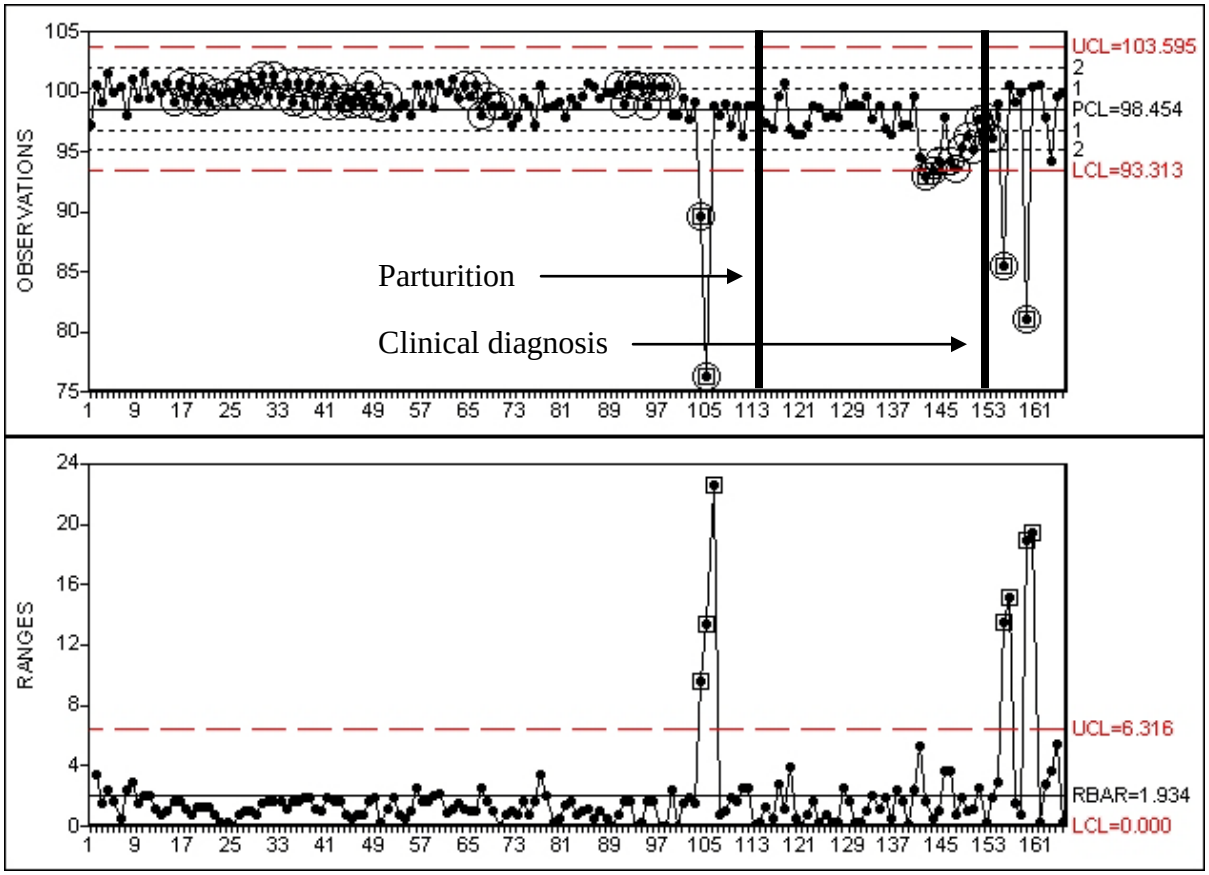


Figure 3.4. Statistical process control analysis of data taken from microchips implanted at the base of the ear in cow # 7713 throughout the dry and fresh periods. In the top graph, ear temperature is shown on the Y axis and the mean is represented by the line labeled PCL. Upper control limit (UCL) and lower control limit (LCL) are also depicted at the 3 sigma levels. The dashed lines represent sigma levels as labeled. The time of parturition and the time of clinical diagnosis of metritis are also depicted as perpendicular solid lines. In the bottom graph, the range of the data is depicted. Points above the UCL are considered to be outliers (in this data set they depict both parturition and a clinical case of metritis).

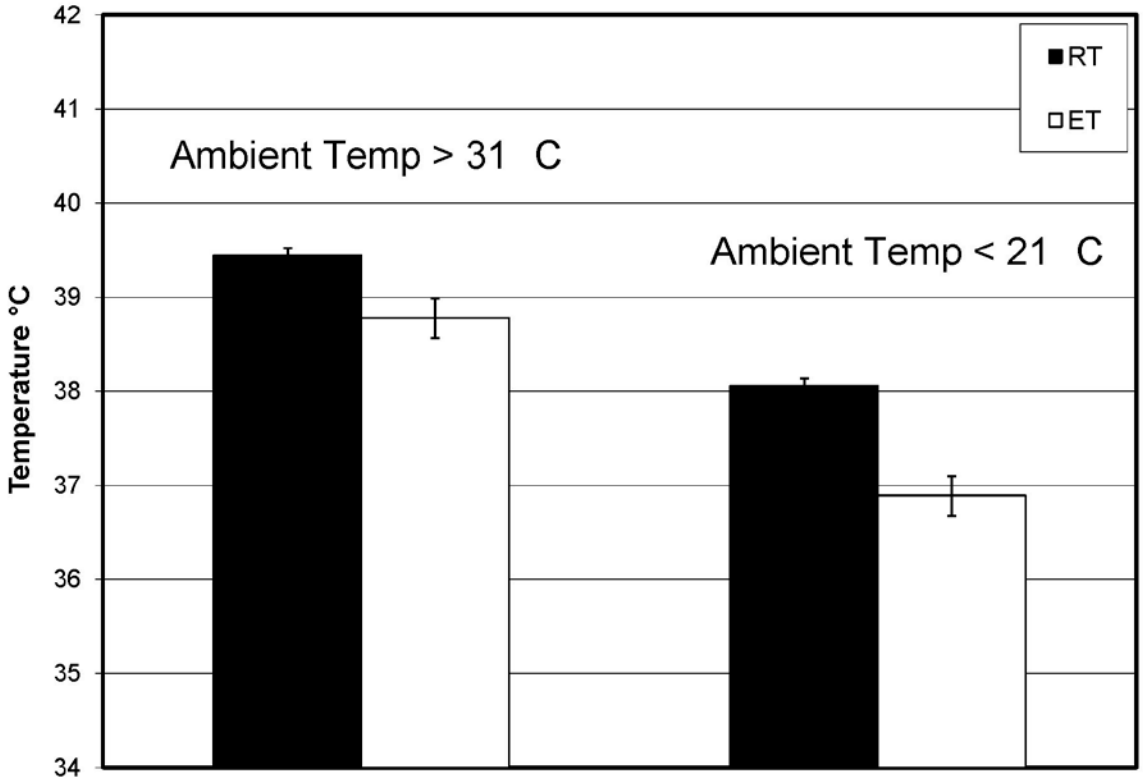


Figure 3.5. Mean daily temperature readings for rectal (RT) and radio-frequency microchip (ET: located under the scutiform cartilage of the left ear) temperatures for periods where the ambient temperature was greater than 31 °C or less than 21 °C. Data was collected from 41 cows during their dry period, resulting in a total of 2200 data points for both RT and ET.

