# MANAGEMENT STRATEGIES TO REDUCE EFFECTS OF THERMAL STRESS ON LACTATING DAIRY CATTLE

by

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# DEDICATION

To my father, Arnaldo Burgos.
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raising me with the morals and ethics to be a better person,
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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ADF | acid detergent fiber |
| AT | ambient temperature |
| BCS | body condition score |
| BGHI | black globe humidity index |
| BGT | black globe temperature |
| BMEC | bovine mammary epithelial cells |
| BSA | bovine serum albumin |
| BW | body weight |
| C | control |
| d | day |
| DIM | days in milk |
| DMI | dry matter intake |
| ECM | energy correcter milk |
| EGF | epidermal growth factor |
| EVHL | evaporative heat loss |
| FCM | fat corrected milk |
| g | grams |
| GM | growth media |
| h or hr | hour |
| HR | heart rate |
| HS | heat stress/heat shock |
| HSF | heat shock factor |
| Hsp | heat shock protein |
| IGF | insulin-like growth factor |
| kg | kilogram |
| KK | korral kool |
| $m^2$ | meter squared |
| MBT | mean body temperature |
| MY | milk yield |
| NDF | neutral detergent fiber |
| NEBAL | negative energy balance |
| NEFA | non-esterified fatty acids |
| P | period |
| PG | prostaglandin |
| PCR | polymerase chain reaction |
| R | rump |
| RH | relative humidity |
| RR | respiration rate |
| RT | reverse transcription or rectal temperature |
| S | shoulder |
| SH | shaved |
| ST | skin surface temperature or shade tracker |
| STHI | surface temperature humidity index |
| SU | supplemented |
| T | tail head |
| $T_{bg}$ | temperature black globe |

LIST OF ABBREVIATIONS- *continued*

| | |
|---|---|
| $T_{db}$ or $t_d$ | temperature dry bulb |
| $T_{dp}$ | temperature dew point |
| THI | temperature humidity index |
| TN | thermal neutral |
| TNZ | thermal neutral zone |
| TRT | treatment |
| TS | thermal stress |
| $T_{wb}$ or $t_{wb}$ | temperature wet bulb |
| USH | unshaved |
| VFA | volatile fatty acids |
| WBGT | wet bulb globe temperature |

## ABSTRACT

Two strategies to reduce impact of heat stress on high producing dairy cows were examined. The first was to recalculate the temperature-humidity index (**THI**) using high producing dairy cows under diurnal summer conditions. This re-evaluation confirmed that current THI values underestimate the severity of heat stress levels. Therefore, cooling of dairy cattle during warm summer months should begin at a THI of 68. Previously, a THI equal to or greater than 72 has been used to define onset of heat stress. This study demonstrated that a THI greater than or equal to 68 is sufficient to increase body heat storage, respiration rate, skin evaporative heat loss, declines in feed intake and milk yield.

A second objective involved three studies carried out to evaluate use of niacin in dairy cow rations to improve evaporative heat loss and resistance to heat stress. Niacin is known to cause intense vasodilation in human and lab species. We hypothesized that increasing vasodilation would improve evaporative heat loss in dairy cows. In the first niacin study, supplementation of lactating dairy cows with an encapsulated rumen by-pass form of niacin (**NIASHURE™**; Balchem Corporation, New Hampton, NY**)** at a dose of 12 g/d proved effective in alleviating some affects of heat stress during mild thermal stress. We hypothesized that encapsulated niacin would induce vasodilation effects documented in humans and lab animals increasing evaporative heat loss.

Past research demonstrated that the possible mechanism for vasodilation affects seen by niacin were most likely due to prostaglandin D secretions. Niacin may act through increased prostaglandin D and E production and secretion by Langerhans cells which then act upon vascular endothelial prostaglandin D receptors to increase vasodilation.

No studies have evaluated impact of encapsulated niacin on milk yield and composition during periods of thermal stress under commercial dairy conditions. The objective of the last study

was to examine the effects of encapsulated niacin during heat stress on milk production and composition as well as core body temperatures under commercial conditions.

# INTRODUCTION

During warm summer months milk production can decrease between 10 to 35% and this is a costly issue in the dairy industry (St. Pierre et al., 2003). The reduced milk yield is a result of increased body temperature induced-decline in feed intake as well as alterations in endocrine profiles, energy metabolism (Baumgard and Rhoads, 2007) and other unidentified factors (Collier et al., 2008). The economic impact of heat stress on the U.S. dairy industry has averaged annual losses of over $900 million as a result of reduced performance and increased incidence of disease (St. Pierre et al., 2003). Over the last 60 years there have been many attempts to develop a "heat index" which would provide estimates of cooling needs for domestic animals (Thom, 1958; Berry et al., 1964; Wiersma et al., 1990; Whittier et al., 1993, Armstrong, 1994).

The current THI was originally developed by Thom (1958) and extended to cattle by Berry et al (1964) is currently used to estimate cooling requirements of dairy cattle in order to improve the efficiency of management strategies to alleviate heat stress. Previous studies have shown that milk production can be reduced significantly (10 to 35%) when the THI exceeds 72 (Thatcher et al., 1974; Schneider et al., 1984). However, as pointed out by Berman (2005) the supporting data for these designations are not published. Several lines of evidence indicate that THI predictions of milk yield losses to varying THI values are currently underestimating the severity of heat stress on physiological responses in Holstein cattle (Linvill and Pardue, 1992; Holter et al., 1996; Ravagnolo et al., 2000; West et al., 2003; Berman, 2005). This is due in part to the fact that current high yielding cattle are producing more heat (Collier et al. 2006). Also, radiant heat load and/or convection effects were not evaluated by Berry et al. (1964). Thus, the THI index needs to be reevaluated.