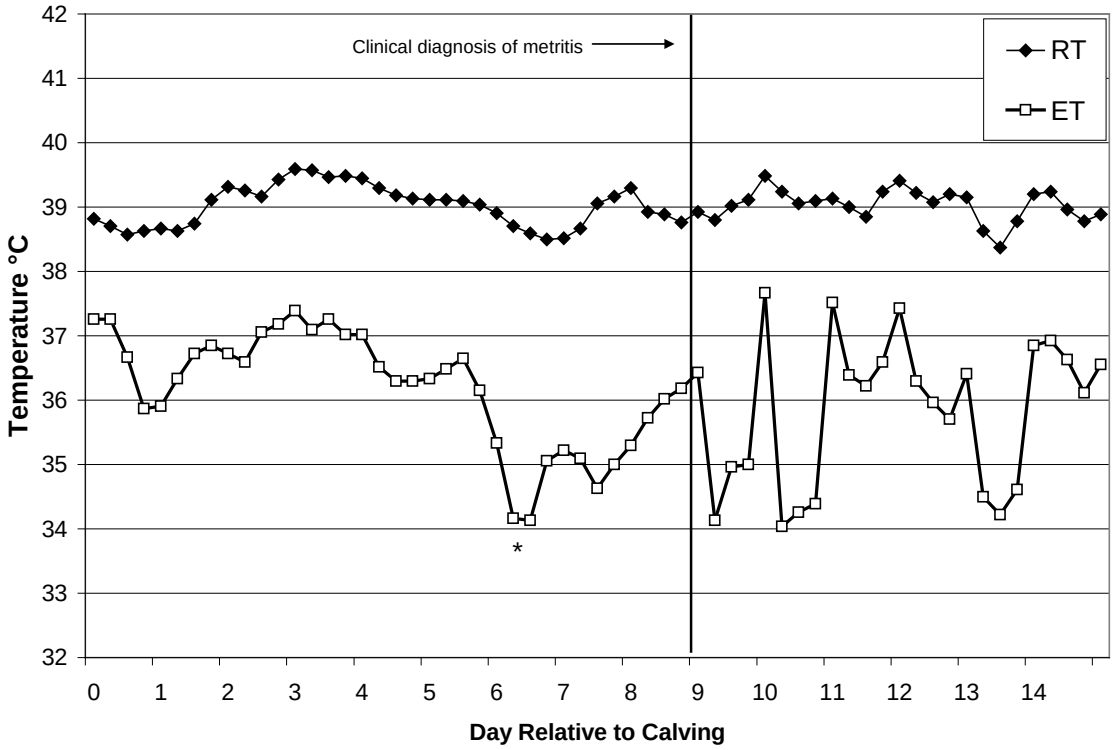


Figure 3.6. Mean rectal (RT) and radio-frequency microchip (ET: located under the scutiform cartilage of the left ear) temperatures for 14 d post-calving for cow number 7713. A clinical diagnosis of metritis was given 9 d post-calving, while a significant deviation from mean ET temperature occurred 6 d post-calving.

CHAPTER 4

CORRELATION OF RECTAL TEMPERATURE AND PERIPHERAL TEMPERATURE FROM IMPLANTABLE RADIO-FREQUENCY MICROCHIPS IN COWS DURING ESTRUS

Abstract

In a sustainable animal agriculture system it critical that animals become pregnant in order to support consistent production and allow for selective culling. The estimated annual monetary loss attributed to poor reproductive performance to the dairy industry alone is $300 million. Traditional methods of identifying animals in estrus are labor intensive and often suboptimal due to the narrow time window in which cattle exhibit estrus behavior. Previous work has shown that body temperature rises during estrus and can be used as a tool to identify animals in heat. Injectable radio frequency implants (RFI) with the capacity to remotely monitor temperature at the site of implantation are commercially available. Basal temperatures recorded from these RFI exhibited a positive correlation with basal rectal temperatures in steers and cows. The hypothesis of this study was that temperature measured by a RFI, implanted under the scutiform cartilage of the ear of cows, would be positively correlated to rectal temperature during estrus and that the resulting data would allow for the identification of animals in heat. Multiparous cows (n = 32) were implanted late in pregnancy to provide time for implant adaptation and initial monitoring. Upon eligibility for breeding, cows were enrolled in a synchronized ovulation program and rectal (RT) and implant temperatures (ET) were recorded every 6 h for 5 d before and 5 d after expected time of estrus. Temperature data were also

collected from cows that did not become pregnant upon first service. In addition, 4 cows were enrolled in an increased frequency data collection period where temperatures were taken every 1 h for 1 wk centered on the day of expected estrus. Rectal temperature and ET were positively correlated when temperatures were measured every 6 h (6H) ($r = 0.52$, $P < 0.001$) as well as every 1 h (1H) ($r = 0.45$, $P < 0.001$). Rectal temperature increased by roughly 0.6 °C around estrus using 6H, but ET did not increase significantly during that time, but the patterns of RT and ET were similar. There was very little variation in RT using 6H, but sharp drops in ET using 1H coincided with cows leaving the barn for the morning milking and spending a period of time outside for exercise in the morning. The results of this study indicate that ear temperature monitoring via a RFI is not adequate to detect cattle experiencing estrus. There is, however, a need for more research using these implants directed toward increased frequency of data collection and differing ambient temperature conditions.

Introduction

Failure to get cows pregnant increases the costs associated with artificial insemination, veterinary pregnancy checks, and synchronization programs, and may increase the need to purchase replacement animals. Accurate estrus detection is a critical factor in attaining a high pregnancy rate. Up to 50% of estrus behavior goes undetected (Hurnik et al., 1975; Stevenson and Britt, 1977), and the estimated loss to U.S. dairy producers each year due to failure to detect estrus is approximately $300 million (Senger, 1994). Traditionally, visual observation is used to detect animals exhibiting estrus

behavior. Standing to be mounted is the most reliable behavior in determining when cows are receptive to breeding (Glencross et al., 1980). The use of visual observation, however, is labor intensive and typically can not be performed 24h/d. Cows exhibit estrus behavior for a short period of time (eg. 5.7 h; At-Taras and Spahr, 2001 and 7.1 h; Dransfield et al., 1998). Further, cows exhibit roughly twice as much mounting behavior in cold weather when compared to hot weather (Pennington et al., 1985), which makes visual observation less effective in the summer months relative to winter months.

There are various methods, besides visual observation, in use today that increase the ability to identify animals that are receptive to breeding. One physiological method is monitoring fluctuations in body temperature. Kyle and coworkers (1997) reported an 89% estrus detection rate using temperature-sensing vaginal implants, where temperature was elevated for 6.5 h during ovulation. Temperature sensing is useful in detecting estrus in cows that ovulate in the absence of observed estrous behavior. For example, Nieuwenhuizen et al. (1979), reported that 8 of 20 animals did not show estrous behavior but did exhibit an increase in body temperature and ovulation.

The practical use of body temperature to identify ovulation is restricted by the need for increased labor to gather enough data to detect the increase in temperature. A system has been developed that uses injectable microchips (Digital Angel Corp., St. Paul, MN) to monitor temperature in real time via radio frequency readings. Temperature readings from these microchips were positively correlated with rectal temperatures of healthy steers and negatively correlated with rectal temperatures of steers during a fever caused by a lipopolysacharride challenge (Reid et al., 2005). The objectives of the current study were to determine if body temperature recorded from a microchip

implanted under the scutiform cartilage of the ear is correlated with rectal temperature during estrus in dairy cows and to determine if time of ovulation could be identified using the data collected.

Materials and Methods

Animal Description and Sampling Procedure

The use of animals in this experiment was approved by the University of Illinois Institutional Animal Care and Use Committee. Thirty two multiparous Holstein cows were used in this study. The animals were part a concurrent experiment examining photoperiodic effects on feed intake, subsequent milk production, behavior, and immune function during a short dry period (Velasco et al., 2008). In short, one week prior to dry off, cows were moved into one bay of a mechanically ventilated, free stall barn with 4 bays, with each bay containing 10 stalls. Upon entering the facility, the cows were implanted with a BioThermo microchip (Digital Angel Corp., Saint Paul, MN) at the base of the left ear under the scutiform cartilage. The microchips were 2x12 mm and delivered via a pre-packaged sterile positive displacement syringe. The microchips are part of a passive full-duplex, ISO 11784 and 11785 compliant system that operates at 134.2 kHz radio-frequency. The cows were observed daily for 1 wk for inflammation around injection sites (no inflammation was observed). Groups of cows were dried off weekly from May 26, 2005 to September 1, 2005 and randomly assigned to their photoperiodic treatment. Upon calving, cows were moved to a mechanically ventilated tie-stall barn where they stayed for approximately 7 weeks. The cows were then moved

to a free stall barn with an open exercise lot for the remainder of their lactation. Cows had ad libitum access to feed and water throughout the study.

Cows were enrolled in a PreSynch-OvSynch estrus synchronization program (described by Thatcher et al., 2002) starting on d 32 of lactation. For the PreSynch portion of the program, cows received a 5 mL injection of prostaglandin ($PGF_{2\alpha}$; dinoprost tromethamine, Lutalyse®, Pfizer Animal Health, Kalamazoo, MI) on d 32 and 46 of lactation. The OvSynch portion of the program consisted of a 2 mL injection of GnRH (GnRH; Cystorelin®, Merial LTD, Duluth, GA) on d 60 of laction, a 5 mL injection of $PGF_{2\alpha}$ on d 67 of lactation, a 2 mL injection of GnRH on d 70 of lactation, and ended with breeding on d 70 of lactation. On the first day of the OvSynch program, cows were fitted with an electronic timed-mount heat detection device (TattleTale, Microdine Co., LLC, Savannah, MO) and the devices were checked at 0800 and 2000 h daily to check for estrus activity. Blood was also taken via coccygeal venipuncture on the day of first breeding following the OvSync program, 21 d post-breeding, and any time that the TattleTale device was triggered, to analyze plasma progesterone concentration to determine pregnancy status.

Rectal (RT) and radio frequency microchip (ET) temperatures were taken at 0200 h, 0800 h, 1400 h and 2000 h each day. Rectal and ET temperatures were taken 5 d prior to and 5 d following expected estrus. Expected estrus was determined to be on the day of the first breeding following the OvSynch protocol and 21 d post-breeding for cows that did not become pregnant during their first breeding period (temperatures were collected during both periods for all animals, regardless of pregnancy status, as that could not be determined until after 35 d of pregnancy). In addition, a group of 13 cows were used to

determine if increased temperature collection frequency would increase the accuracy of determining estrus using body temperature. The cows were moved from the free stall facility into a mechanically ventilated tie-stall barn to aid in frequent temperature collection. Cows were moved from the tie-stall facility twice daily (0500 and 1600 h) to be milked, thus exposing them to ambient air temperature. Rectal and ET temperatures were taken hourly for 3 d prior to expected estrus and 3 d after expected estrus. Of the 13 cows, 3 were enrolled in the PreSynch-OvSynch program and 11 were expected to be pregnant or experiencing a natural heat. Nine of the 13 cows were later determined to be pregnant, leaving 4 cows to be used for temperature data during estrus.

Rectal temperatures were collected throughout the study with a digital thermometer with a 10 cm long, round end probe connected to a digital readout (model M500, GLA Agricultural Products, San Luis Obispo, CA). Microchip queries were performed with a handheld scanner that provided chip ID number and current temperature reading (Biothermo Pocket EX Reader, Digital Angel Corp., St. Paul, MN).

Statistical Analysis

Rectal temperature and microchip data were analyzed using the PROC MEANS and PROC CORR functions in SAS (version 9.1, SAS Institute Inc., Cary, NC). Results are expressed as Pearson's Correlation coefficients (r). P values less than 0.10 were considered to represent a likely correlation and p values less than 0.05 were considered to indicate a statistically significant correlation. For the period of increased frequency of temperature collection, the 9 cows that were determined to be pregnant were used to determine a correlation between ambient temperature and ET. This was done by

calculating a regression line ($y = 0.41x + 93$) and performing an axis transformation to remove the correlation between the two variables. The transformation matrix used to account for the regression line was:

$$T = \begin{bmatrix} 0.919 & -0.395 \\ 0.395 & 0.919 \end{bmatrix}$$

Results

Temperature Collection Every 6 Hours

Estrus detection using the TattleTale indicators resulted in the observation of mounting behavior in 25 of 32 cows during the synchronized ovulation, and 12 out of 17 of the cows that were determined to not be pregnant by veterinary staff. During the period before and after expected estrus, RT were 38.7 ±0.1, 38.4 ±0.1, 38.7 ±0.1, and 38.8 ±0.1 and ET were 36.6 ±0.2, 36.0 ±0.2, 37.1 ±0.2, and 37.6 ±0.2 °C for 0200, 0800, 1400, and 2000 h respectively (Figure 4.1). The Pearson correlation coefficient for RT and ET during the collection period was 0.52 ($P < 0.001$). Rectal and ET temperatures taken for 10 time periods before and after expected day of estrus are shown in Figure 4.2. A significant elevation in RT was observed at estrus, whereas ET did not exhibit a significant rise around expected estrus.

Temperature Collection Every Hour

In an effort to determine if the frequency of data collection influenced the utility of ET as an indicator of estrus, hourly measurements of RT and ET were made during

one estrous cycle. Mean values for RT and ET throughout the day of estrus are presented in Figure 4.3, whereas mean RT and ET for the period from one day before to one day after expected estrus are presented in Figure 4.4. As in the 6 h frequency collection period, RT and ET were positively correlated ($r = 0.45$. $P < 0.001$). In addition, RT and ET were positively correlated with ambient temperature ($r = 0.35$ and 0.45 respectively, $P < 0.001$ for both). Although ambient temperature was significant in the statistical model, the attempt to form a regression line to reduce variation failed to identify animals experiencing estrus as identified by an increase in either RT or ET. Increasing the frequency of data collection from every 6 h to every 1 h did not result in a significant change in ET during expected estrus.

Discussion

During the first part of this study, mean RT and ET followed the same daily pattern with a nadir at the 0800 h time point. Rectal and ET were also positively correlated with one another. These data are slightly different when compared with previous research (Reid et al., 2006), in that both RT and ET had a relatively higher value at the 2000 h time point in this study, where the 2100 and 0300 h temperatures were relatively similar in the work by Reid et al. While the dusk-dawn pattern of both RT and ET agrees with previous work (Piccione et al., 2003), it is important to note that the animals in the previous study (Reid et al., 2006) were observed in early lactation and during spring/summer, while the timing of this study was in late summer/fall. There may be ambient temperature effects occurring that alter the body temperature during this time, causing subtle changes in body temperature patterns. Rectal temperature did rise by

roughly 0.6 °C around expected estrus (Figure 4.2), which confirms previous research (Wrenn et al., 1958; Redden et al., 1993; Piccione et al., 2003). Ear temperature followed a similar pattern to RT, but did not have a distinct peak around expected estrus (Figure 4.2). At a measurement frequency of every 6 h, it was not possible to use ET to determine estrus. The time a cow spends in estrus has been reported to be less than 6 h (At-Taras and Spahr, 2000), and the sampling frequency in this study was most likely not frequent enough to catch enough animals in heat.

The second group of animals in this study were subjected to a higher frequency of temperature measurement (1 per h). These animals were observed in December, when ambient temperature outside of the barn was occasionally below freezing. The animals were required to walk outside to the milking parlor and back twice daily during the data collection period. Mean RT had very little variation throughout the day (Figure 4.3), whereas mean ET dropped considerably after the morning milking and a subsequent exercise time while the barn was being cleaned. This is also seen in Figure 4.4 where there are large drops in ET at samples -15 and 10 corresponded to when all of the cows were milked and outside for their exercise period. There are also two sharp increases in ET roughly 10 h after each nadir. These increases are at the time when the cows would have been in the milking parlor for the afternoon milking. It is possible that either an increase in activity in relatively warmer ambient temperatures, an increase in holding area/parlor ambient temperature (not measured), or a combination of both, could have caused ET to increase at those time points. There was a small increase in RT around time of expected estrus, and aside from the nadir during the morning milking/exercise ET followed a similar pattern. However, changes in neither RT and ET were robust enough

to detect a significant difference well enough to identify animals experiencing estrus, which may have been due to the small sample size (4 animals).

Implications

The data from the first part of this study suggest that an increase in rectal temperature might be used as an indicator of estrus, but peripheral temperature (measured at the base of the ear via radio-frequency microchip) is not a good predictor of estrus in lactating cows when measured at 6 h intervals. The second part of this study showed that ambient temperature plays a pivotal role in determining peripheral temperature and that any attempt to use peripheral temperature to identify changes in biological status must take ambient temperature into account. Further research is needed to determine if high ambient temperature causes similar changes in peripheral temperatures during the estrus period, and if increasing the frequency of temperature collection increases the ability to detect estrus in cattle using peripherally implanted microchips.