In addition, it is clear that above the thermal neutral zone the primary route of heat loss for domestic animals is insensible or evaporative heat loss because sensible routes of heat loss

(conduction, convection and radiation) require a thermal gradient which disappears as environmental heat loads approach the animals body temperature (Collier et al., 2006). Increasing heat dissipation (the transfer of body heat from the core to the surface) via enhanced peripheral vasomotor function and evaporative heat loss may alleviate some of the decrease in dry matter intake and thus milk production. One possible method evaluated in research is the supplementation of encapsulated niacin to lactating dairy cows during periods of thermal stress. Niacin is known to cause vasodilation (Di Constanzo et al., 1997) which could lead to improved sweating rate and evaporative heat loss. However, free niacin is metabolized in the rumen and does not reach the small intestine (Campbell et al., 1994). Balchem Corp has recently developed a process to encapsulate niacin and improve delivery of niacin to the small intestine (Kung et al. 2003). Therefore, feeding encapsulated niacin, (NIASHURE™) may represent a management opportunity for dairy producers to provide improved thermal resistance to their cattle during warm summer months. The general objectives of this research were then; to reevaluate THI utilizing high producing dairy cows and to evaluate use of encapsulated niacin in diets of lactating dairy cows on heat dissipation and resistance to thermal stress.

# CHAPTER ONE: LITERATURE REVIEW

## Stress

Stress is defined as the magnitude of forces outside of the body resulting in a displacement of its systems from their resting or ground state (Yousef, 1985). Therefore, elevated ambient temperatures cause an animal to adjust in order for the animal to prevent physiological dysfunction and allow the animal to live in its environment (Kadzere et al., 2002). Heat stress is a result of the combination of high ambient temperatures, relative humidity, and radiant energy inhibiting the dissipation of heat from the animal to its environment (West et al., 1999). Environmental factors along with metabolic heat production prevent the animal from maintaining thermal balance or homeostasis, thus body temperatures rise and the animal must compensate and adapt in order to reestablish homeostasis (West et al., 1999). Direct effects of heat stress on cows include reducing feed intake, increasing water intake, altering metabolic rates, increasing maintenance requirements, increasing evaporated water loss, increasing respiration rates, and increasing body temperatures (Armstrong, 1994). Due to these effects, heat stress has a severe economic impact on the dairy industry because of reduced performance, reproduction and associated increases in mortality (St. Pierre et al., 2003). Therefore, it is vital to determine the threshold at which an animal's production begins to decline.

## Thermal Neutral Zone

Animal performance is maximized when environmental conditions do not impact their basal metabolic rate. This range of environmental temperatures is called the zone of thermal comfort or thermoneutral zone (**TNZ**; Armstrong et al., 1993; St. Pierre et al., 2003). The TNZ varies depending upon an animal's physiological status, (age, degree of

insulation and level of production) relative humidity of the environment, velocity of wind speed, ambient temperature, and the degree of solar radiation (NRC, 1981). When a combination of environmental factors result in conditions which exceed the thermoneutral zone of the animal, an imbalance, in the heat exchange between the cow and its surrounding environment causeses increasing amounts of heat storage (St. Pierre et al., 2003). For lactating dairy cattle the TNZ ranges from 1.7° to 21°C and is dependent upon breed, degree of acclimation, milk production, and/or dry matter intake (DMI; Johnson and Vanjonack, 1975). Milk production averages have doubled per cow in the United States since the 1950's, thus the TNZ has shifted downward as cows become more heat sensitive and cold tolerant (Collier et al., 2006). The mechanisms by which an animal can alleviate effects beyond their TNZ become minimal as the stressors increase. Understanding and identifying these mechanisms are important in order to resolve the challenges currently associated with the economic losses of heat stress.

**Heat Dissipation**

Typically, a mammal maintains its core body temperature above the ambient temperature in order to facilitate heat dissipation from the core of the body through four basic routes of heat exchange (conduction, convection, radiation and evaporation; Collier et al., 2006). The sensible routes of heat loss are conduction, convection, and radiation which all require a thermal gradient in order to aid in heat dissipation. Evaporation is considered an insensible form of heat loss therefore requiring a vapor/pressure gradient (Collier et al., 2006).

The heat flow from one surface in direct contact with another surface is considered conduction. Only 20% of a dairy cow's surface area is available for heat dissipation through conduction and that only occurs if the animal is lying down. If the animal is standing, there is essentially no heat lost by this route of energy exchange. The direct contact with a cooler surface allows the animal to transfer heat energy to the surface and the rate of energy flow is directly affected by the size of the temperature gradient between the animal and the surface it is lying on. Therefore, heat stressed animals tend to seek cool wet surfaces to lie on if possible.

The transfer of heat from a stationary or fixed surface to air movement or water is considered convection. Factors influencing convection are size of the gradient, velocity of air or water movement, and degree of insulation such as fat, fur or wool which would impede heat transfer to the skin surface (fat) or air or water flow over the surface (hair or wool; Kadzere et al., 2002).