Figures

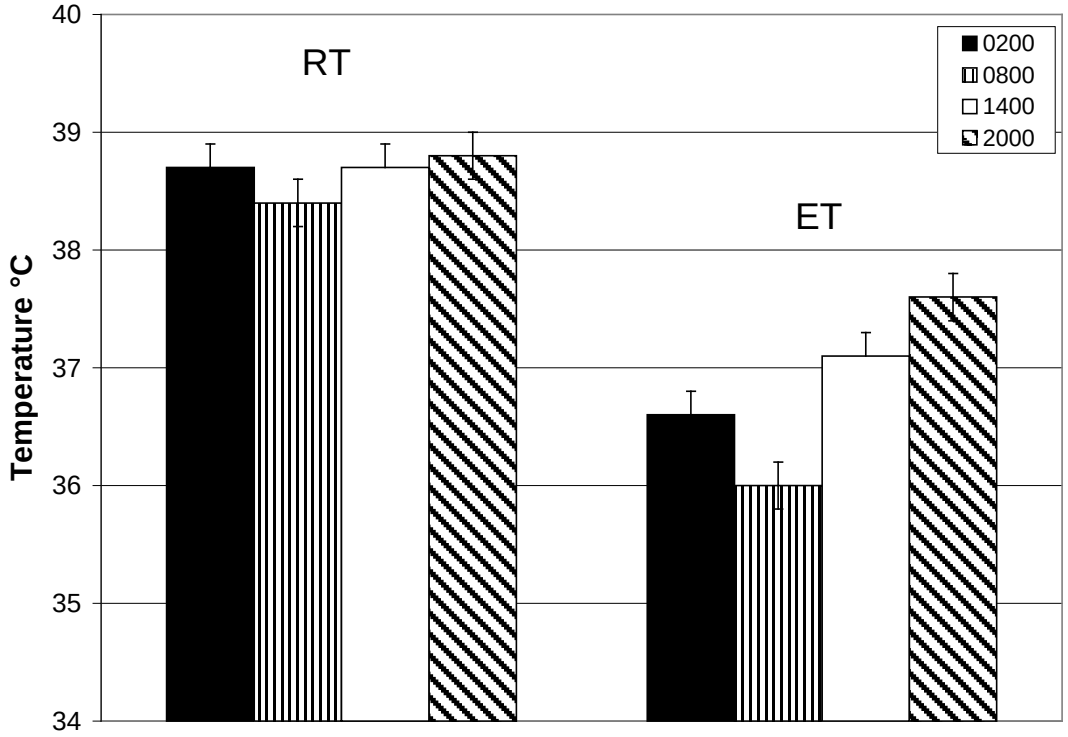


Figure 4.1. Rectal temperature (RT) and temperature recorded from subcutaneous radio frequency implants (ET) from 32 cows at 4 different time points during the day for the period from 7 d before to 7 d after expected estrus.

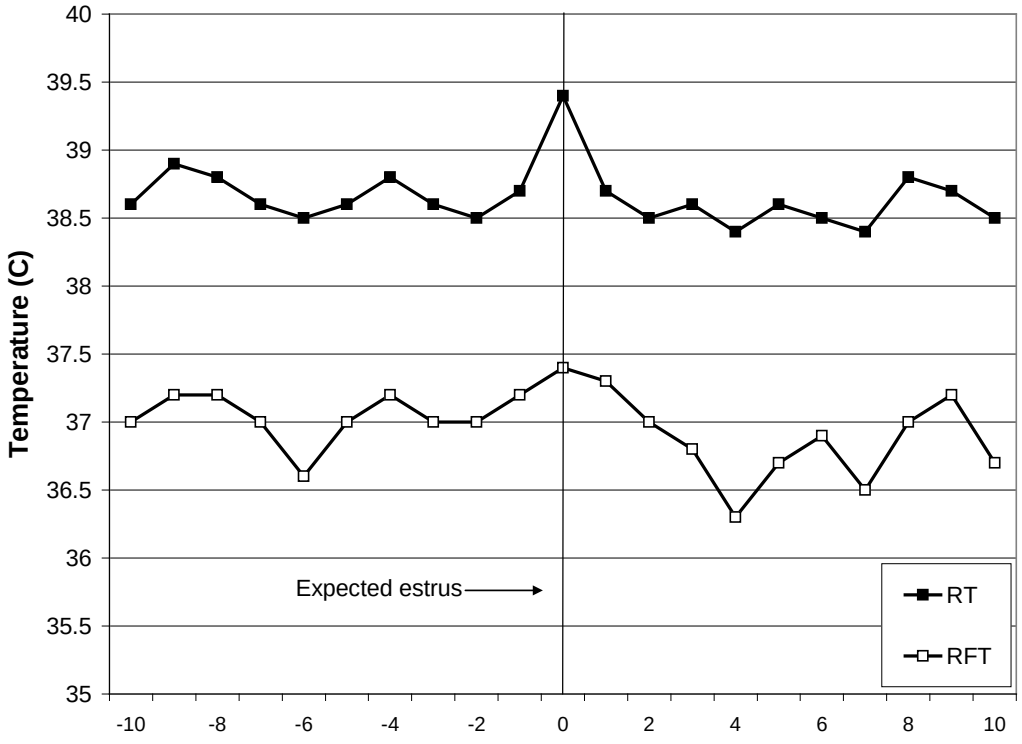


Figure 4.2. Rectal temperature (RT) and temperature recorded from subcutaneous radio frequency implants (ET) from mature cows during a synchronized estrus. Each point represents the estimated mean temperature for 32 cows. Each estimate was generated by readings collected at 6 h intervals each day. Timepoints on the x axis are collection points relative to expected estrus. Each point represents 6 h.

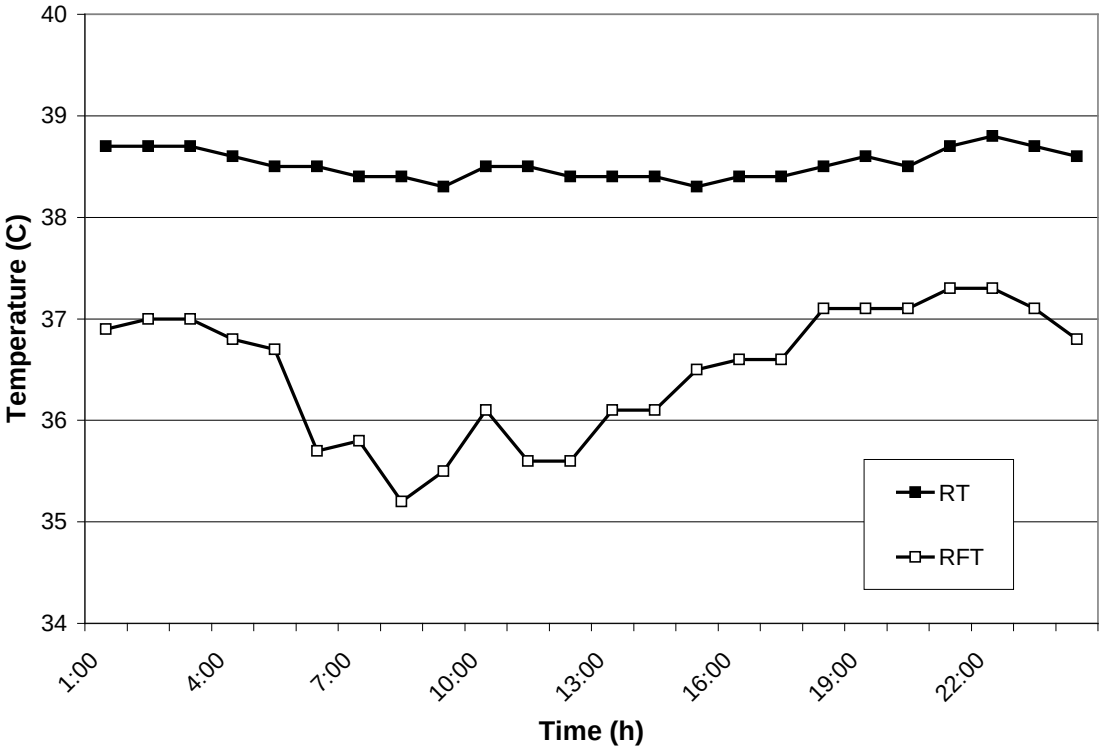


Figure 4.3. Mean hourly rectal temperature (RT) and temperatures recorded from subcutaneous radio frequency implants (ET) from mature cows during a synchronized estrus (3 cows) or a natural estrus (1 cow). Hourly recordings were taken for 7 d before and 7 d after expected estrus.

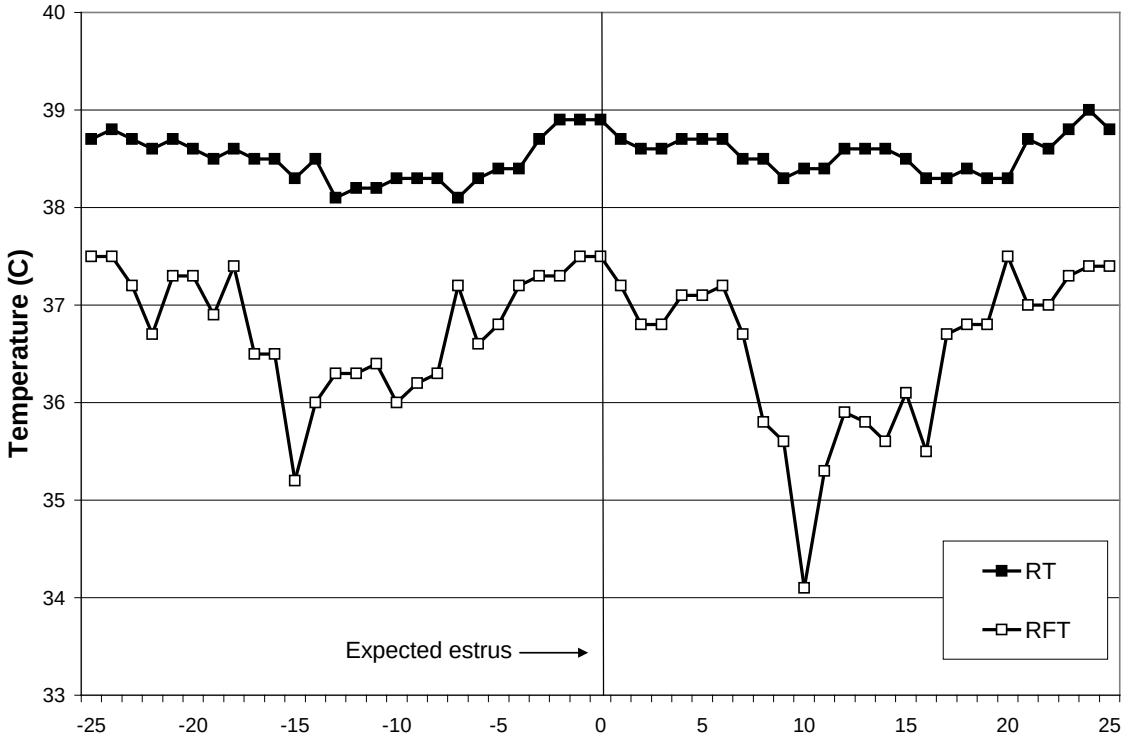


Figure 4.4. Mean hourly rectal temperature (RT) and temperatures recorded from subcutaneous radio frequency implants (ET) from mature cows during a synchronized estrus (3 cows) or a natural estrus (1 cow). Estimates are shown for 24 h before and 24 h after expected estrus. Each time point on the x axis represents 1 h.

CHAPTER 5

CORRELATION OF RECTAL TEMPERATURE AND PERIPHERAL TEMPERATURE FROM IMPLANTABLE RADIO-FREQUENCY MICROCHIPS IN HEAT STRESSED STEERS DURING A LIPOPOLYSACCHARIDE CHALLENGE

Abstract

Heat stress in livestock reduces production and profit for livestock owners by reducing feed intake. Heat stress also causes an increase in body temperature, which makes it difficult to identify sick animals in systems using rectal temperature as a tool to assess animal health. A radio-frequency microchip device has been developed that provides real-time monitoring of body temperature. Temperatures collected from these microchips (RFT) have been shown to be positively correlated with rectal temperature (RT) in cattle during thermoneutral periods and periods of high ambient temperature. However, these microchips were negatively correlated with RT during fever caused by lipopolysaccharide (LPS) administration. The hypothesis of the present study was that RFT would be positively correlated with RT under high ambient temperature conditions and negatively correlated with RT during a LPS challenge under high ambient temperature. To test this hypothesis, four weaned steers (127 ±7 kg) were housed in controlled environment chambers with individual stalls (2 steers per chamber), implanted with the microchips, and allowed 2 wk to acclimate. One chamber remained at 20 °C, the other was increased to 34 °C starting at 0800 h for a period of 48 h. LPS was administered by IV injection to all steers at 1000 h on d 2. The steers were then given a 2

wk adjustment period at 20 °C, and the ambient temperature was increased in the opposite chamber, resulting in a crossover statistical design with each steer serving as its own control. Rectal and microchip temperatures were logged at 5 min intervals. Ambient temperature was also recorded every 5 min and was included as a covariate in the statistical model. Pearson correlation coefficients for RFT and RT were 0.3 ($P < 0.01$) during heat stress, 0.20 ($P < 0.05$) during heat stress with LPS challenge, 0.34 ($P < 0.01$) during the thermoneutral period, and -0.42 ($P < 0.01$) during the thermoneutral period with LPS challenge. Individual response varied; some animals exhibited negative correlation while others exhibited positive correlation. These data do not support our hypothesis and suggest that individual response be considered when identifying models for use of RFT in temperature monitoring.

Introduction

It is estimated that heat stress costs the U.S. livestock industry approximately $2.5 billion per year due to decreased performance, increased mortality and decreased reproduction, and roughly $900 million of that loss is accounted for in the dairy industry (St-Pierre et al., 2003). Factors that affect the heat stress losses on dairy farms include a reduction in DMI (West, 1994), suppression of estrus behavior (Hansen et al., 2001), decreased fertility (Folman et al., 1983) and an increase in the incidence of mastitis (Giesecke, 1985). Methods of heat stress abatement include environmental modification, such as blocking radiant heat, the use of fans and soakers, genetic selection for animals that are less prone to exhibit the negative effects of heat stress, and altering nutrition to

decrease heat production and maximize DMI (Beede and Collier, 1986). The use of sprinkler/fan systems has been shown to increase milk production by up to 3.6 kg/cow/d in hot weather(Turner et al., 1992 and Her et al., 1988). The practical use of fan or sprinkler/fan systems must take into consideration the cost of water and electricity compared to the improvement in production and cow comfort. Using a temperature-humidity index (THI; Mayer et al., 1999) researchers have identified that a loss of 0.2 kg milk/d per unit of THI over 72 (Ravagnolo et al., 2000) or 0.41 kg milk/d per unit THI over 69 (Bouraoui et al., 2002), which stimulates some debate on the topic. Using a physiological monitor could provide more accurate timing to a heat abatement system that is designed to turn on when the cows become stressed, thus conserving water and electricity to times of need. At high THI (93 ± 3.1) an increase in rectal temperature (RT) of 0.47 °C has been reported (Srikandakumar and Johnson, 2004). Increases in RT due to heat stress can interfere with disease monitoring programs that use RT to identify animals experiencing a health event. Thus, a system that measures body temperature with high frequency may be more accurate in distinguishing between high body temperature due to environmental heat stress and an immune system challenge.

Currently, RT measurement is labor intensive and thus costly. However, a system exists that uses injectable microchip devices to monitor temperature in real time via radio frequency readings (Digital Angel Corp., St. Paul, MN). Temperature recordings from these microchips are positively correlated with RT in dairy cows during periods of high ambient temperature (> 31 °C; Reid et al., 2006). Readings from the microchips were positively correlated with RT in steers during thermoneutral periods but negatively correlated with RT during fever induced by lipopolysacharride (LPS) challenge (Reid et

al., 2005). The objectives of this study were to determine if readings from amicrochip implant, placed at the base of the ear in steers, were positively or negatively correlated to rectal temperature during periods of high ambient temperature and during a LPS challenge during heat stress.

Materials and Methods

Animal Description and Sampling Procedure

The use of animals in this experiment was approved by the University of Illinois Institutional Animal Care and Use Committee. Four weaned Holstein steers (127 ± 7 kg) were used in the study. The steers were moved from outdoor housing to an indoor controlled environmental facility and housed in two chambers, with each chamber accommodating two steers. The chambers measured 2.74 m (l) x 2.16 m (w) x 2.41 m (h) and each was equipped with a ventilation fan, three circulating fans, and a cooling unit. Supplemental heat during heat stress periods was provided by two electric space heaters positioned near the circulating fans. Inside each chamber, the steers were housed in painted wood crates measuring 0.8 x 1.5 m and 1.4 m high. Each crate had a grate in the rear for manure to pass through into a collection vessel below. Fresh feed and water were provided and manure was removed daily at 0800 h. The steers were allowed ad libitum access to pelleted alfalfa hay and water and were fed 2.3 kg/head/d of a grain mix to meet nutrient requirements (NRC, 2001).

Upon transfer to the chambers, the steers were implanted with a BioThermo microchip (Digital Angel Corp., Saint Paul, MN) at the base of the left ear under the

scutiform cartilage. The microchips were 2x12 mm and delivered via a pre-packaged sterile positive displacement syringe. The Biothermo microchips are part of a passive full-duplex, ISO 11784 and 11785 compliant system that operates at 134.2 kHz radio-frequency. The steers were observed daily for 1 wk for inflammation around injection sites. The steers were allowed 14 d after the move to acclimate to the environment at a thermoneutral temperature (20.7 +/- 0.7 °C).

A crossover design was utilized and after the initial acclimation period, the steers in chamber 1 were exposed to increasing ambient temperature starting at 0900 h and continuing for 47 h. The ambient temperature was maintained at 32 +/- 1.2 °C until 1900 h when it was decreased (27.1 +/- 1.1 °C) to simulate night time cooling. The temperature was again increased starting at 0900 h the following day and remained high until 0000 h when it was returned to thermoneutral. The steers in chamber 2 were maintained at thermoneutral temperature throughout period one. The steers remained at thermoneutrality for 14 d to re-acclimate and reduce any residual effects and the treatments were switched for each chamber following the same protocol as in period 1. Throughout the study, ambient temperature and humidity were logged every 5 min using HOBO data loggers (Onset Corp., Pocasset, MA).