Heat exchange by radiation is the net amount of heat lost or gained through emission or absorption of infrared radiant energy (Kadzere et al., 2002). Radiant heat loss or gain occurs at the speed of light and is therefore instantly felt by the animal. Increased levels of relative humidity and clouds can inhibit heat dissipation by radiation but also tend to reduce incoming solar radiation (Fuquay, 1981). During periods of high ambient temperatures such as during summer months there is a net heat gain from radiation during daytime hours and net heat loss from radiation at night when the animal is radiating to the cooler night sky; however, there is little heat gain or loss from conduction or convection (Fuquay, 1981).

Evaporation is driven by a vapor pressure gradient, therefore, when the dew point temperature around the animal is lower than the temperature of the evaporative surfaces of the animal or if there is an increase in air velocity and a low humidity level around the animal, evaporative heat loss can occur (Fuquay, 1981). Evaporative heat loss from cattle occurs by two routes; skin and surfaces of the respiratory tract (Bligh and Johnson, 1973). When ambient temperatures approach the surface temperature of the animal, heat loss by sensible means (radiation, conduction and convection) is lost because it depends on a temperature gradient and the only route of heat loss left is via evaporation and if the ambient temperature rises above the body temperature heat flow will reverse and the animal will become a heat sink (Collier et al., 2006). Evaporation is decreased when high levels of humidity are present due to heat dissipation being challenged as environmental temperatures nears the body temperature of the animal (West, 1994). Thus, when cattle are above their thermoneutral zone the primary route of heat loss is evaporative. Unfortunately, cattle are not capable of high sweating rates. For example, cattle can only dissipate 105% of metabolic heat production where as humans can dissipate 190% because of their superior ability to sweat (Bianca, 1965; Bohmanova et al., 2007). Thus, dairy cattle are susceptible to increased environmental heat loads.

As discussed above, environmental factors influence the routes of heat dissipation available to the animal. Not only is it a single factor from the environment that can affect the dissipation of heat but rather a combination of environmental factors as well. Utilizing certain calculations such as THI, black globe humidity index and wet bulb glob

temperature are important in aiding management to implement different technologies to try and alleviate some if not all of the heat stress.

**Temperature Humidity Index (THI)**

THI is a value associated with different levels of thermal stress and combines the effects of humidity and temperature (Bohmanova et al., 2007; West, 2003). The initial purpose of this index was to be used as a weather safety index in order to monitor and reduce heat-stress losses (Bohmanova et al., 2007). Tolerance of heat stress differs between species and the sensitivity to ambient temperatures and air humidity (Bohmanova et al., 2007; West, 2003).

Evaporative heat loss through the skin and lungs is impacted by the water vapor content in the air (Bohmanova et al., 2007). When ambient temperatures increase outside of the TNZ, moisture in the air is an important determinant in an animal's thermoregulatory system (Bohmanova et al., 2007). Water vapor content is measured by relative humidity (RH), wet bulb temperature ($T_{wb}$) or dew point temperatures ($T_{dp}$) (Bohmanova et al., 2007). Relative humidity determines the saturation of the air at a certain air temperature and $T_{dp}$ measures water vapor content (Bohmanova et al., 2007). Wet bulb temperature represents the equilibrium temperature of a thermometer covered with a wet cloth with pure water (Bohmanova et al., 2007). The temperature that the air has to be cooled in order for saturation to occur is determined by $T_{dp}$ (Bohmanova et al., 2007). There are many proposed THI calculations however different weightings are placed on various variables (Bohmanova et al., 2007). Calculations implementing $T_{wb}$ have been proposed by Thom, 1958; Bianca, 1965; NRC, 1971), $T_{dp}$ (NRC, 1971;

Yousef, 1985), or RH (NRC, 1971); however none of the calculations have been specifically designed for lactating dairy cows.

The Temperature Humidity Index (THI = $t_{db}$ + .36$t_{dp}$ + 41.5, where $t_d$ = dry-bulb temperature, $^0$C and $t_{dp}$ = dew point temperature, $^\circ$C) was originally developed by Thom (1958) and in 1964 Berry and colleagues extended its use to cattle. Currently it is widely used in computerized cooling systems to estimate cooling requirements of dairy cattle. The Livestock Conservation Institute categorized the THI values into mild, moderate and severe stress levels for cattle (Whittier, 1993; Armstrong, 1994). Berman (2005) reported that the data supporting these results were not clearly understood for today's industry standards. For example, the index is based on a retrospective analysis of studies carried out at The University of Missouri in the 1950's and early 1960's on a total of 56 cows averaging 15.5 kg/d, (range 2.7-31.8 kg/d). In contrast, average production per cow in the United States is presently over 30 kg/d with many cows producing above 50 kg/d at peak lactation. The elevated milk production observed from past years to recently, increases the sensitivity of cattle to environmental thermal stress and reduces the tolerance or "threshold temperature" at which milk yields begin to decrease (Berman, 2005). This is due to the increases in metabolic heat production as the production level of a cow is also increased. For example, cows producing 18.5 and 31.6 kg/d of milk demonstrated increased heat production, of 27.3 and 48.5% over heat production in non-lactating cows (Purwanto et al., 1990). Berman (2005) reported that increasing milk production from 35 to 45 kg/d decreased threshold temperature for tolerance of heat

stress by 5°C. Furthermore, potential effects of infrared heat load from metal roof's or convection effects were not evaluated in the research conducted by Berry et al., (1964).