Rectal temperature was collected with a digital thermometer with a 10 cm long, round end probe connected to a digital readout (model M500, GLA Agricultural Products, San Luis Obispo, CA). Microchip queries were performed with a handheld scanner that provided chip ID number and current temperature reading (Biothermo Pocket EX Reader, Digital Angel Corp., St. Paul, MN). Temperature collection (RT and RFT) started at 0700 h at the beginning of each period and continued for 48 h. The

frequency of collection was every 5 min from 0800 h to 1300 h, every 15 min from 1300 h to 2000 h, and every 30 min from 2000 h to 0800 h the following morning. On day two of the period, temperature collection frequency was the same as day one and LPS was administered into the jugular vein at 1000 h at a dose of 0.1 μg/kg BW (*E. coli* 055:B5; reconstituted with 0.9% saline solution, Sigma-Aldrich, St. Louis, MO). Respiration rate, skin tent duration, eyeball recession, and recumbancy were also monitored every 4 h from 1000 h on day one to 1800 h on day two to monitor heat stress and hydration status.

Statistical Analysis

Rectal temperature and microchip temperature (ET) were analyzed using the PROC MEANS, PROC MIXED, and PROC CORR functions in SAS (version 9.1, SAS Institute Inc., Cary, NC). The analysis of the crossover design was performed using PROC MIXED with repeated measures and the model included time of day, period of study, and presence of heat stress. To determine correlations during each period, the data set was broken into periods (i.e. thermoneutral no LPS, thermoneutral after LPS, heat stress no LPS, and heat stress after LPS). The correlation results are expressed as Pearson's Correlation coefficients (*r*). P values less than 0.10 were considered to suggest a likely correlation and p values less than 0.05 were considered to indicate a statistically significant correlation.

Results

For the duration of the study, steers at thermoneutral temperature averaged 15 breaths per min and did not show and signs of dehydration. Steers under high ambient

temperature averaged 25 breaths per minute, had mean skin tent durations of 2 s and showed some signs of eyeball recession (approximately 2 mm) by the end of 2 days of heat stress; all indicators of heat stress returned to normal within 4 h of reducing ambient temperature. A reduction in water consumption was observed, but not measured.

Mean temperatures during the thermoneutral period were 38.4 (+/- 0.2) and 39.1 (+/- 0.1) °C for ET and RT, respectively (Figure 5.1). Mean temperatures during the high ambient temperature period for ET and RT were 39.2 (+/- 0.2) and 40.0 (+/- 0.1) °C (Figure 5.1). The mean ET and RT for the period before LPS administration were 38.5 (+/- 0.5) and 38.9 (+/- 0.3) °C under thermoneutral conditions, respectively (Figure 5.2). Rectal temperature and ET were also positively correlated during that period ($r = 0.34$, $P < 0.01$). During thermoneutral conditions and during the LPS challenge (1000 h to 1200 h) mean RT and ET temperatures were 39.4 (+/- 0.4) and 37.6 (+/- 0.9)°C respectively (Figure 5.2) and were negatively correlated ($r = -0.42$, $P < 0.01$). The mean RT and ET were 39.4 (+/- 0.1) and 38.6 (+/- 0.2) °C (Figure 5.2) under thermoneutral conditions following the LPS challenge (1200 h to 0800 h the following day). The mean ET and RT for the period before LPS administration were 39.3 (+/- 0.6) and 40.0 (+/- 0.7) °C under high ambient temperature, respectively (Figure 5.2). Rectal temperature and ET were also positively correlated during that period ($r = 0.30$, $P < 0.01$). During high ambient temperature during the LPS challenge (1000 h to 1200 h), mean RT and ET were 40.4 (+/- 0.6) and 39.1 (+/- 1.1) °C, respectively (Figure 5.2) and were negatively correlated ($r = -0.20$, $P < 0.05$). The mean RT and ET were 40.0 (+/- 0.3) and 39.4 (+/- 0.5) °C (Figure 5.2) under high ambient temperature following the LPS challenge (1200 h to 0800 h the following day).

The weak correlations between ET and RT during LPS challenge and under high ambient temperature are illustrated by looking at individual animal temperature data. Three of the 4 steers exhibited a negative correlation (data from steer number 7860 shown in Figure 5.3) and one steer exhibited a positive correlation between ET and RT (data from steer number 7769 shown in Figure 5.4). In addition, the duration of the negative correlation between ET and RT differed for thermoneutral and heat stress periods (as depicted by data from steer number 7860; Figure 5.3). For steer number 7860, the drop in ET after LPS administration lasted roughly 1.5 h during heat stress, while it lasted for roughly 6 h under thermoneutral conditions.

Discussion

Rectal and RFT temperatures were positively correlated during thermoneutral ambient temperature and were negatively correlated during an LPS challenge under thermoneutral conditions. This concurs with previous research reporting similar relationships between rectal and ET during thermoneutral conditions and a LPS challenge in steers (Reid et al., 2005). Mean rectal temperature during the high ambient temperature period was 1.1 °C higher when compared with thermoneutral conditions. This increase was more than the 0.47 °C increase in rectal temperature reported by Srikandakumar and Johnson (2004). The large increase in rectal temperature in this study could be attributed to the acute increase in ambient temperature (1 h to get from thermoneutral to high ambient temperature zone). Rectal temperature and ET were also positively correlated during high ambient temperature periods. This is consistent with the relationship between RT and ET temperatures that also exhibited a positive correlation

during high ambient temperature (> 31 °C) in periparturient cows (Reid et al., 2006). Collectively, these results suggest that the steers were heat stressed, and that ET consistently reflected the body temperature of the animal.

In the present study three of the steers had a negative correlation between RT and ET during an LPS challenge under heat stress. All four steers exhibited an increase in RT during the LPS challenge. This suggests that a febrile response was initiated, but was regulated differently in one steer. This could be associated with the fact that temperature regulation is controlled differently during an immune response and heat stress. During an immune response, cytokines are released, altering central mechanisms that control heat loss and retention (Lazarus et al., 1993; De Benedetti et al., 1997), causing an increase in body temperature (Bligh, 1982). An innate immune response also includes the release of angiotensin from the liver, which is responsible for vasoconstriction (Charmandari et al., 2005). The resulting reduction in peripheral blood flow, induced by the immune response is most likely the cause of decreased temperature at the periphery observed during this study. In contrast, during a heat stress response circulating vasopressin concentrations are increased (Harikai et al., 2003) resulting in an increase in peripheral blood flow in an attempt to increase heat dissipation. It is possible that 3 of the steers' homeostatic mechanisms perceived the LPS induced immune challenge as a priority over the heat stress response, while the fourth steer perceived the heat stress as a priority and continued with heat abatement strategy. In support of this theory is a study by Lacetera and coworkers (2006) who showed a difference in immune cell function between two breeds of cattle during heat stress, indicating that different genetic profiles can affect the immune system response. Further research utilizing a similar experimental model and

Bos indicus and *Bos taurus* cattle could provide insight into the differences that genotypic variation plays during simultaneous heat stress and immune system stimulation.

Implications

The results of the present study suggest that RFT can be used to collect data potentially useful to identify animals experiencing heat stress and to use that information to manage heat mitigation strategies. Radio-frequency temperature collection also appears to be a promising technology for identifying animals experiencing a fever event, but these data show the importance of individual data collection, as individual animals respond to environmental and pathogenic challenges in unique ways. The duration of the negative correlation between RFT and rectal temperature (shorter duration under heat stress) should be considered when determining optimal data collection frequency. Future research is needed to determine how RFT responds to naturally occurring pathogenic insults and the frequency of data collection needed to identify animals during disease events.