Management time intervals are an additional factor in determining THI values. The time interval involved in the original THI predictions by Berry et al. (1964) was two weeks. In other words, the milk yield response to a given THI was the average yield in the second week at a given environmental heat load. However, this time lag is physically unacceptable and current dairy producers need real time estimates of cooling needs in order to prevent present and future production losses. Collier and colleagues (1981) and Spiers and co-workers (2004) indicated that effects of a given temperature on milk yield were maximal between 24 and 48 hours following a stress. Additionally it has been reported that ambient weather conditions 2 d prior to milk yield measurement had the greatest correlation to reductions in production and dry matter intake (West et al., 2003). Furthermore, Linvill and Pardue (1992) indicate that the total number of hours when THI exceeded 72 or 80 over a 4 d interval had the highest correlation with milk yield. Collectively, these results demonstrate that current THI values for lactating dairy cows underestimate the size of the thermal load as well as the impact of given thermal loads on animal productivity. In addition, there is an inappropriate time interval associated with cooling management decisions. Practically, if producers can avoid an acute (i.e. 48 hr) decline in production this will probably result in maintaining milk yield in the long run (i.e. two weeks later). Specifically, the time frame for utilizing THI values to reduce milk yield losses needs to be shortened. New studies need to be conducted utilizing high producing dairy cows and including radiant energy impacts on animal performance.

Furthermore, impacts of a given THI on milk yield within 48 hours need to be identified. This will provide meaningful data to producers who need this information to make immediate cooling decisions to improve cow comfort, animal well-being and to maintain current and long-term production.

A final component of the current THI index is the pattern of stress application. In the original work by Berry et al. (1964), cows were exposed to given THI conditions continuously (no daily fluctuations) for the entire two week period. This is obviously not what occurs under natural/practical management conditions where temperatures cycle (rise and fall) during a normal day. As a consequence, we presently do not know how to assess the true THI value. For example, is it the average, the peak or minimum which is important? Alternatively, is it a given number of hours above an arbitrary THI value which is most critical? Holter et al. (1996) reported that minimum THI was more closely correlated with reduced feed intake than maximum THI. Ravagnolo et al (2000) evaluated test day yields and found a decrease of 0.2 kg milk per unit increase in THI above 72 when THI was composed of maximum temperature and minimum humidity. A designed study where temperature and humidity are controlled in a circadian manner, similar to natural environmental conditions, has never been conducted. West et al. (2003) evaluated feed intake and milk yield under natural conditions and found that mean THI two days earlier had the greatest effect on both intake and yield. However, they were working under natural conditions and could not quantify the relationship between THI and milk yield.

**Wet Bulb Globe Temperature (WBGT)**

In the 1950's the WBGT was constructed in order to control serious outbreaks of heat illness in training camps of the United States Army and Marine Corps (Budd, 2008). The WBGT is calculated from the wet bulb temperature ($T_{wb}$) the black globe temperature (BGT), and sometimes the dew point temperature ($T_{db}$), (Budd, 2008). Using these three variables the BGT represents the ambient temperature plus any incoming infrared heat load and any cooling effects of wind speed while $T_{wb}$ represents the ease of evaporation (Budd, 2008). The concept is that while radiant heat warms the BGT above $T_{db}$ and the wind cools it down towards the $T_{db}$, therefore the WBGT measures the combined effect of radiant heat, $T_{db}$, and wind speed (Budd, 2008). The purpose of using WBGT is to overcome the limitations of wet bulb temperature alone (Lee, 1980). Combining these effects in one index helps WBGT correspond with the "effective" temperature very closely (Lee, 1980).

**Black Globe Humidity Index (BGHI)**

The BGHI is a single number or value that takes into account $T_{db}$, RH, net radiation, and air movement (Buffington et al., 1981). Instead of inserting the $T_{db}$ into the THI equation, the black globe temperature ($T_{bg}$) is inserted creating the BGHI (Buffington et al., 1981). Researchers have shown BGHI is significantly higher in areas with no shade than areas with shade, demonstrating the effects of radiation (Buffington et al., 1981). The BGHI is considered a comfort index for cows because of the cumulative effects of $T_{db}$, RH, net radiation, and air movement (Buffington et al., 1981). When identifying a measurement for cow comfort and production BGHI is a more accurate

indicator than THI under heat stressed conditions with solar radiation (Buffington et al., 1981). In areas of low to moderate thermal radiation, THI or BGHI give similar estimates of cow comfort (Buffington et al., 1981). Rectal temperature increases and milk yield decreases have a higher correlation to BGHI than THI (Buffington et al., 1981).

The effects of radiant heat load can be evaluated using the Black Globe Humidity Index (BGHI = $t_{bg}$ + .36$t_{dp}$ + 41.5 where $t_{bg}$ = black globe temperature $^{\circ}$C and $t_{dp}$ = dew point temperature, $^{\circ}$C), developed by Buffington et al. (1981). These investigators demonstrated that BGHI had a higher correlation to rectal temperature increases and milk yield decreases than THI. They also pointed out that the correlation of BGHI to milk yield was greater ($r^2$ = .36) under conditions of high solar radiation (no shade) than under a shade structure ($r^2$= .23). However, milk yields in this study were also low (average 15 kg/cow). Therefore, correlations of BGHI to milk yield under shade structures might be higher with higher producing dairy cows (which are more sensitive to increased heat loads).

Along with having environmental measurements to help producers alleviate heat stress there are other parameters that can be used. The physiological parameters of an animal can indicate if an animal is undergoing heat stress or in perhaps their TNZ, therefore by evaluating these changes we can get a better understanding of the mechanisms behind heat stress.

**Parameters indicative of heat stress**

The upper limit of ambient temperature where Holstein cattle can maintain homeothermy or a stable RT is between 25 to 26°C (Berman et al., 1985). When ambient temperatures are increased from 18 to 29°C, heat intolerant cows illustrated a 1.4°C increase in body temperature and a 4 kg decrease in daily milk yields compared to 0.7°C increase and a 2 kg decrease in daily milk yields for heat tolerant cows (Morrison, 2000). During heat stress, respiration rates have been used as one standard as an indicator of heat stress, and cows experiencing a respiration rate between 80-90 breaths/min are classified as cows undergoing heat stress (Stowell, 2000). Cows provided shade had lower RT (38.9 vs. 39.4°C) to cows not provided with shade, they also had decreased RR (54 vs. 82 bpm), and a 10% increase in milk yields (Collier et al., 2006). Milk yields have shown a significant effect on RT however, only accounted for 9% of the variance (Berman et al., 1985). The ST is significantly correlated to RR and RT in dairy cows (Sampson et al., 2004). Black globe temperatures greater than 35°C increased RT along with RR (Collier et al., 1981).

During heat stress, cows will spend the majority of the daytime inactive and under a shade structure or natural shade; while RT and RR increase progressively throughout the day (Seath and Miller, 1947a, 1947b, 1947c). The rise in RT and RR can be reduced by sprinkling water on the animals to increase evaporative heat loss (Seath and Miller, 1947b). Breed effects are also apparent when evaluating effects of environment on cattle when given the options of remaining under a water shower, Holsteins remained under the water 9% more than Jersey cows, illustrating that Holstein breed is not as efficient at

maintaining homeothermy and therefore relies on the assistance of evaporation more heavily (Miller et al., 1951).

Oscillations in body temperatures can be due to passive shifts in heat which can be caused from environmental or internal changes in thermal load (Verwoerd et al., 2006). These changes may come with lag times as a result of thermal inertia or because of shifts in adjustments of thermoregulation (Verwoerd et al., 2006). Hypothesis of episodic changes in core body temperature have been thought to be due to changes in the thermal environment (Verwoerd et al., 2006). Researchers found a marked oscillation of core body temperature during the early morning hours, although not completely understood when comparing to non-lactating cows, it is thought that this may be caused by metabolic effects of milk production and/or the schedules of milking times (Verwoerd et al., 2006). When core body temperatures peak following THI peak with a time delay it is because of thermal inertia associated with limited heat transfer rate and the body mass of the animal (Verwoerd et al., 2006). The idea of a trigger level of THI has also been discussed, in which the normal temperature regulation mechanisms of the animal are not able to function and this can be due to the rise in body temperature being almost three times the normal range of variation in more severe episodes (Verwoerd et al., 2006). Body temperatures have also shown to rise sharply when animals are lying down due to less body surface area that is available for convective or evaporative cooling (Hillman et al., 2001a).

The majority of heat loss in dairy cows during elevated temperatures resulting in heat stress is evaporative heat loss (Gebremedhin and Wu, 2001). Hillman et al, 2005

and Gebremedhin et al. 2008 evaluated factors regulating evaporative heat loss in cattle and found that hair coat qualities, skin temperature and wind speed were major factors affecting evaporative heat loss from skin. During heat stress cows can experience two routes of autonomic responses such as sweating and panting (Gebremedhin et al., 2008). Heat loss through evaporation from skin temperature is result of sweating (Gebremedhin et al., 2008). Panting increases air velocity in the respiratory tract which increases evaporative heat loss (Gebremedhin et al., 2008). Other factors that affect evaporative cooling are ambient temperature, velocity of wind, relative humidity, thermal and solar radiation (Gebremedhin et al., 2008). Research on accurate measures of evaporative heat loss is limited as past research was conducted in the 1950's using dairy and feedlot cattle. Results comparing sweating rates have been reported as inconclusive or non-comparable due to incomparability of methods of measurement and environmental conditions that were implemented when data was collected (Gebremedhin et al., 2008). Holstein cows under direct sunlight with black hair coats had increased surface temperatures by 4.8°C compared to white hair coated cows by 0.7°C (Hillman et al., 2001a). Researchers reported that differences were due to the solar absorptivity by black hair coated animals compared to the white hair coated (Hillman et al., 2001a). They also found Holstein cows with black hair coats had significantly elevated sweating rate levels compared to Holstein cows with white hair coats (Hillman et al., 2001a).

**Glucose Metabolism**

As discussed previously, during heat stress milk yield and DMI are reduced however, the maintenance requirements or costs of the animals are increased by up to

30% (Morrison, 1983; Fox and Tylutki, 1998). Due to the decrease in DMI the energy made available to the animal in the diet is reduced as well causing the animal to experience a negative energy balance (NEBAL) as energy utilization is increased (Moore et al., 2005).