Figures

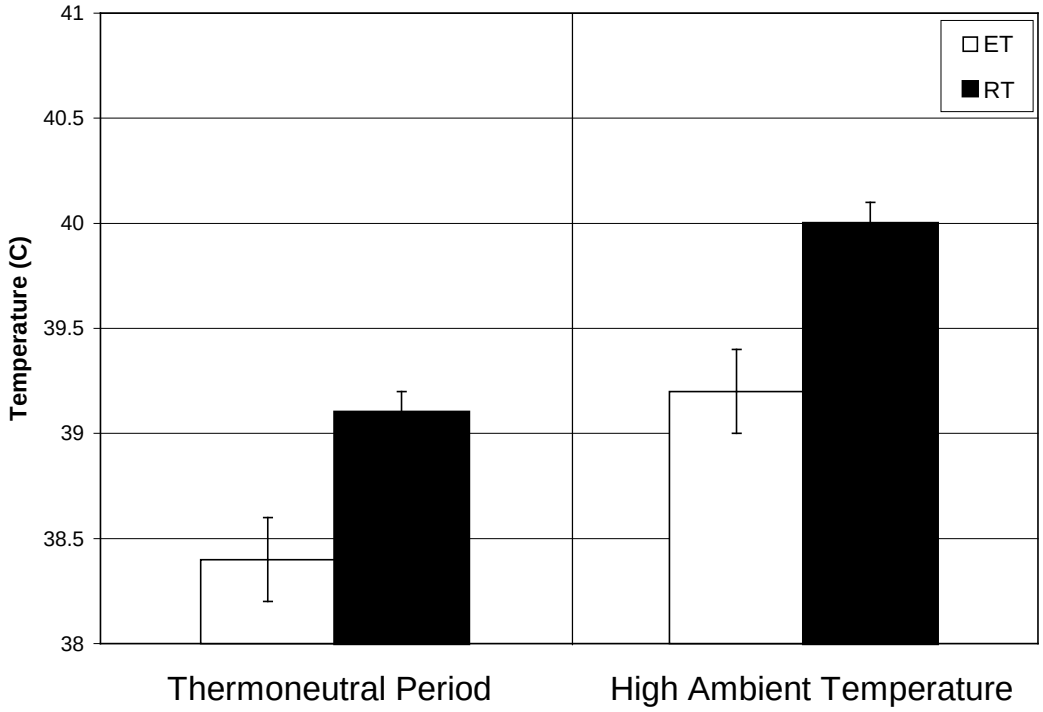


Figure 5.1. Mean rectal (RT) and radio-frequency microchip (ET: located under the scutiform cartilage of the left ear) temperatures taken during periods of thermoneutral (20 °C) and high (34 °C) ambient temperature.

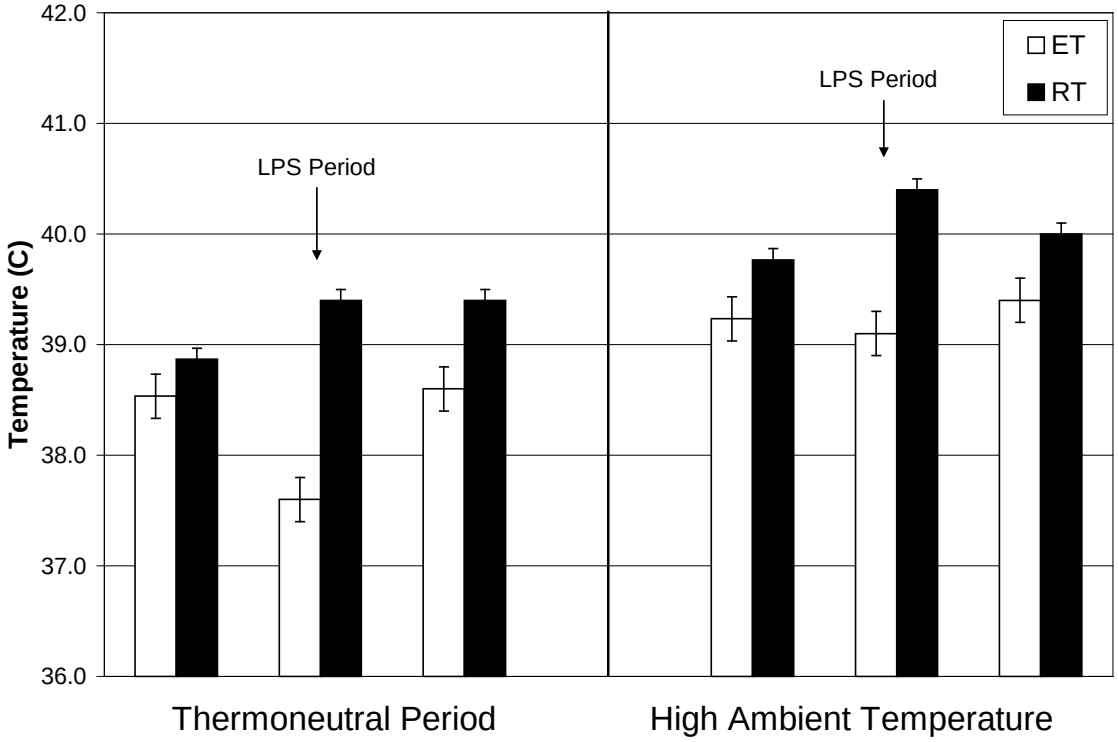


Figure 5.2. Mean rectal (RT) and radio-frequency microchip (ET: located under the scutiform cartilage of the left ear) temperatures in steers before, during, and after a lipopolysaccharide challenge (LPS Period) and during periods of thermoneutral (20 °C) and high (34 °C) ambient temperature.

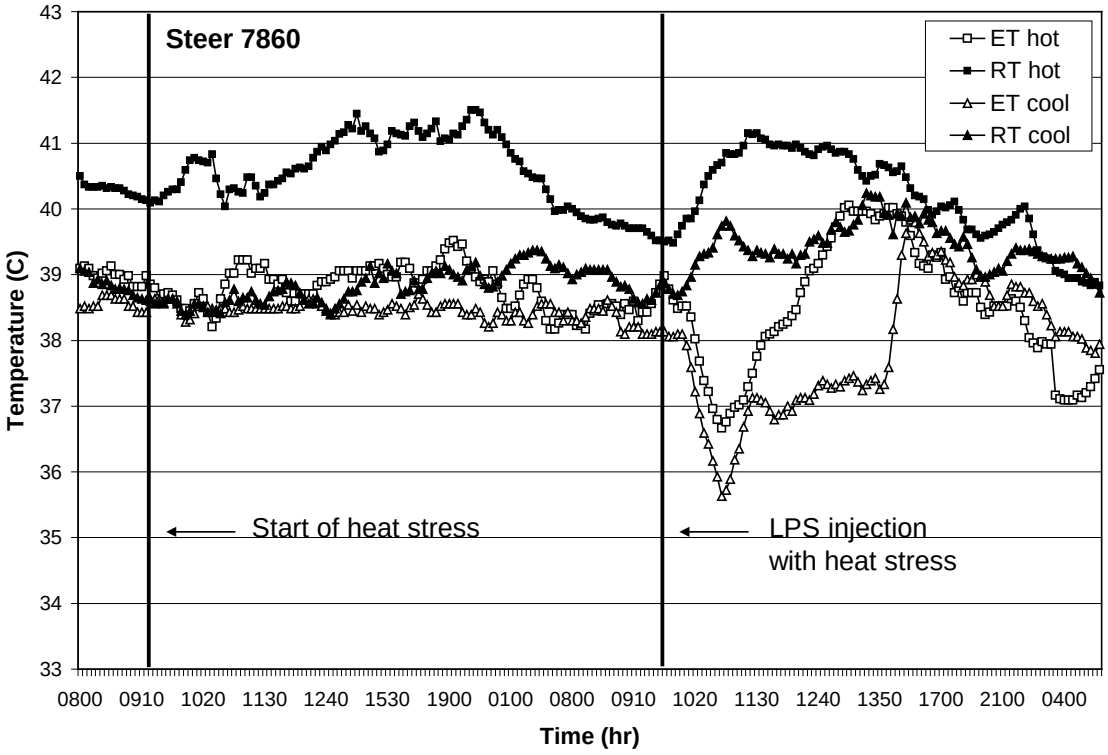


Figure 5.3. Complete data set for steer number 7860 displayed as a 3-point running average. Rectal (RT) and radio-frequency microchip (ET: located under the scutiform cartilage of the left ear) temperatures for two 2-day periods (cool = thermoneutral ambient temperature, 20 °C; hot = high ambient temperature 34 °C). The ambient temperature rise during the hot period was begun at 0900 h on the first day of the period and was reduced to 27 C at 2000 h to simulate night time cooling. Lipopolysaccharide (LPS) was administered on the second day of each period (at 1000 h) to stimulate a fever response.