Metabolizable energy available to the ruminant is in the form of volatile fatty acids (VFA's) produced via rumen fermentation from carbohydrate metabolism (Kelly et al., 1967). During heat stress the amount of VFA's produced is decreased due to lowered ruminal contractions of VFA's thus a reduction in DMI (Schneider et al., 1984; Beede and Collier, 1986; Gengler et al., 1970). The reductions of VFA's reported are not entirely due to the decrease in DMI. Past research has shown that concentrations are restored partially when the orts of cattle subjected to heat stress were force fed through rumen cannulae in order to maintain DMI levels equal to those animals undergoing a thermoneutral environment (Kelly et al., 1967). In a normal fed state, ruminant animals such as dairy cows receive the majority of the glucose through gluconeogenesis via the liver. This is primarily due to its inefficiency to absorb dietary glucose in the digestive tract. The three VFA's produced in the rumen are acetate, butyrate, and lastly, propionate. All three can be converted into energy for the animal however; propionate is the only precursor for gluconeogenesis. Research has shown that when cows are exposed to heat stress there is an increase in the rate of glucose disposal in heat stressed animals compared to cows pair fed in a thermoneutral environment (Wheelock et al., 2006). The same group also showed increased sensitivity to insulin in response to glucose challenges when comparing to cows being under fed. Pair fed animals compared to heat stressed

cows fed ad libitum had similar glucose patterns regardless of both decreasing glucose concentrations by seven percent (Rhoads et al., 2007). These similar glucose patterns seen in the pair fed and heat stressed groups could be possible due to the decrease in glucose concentrations as a result of decreased hepatic propionate delivery (Rhoads et al., 2007). Measuring non esterified fatty acids (NEFA's) have been indicative of lipolysis in dairy cows. When cows are heat stressed the normal response of increasing NEFA concentrations is not seen rather the NEFA concentrations are decreased compared to thermoneutral pair fed cows, thus resulting in an animal that is hypersensitive to insulin (Rhoads et al., 2007). Although adipose tissue is not being mobilized as indicated by NEFA's during heat stress, the expectation of increasing fatty acid oxidation is not observed as well (Shwartz et al., 2008). Reports have been made that heat stress could be affecting the cellular physiology and systemic metabolism however, identifying the direct and indirect effects of thermal load are challenging (Rhoads et al., 2007).

Plasma NEFA concentrations have been shown to be increased in plasma during warm months or seasons compared to cows in the cool months/seasons most likely due to the decrease in DMI (Skaar et al., 1989).

**Heat Shock Response**

During heat stress there is an altered gene expression response that is caused by a cascade of protein activation (Sonna et al., 2002; Collier et al., 2006). This response in gene expression is under the regulation of heat shock transcription factor (**HSF**; Pirkkala et al., 2001; Page et al., 2006). The main function of heat shock proteins (**Hsp**) during heat stress is to protect the cell, and this has been illustrated by Hsp over expression

protecting against hyperthermia, circulatory shock, and cerebral ischemia during stages of heat stroke (Lee et al., 2006). When the cell is exposed to increased temperatures as would define heat stress abnormalities occur in the function of the cell (Sonna et al., 2002). These include inhibition of protein synthesis, irregularities in protein function and structure, morphological alterations; metabolism shifts, changes in cell membrane dynamics and fluidity, and decreases in cell proliferation (Sonna et al., 2002). When these changes occur we begin to see the effects of heat shock response and the end result of the cell which is survival and acclimation or cell death (Lanks, 1986; Lindquist, 1986). The first responders during heat shock are the HSF's which are a family of transcription factors whose primary function is to coordinate the cellular response to heat stress and alter the expression of genes, in our case, Hsp's (Akerfelt et al., 2007; Page et al., 2006). Heat shock transcription factor-1 (HSF-1) is the primary factor responsible for the action of Hsp gene expression during heat stress (Pirkkala et al., 2001). In a normal non-stressed cell, it is believed HSF-1 is folded in the cell as a monomer bound to Hsp in the cytoplasm, when heat stress is induced, the HSF-1 and Hsp disassociate, then unfolding and binding to two HSF-1 monomers in order to form a trimer before translocating into the nucleus (Collier et al., 2008). In the nucleus, homotrimeric HSF-1 can then bind promoters containing heat shock elements in order to initiate the activation of heat stress gene transcription (Collier et al., 2008). Studies analyzed using bovine mammary epithelial cells (**BMEC**) it has been shown that during heat stress (42°C) , the BMEC showed regression of ductal branches and a decrease in cell growth; along with a reduction in gene expression for genes that are known to be associated with protein

synthesis and metabolism of the cell (Collier et al., 2006). In this study, they reported a elevation of Hsp 70 gene expression in BMEC for 4 hours at 42°C prior to returning to basal levels 8 hours later, they reported this due to thermotolerance and the activation of genes associated with apoptosis (Collier et al., 2006).

Prostaglandin $A_1$ (**$PGA_1$**) has been shown to induce HSP synthesis in mammalian cells in turn protecting the cell against stressors and prostaglandin $E_1$ (**$PGE_1$**) is known during a fever to alter the hypothalamic set point (Collier et al., 2007). The effects of $PGA_1$ and $PGE_1$ on the gene expression of Hsp 70 using BMEC were evaluated. The addition of $PGA_1$ to the cell culture media increased thermotolerance of BMEC in part by increasing Hsp 70 gene expression 150-fold over controls during an 8 hour period at 42°C (Collier et al., 2007). As discussed earlier, niacin has been shown to induce vasodilation caused by acting through prostaglandin D production by the epidermal Langerhans cells (Benyo et al., 2006; Maciejewski et al., 2006) and vascular endothelial prostaglandin $D_2$ receptors (Cheng et al., 2006). The response of Hsp's to niacin and prostaglandin D have not been studied however, research associated with the vasodilation affect and prostaglandin in the presence of niacin has been reported and therefore, the concept of prostaglandin D and niacin alone or in combination should increase Hsp response during heat stress.

**Heat stress and milk production**

During heat stress months milk production in dairy cows decreases between 10 to 25% (Thatcher et al., 1974; Schneider et al., 1984). When milk production increases the sensitivity of cows to heat stress also rises, reducing the "threshold temperature" at which

milk loss occurs and is caused by metabolic heat production associated with increased milk production (Berman, 2005). The ambient temperature when milk production decreases is dependent upon the animals' degree of acclimation, daily milk production, amount of air movement, and RH (Fuquay, 1981). Research has shown heat production from cows producing 18.5 and 31.6 kg/d of milk was 27.3 and 48.5% higher than that of non-lactating cows (Purwanto et al., 1990). The threshold temperature for heat stress is decreased by 5°C when milk production increases from 35 to 45 kg/d (Berman, 2005).

As stated earlier, the effects of a given temperature humidity combination on milk yield are maximal between 24 and 48 hours after heat stress has been initiated (Collier et al., 1981; West et al., 2003; Spiers et al., 2004). When THI exceeds 72 or 80 over a four d interval there is an associated negative correlation with milk yield production (Linville and Pardue, 1992). Ravagnolo et al., (2000) evaluated test day milk yields resulting in a decrease of 0.2 kg milk per unit increase of THI above 72 when composed of maximum temperature and minimum RH. Others have reported a reduction in milk yields of 0.26 kg per unit increase of THI above 72 (Johnson et al., 1962). Implementation of environmental modifications, such as evaporative coolers, milk production reductions are not as drastic compared to heat stress without any alleviation (Flamenbaum et al., 1986).

Stage of lactation has been dependent upon the severity of milk loss during heat stress (Maust et al., 1972). Mid lactation (100-180 days in milk; **DIM**) cows are more adverse affects are more measurable by increased heat stress followed by late lactation (180 to 260 DIM) intermediate and early (<100 DIM) lactation cows (Maust et al., 1972). Reasons behind early lactation cows being least affected is potentially due to cows

utilizing body reserves in order to quickly offset the impact of heat stress (Maust et al., 1972) or because it is early lactation and milk yield is still rising it is more difficult to predict what a cow would have produced had she not been stressed. Primiparous cows are not as vulnerable to heat stress as multiparous cows with increased levels of milk production because multiparous cows have greater body weight, feed intake, metabolic activity and greater milk yields than primiparous cows (West, 1994). Therefore, the main cause of milk production losses is due to a decrease in dry matter intake.

**Heat stress and dry matter intake (DMI)**

Negative impacts of heat stress can be attributed to a reduction in DMI and endocrine changes within cows including nutrient reabsorption, decreased rumination, and an increase in maintenance requirements (Collier and Beede, 1985; Baumgard et al., 2006). Research on dry matter intake decreases during heat stress have been well documented however, DMI alone is not good indicator of the status of the heat stressed animal (NRC, 1981; Roesler et al., 1997; Holt et al., 2004; Gaughan et al., 2008). Decreases in DMI will be visible at ~25 to 27° C; however this can be influenced by diet composition and will be much more severe when greater than 30°C (Beede and Collier, 1986; Sanchez et al., 1994). Minimum THI is more closely correlated with decreased DMI than maximum THI (Holter et al., 1996). It has been shown that DMI reductions begin when minimum THI exceeds 56 or 57 (Morrison, 2000). When THI was between 71 to 85 DMI decreased by 4.4 kg per d or 22% (Morrison, 2000). Heat stress decreases DMI which may be contributing to the balance of homeothermy because of reduced heat production (West, 1994). In order to balance homeothermy in the animal, decreased DMI

may be a preventative way to reduce heat production (West, 1994). This is often due to the heat production generated from ruminal fermentation and the body's metabolism (Beede and Collier, 1986). At a temperature of 40°C, cows have shown a 20 to 40% decrease in DMI compared to cows in a thermoneutral environment regardless of shading (Sanchez et al., 1994).

Increased ambient temperatures will improve the digestibility of feed due to the reduction in DMI resulting in a slower rate of passage in the animal (Fuquay, 1981). The rate of passage of ingesta is decreased and rumen volume capacity is at its capacity thus, residence time in order to digest dry matter is increased (Beede and Collier, 1986). Wind, velocity, humidity, and radiation are all environmental factors that can affect DMI because of the attempt to maintain homeothermy during natural environmental conditions (Beede and Collier, 1986). When feeding a less palatable diet under heat stressed conditions the rate and extent of DMI reductions would be more severe (Beede and Collier, 1986). In order to prevent or reduce the negative affects of heat stress such as decreases in milk production and dry matter intake, it is important to evaluate the two ways to reduce heat stress by cooling the animal or cooling the environment around the animal.

**Environmental modifications to alleviate heat stress**

Concepts for alleviating heat stress have been narrowed to two strategies: altering the environment around the cow by providing shade in order to decrease exposure to solar radiation and/or evaporative cooling; secondly, directly cooling the cow by using sprinkler or soakers to wet the animal (Knapp and Grummer, 1991).