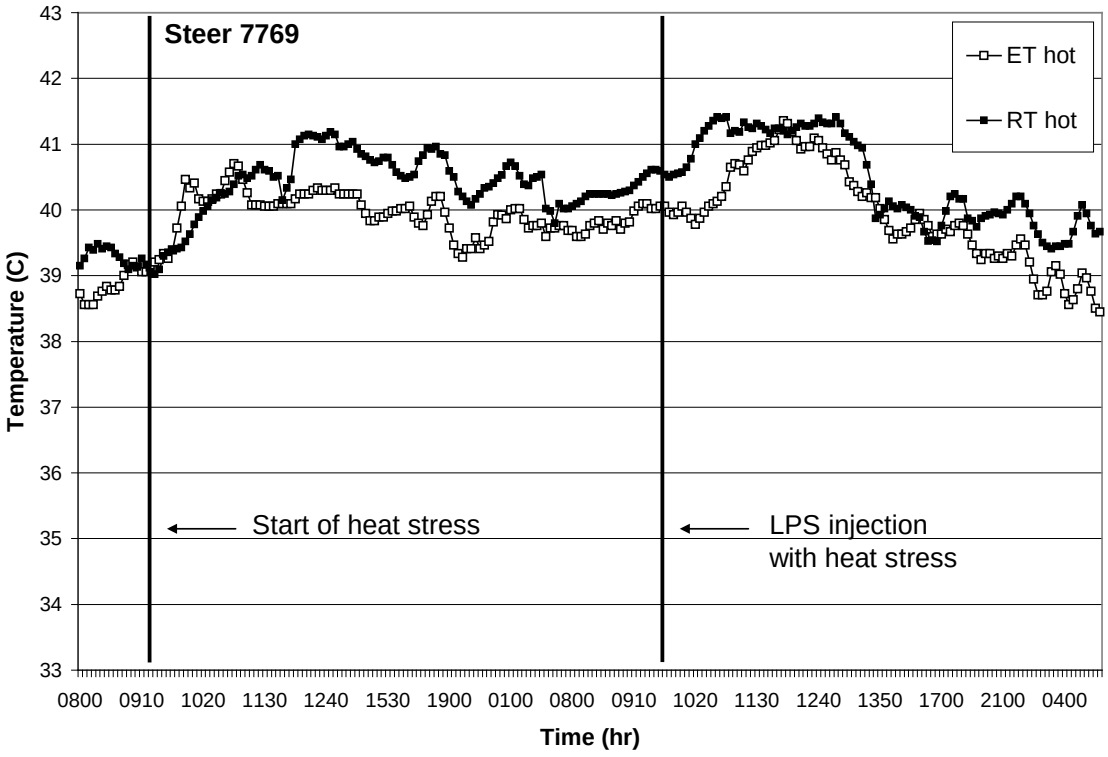


Figure 5.4. Complete data set for steer number 7860 displayed as a 3-point running average. Rectal (RT) and radio-frequency microchip (ET: located under the scutiform cartilage of the left ear) temperatures for a 2-day period at high ambient temperature (20 °C). The ambient temperature rise during the period was begun at 0900 h on the first day of the period. Lipopolysaccharide (LPS) was administered on the second day of the period (at 1000 h) to stimulate a fever response.

CHAPTER 6

OVERALL CONCLUSIONS

As production agriculture continues to head toward larger production facilities with a decreasing labor force, technologies that increase the ability to monitor animals individually without increasing labor cost become necessary to provide proper care. As animals travel through an ever expanding global market it is also advantageous to have the ability to identify movement from location to location to speed the identification of sick animals to prevent disease spread and maintain food security. The development of the microchips used in the experiments described here provide both individual animal identification and the ability to monitor body temperature. The experiments conducted with these microchips have revealed novel results and opportunities to improve the devices.

The results of these studies indicate that the BioThermo microchips correlate well with rectal temperature under thermoneutral conditions. In the first experiment a positive correlation was found between microchip temperature and rectal temperature in young steers at three separate locations (ear, poll, and umbilical fold) under thermoneutral conditions. The positive correlation was similar to that in both dry and lactating cattle not experiencing disease events in the second experiment and the positive correlation continued in those cows before and after estrus in the third experiment. The positive correlation was again found in a second cohort of young steers experiencing thermoneutral ambient temperature in the fourth experiment. There appears to be a repeatable positive correlation between the microchip and rectal temperatures during

periods of thermoneutral ambient temperature and when animals are not experiencing a disease event.

The ability to detect animals in a normal state is less important than being able to determine when animals are experiencing stressors that alter their body temperature. This series of experiments has demonstrated that the microchips will deviate from mean values when changes in temperature regulation occur. Most interesting is the negative correlation of the microchips to rectal temperature during an LPS challenge as seen in the first experiment. The mechanism of that response is thought to be related to an acute stress response that shunts blood to the core, thus reducing temperature at the periphery. This phenomenon was also exhibited in the fourth experiment when LPS was given at thermoneutral ambient temperature. This was also conserved under heat stress for three out of the four steers in the fourth experiment. The fact that one steer exhibited a positive correlation between rectal temperature and the microchip temperature indicates that using microchip temperature for diagnostic purposes requires models that take individual animal differences into account.

The results of these experiments indicate that there may be some value to using the microchips in a real world application. Some animals from the second experiment exhibited a reduction in microchip temperature roughly 24 hours before parturition. This could be used to identify animals needing to move to a maternity pen around calving. After calving there was one clear example of large changes in microchip temperature nearly 2 days before the animal was clinically diagnosed with metritis. Using the data generated during the first 2 weeks of lactation a model was able to identify all animals experiencing health events, although 75% of all animals were flagged. Some animals

exhibited significant changes in microchip temperature around estrus, although not enough to have significant results. There were also clear increases in microchip temperature when ambient temperature was raised beyond the thermoneutral zone. Data generated by these microchips could be used to identify animals experiencing heat stress and could hasten heat abatement strategies.

The microchip system tested is not without flaws. Currently, there is a large amount of labor needed to run the system. Temperature collection must be taken with a handheld wand which requires the user to be within reach of the animal. There is also little or no data collection and analysis software available. The data generated by these experiments indicate that data must be collected much more frequently to provide enough power to provide statistically significant results or develop systems that have higher resolution and/or more accuracy. Taking ear temperature every 6 h resulted in limited success in identifying animals experiencing health events, and attempts to construct a model to identify sick cows was also limited by high variation in the data set. While the data generated in the steer studies indicate that ear temperature collection every 5 min is robust enough to identify a fever event, it must be considered that the fever event in the studies was an acute response. Changes in ear temperature are most likely linked to blood flow at that location. It is assumed that a system that could collect temperature in the range of every second should have the ability to be more responsive to changes in blood flow, and thus having a higher chance of identifying changes in local temperature. It would also be necessary to match the data generated by a microchip to a system that monitors ambient temperature to account for environmental temperature changes.

In defining the needs for future remote temperature monitoring systems, it is important to consider animal management, facilities, and the ability to harvest the implant at slaughter. There are four main types of animal management; confinement, feed lots, pasture-based, and range-based. In confinement facilities animals are limited to movement within a barn or fenced pen with access to cover and generally have centrally located feed and water areas. The defined space could be up to 1000 m x 50 m or more, but typically is much less. On dairy facilities there is also the need for animals to enter a milking facility two or three times daily to harvest the milk. In feed lot systems large areas are fenced off with centrally located feed and water access and generally no or limited cover is provided. These feed lots can often be 1500 m x 1500 m or larger. In pasture based systems small areas of land (typically 10 acres sections) are sectioned off for use and are rotated frequently (generally every other day). In pasture based dairy systems the animals must return to a centrally located milking facility two or three times daily to harvest the milk, so a moveable system or one that could be utilized at the milking facility would need to be developed. In range systems, animals are allowed to graze large tracts of land that can sometimes be 100 km x 100 km or larger. In range systems very little animal management is performed and in some instances the animals are not seen for months at a time. The development of radio-frequency temperature collection systems should take these differences in facility design and management procedures into account, which means read distance will need to vary from 30 m to 50 km or more, employ microchips that have the ability to log the data and upload it at collection points, or offer systems that are suited for a limited number of applications.

There is also a need to harvest the microchips at slaughter to ensure that they do not enter the food chain. It is also necessary to prevent the recovery of the microchip from a live animal to ensure permanent identification. All microchips implanted at 3 locations in the first study were able to be harvested at slaughter. In addition, an attempt was made by University veterinary staff to recover the microchip located at the base of the ear in a live animal. After 2 h of surgery, the microchip was unable to be found, although it was still operational.

The results of these studies indicate that the BioThermo microchip system has great potential for use in identifying animals experiencing a range of biological changes through the use of peripheral body temperature. In order for the system to be utilized in common commercial applications will require the development of longer read distances, more frequent data collection, and better data collection and analysis techniques. More research is needed to develop more robust models that take into account varying ambient conditions and individual animal variation.

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