**Shade**

The simplest way to alleviate heat stress historically has been to provide a shade structure. The vast majority of dairy cattle today are housed under some type of shade structure during warm summer months and although this greatly reduces solar heat load there is still a radiant heat load on animals originating from the metal roof. Prior to the implementation of shade structures, trees were thought to be effective at alleviating heat stress due to the combination of providing shade and evaporation through the leaves (Armstrong et al., 1993). However, crowding of cattle under trees results in damage to the tree roots and eventual loss of the tree. Shade structures have been utilized since the 1960's to provide shade for large numbers of cattle on commercial dairies. Shade structures were primarily assembled out of sheet steel and/or aluminum, because of less maintenance, durability, and economic cost (Armstrong et al., 1993). Using a shade structure alone, could decrease heat load by 30 to 50% if well constructed and designed (Collier et al., 2006). Unfortunately, shade structures alone are not sufficient enough to alleviate the majority of heat stress effects; in fact, they are most commonly used in conjunction with another method such as evaporative cooling. Berman (2005) estimated that the typical shade structure in Israel adds an additional 3$^{\circ}$C of infrared heat load from the metal roof to the effective ambient temperature surrounding animals. In addition, there are varying convection levels under shade structures depending on the use of fans as part of the cooling management system and size of the shade.

**Evaporative Cooling**

As stated earlier, the implementation of a shade structure decreases solar heat load on animals. The environment surrounding the animals can then be further improved via increasing convection with fans or to decrease air temperature by evaporative cooling or by directly cooling the cow by using sprinklers and soakers (Knapp and Grummer, 1991). Using high pressure, fine mist, and large volumes of air evaporative cooling systems can be implemented to evaporate moisture and cool the air surrounding the animal (Collier et al., 2006). A reverse chimney fixed evaporative cooling system mounted onto a conventional shade structure and is manufactured by Korral Kool Inc, in Mesa, AZ (Armstrong et al., 1993). In order to cool the environment around the cow it injects micron sized (30-65 microns @ 300 psi) water droplets in to the air moving downward in the cooler (Ryan et al., 1992; Armstrong, 1994). Curtains are used with these systems due to the fact that they are stationary and can not be moved once mounted on the shade structures which are oriented north to south reducing the sun angle and the shade area under the roof during early mornings and late afternoons. Oscillating fans with misters are another common evaporative cooling system also now marketed by Korral Kool that can be utilized to reduce heat stress effects. The fans operate by using a variable speed water injection (5-15 microns @ 250-1250 psi) into the airstream; they are suspended from the roof of the shade structure and have a variable motion of 270°. Due the range of motion it allows for cooling around and under the shade as the sun changes its angle throughout the day. A photoelectric cell on one system (Shade Tracker) allows the fan system to follow the shadow as the shade moves during the day negating the need for a

curtain. Both of these systems are controlled via computers with variable parameters based on THI conditions operating degree and length of cooling.

Two independent trials were conducted from June 3rd to September 30th of 2004 and 2005 in order to evaluate the effectiveness of a fixed reverse chimney cooler (Korral Kool Coolers) versus an oscillating system (shade tracker), (Burgos et al., 2007). In each trial, 400 multiparous and 100 primiparous Holstein cows were balanced for stage of lactation, parity, and milk yield. They were randomly assigned to one of two cooling treatments; Shade Tracker (**ST**) versus Korral Kool (**KK**). Individual milk yields and pen DMI were collected daily, respiration rates and body surface temperatures were recorded weekly, and milk components, body condition score (**BCS**), and body weights (**BW**) were obtained monthly. In 2004, milk production did not differ between multiparous cows cooled with ST or KK. However, milk yield for primiparous cows housed under KK had a tendency to be elevated compared to cows housed under ST conditions (37.8 vs. 36.7 kg/d). Respiration rates of multiparous cows cooled using ST were increased (60.5 vs. 58.3 bpm); however, RR of primiparous cows did not differ between treatments (59.2 bpm). In 2005, multiparous (42.2 vs. 38.3 kg/d) and primiparous (35.2 vs. 32.7 kg/d) milk yields for cows cooled using the KK system were increased ($P < 0.01$) compared to cows cooled by ST. Changes in body weights were similar between multiparous cows housed under either system (7 kg), however primiparous animals showed an increase (60.5 vs. 19.9 kg) in body weight when housed under KK compared to ST systems. In 2005, multiparous (70.9 vs. 59.3 bpm) cows and primiparous (72.2 vs. 61.3 bpm) cows cooled using ST had increased RR compared to those cooled by KK

coolers. During both studies, ST systems used less electricity (526 vs. 723 and 517 vs. 840 kwh/d; 2004 and 2005, respectively) than KK systems. They also used less water (291 vs. 305 and 290 vs. 460 L/d; 2004 and 2005 respectively, in comparison with KK coolers. The differences in cost for ST and KK systems was 27.30 vs. 36.36 $/d in 2004 and 2005 and 25.95 vs. 42.06 $/d during the 2004 and 2005 studies respectively. Not only is technology important in trying to alleviate heat stress there are also strategies to alleviate metabolic heat production by altering the diet.

**Nutritional strategies to alleviating heat stress**

As discussed earlier, milk production is decreased during period of heat stress; this is not only due to the heat storage excess the animal is receiving but also from a reduction from DMI (Beede and Collier, 1985; Collier et al., 2006). The nutrient requirements of dairy cows are altered during heat stress which results in a need for reformulation of rations. Research has shown that if DMI is reduced not only does recalculation of mineral and water requirements change due to greater sweating but nutrient density of the diet must be altered as well (Collier et al., 2006). During heat stress there is a marked increase in maintenance requirements, this is in part caused by a decrease in rumination and nutrient reabsorption, cumulatively this causes a net decrease in nutrient/energy availability for production (Beede and Collier, 1985; Collier et al., 2006). Dairy cows producing larger quantities of milk have a greater metabolic activity and generate more metabolic and body heat then lower producing dairy cows, thus raising the severity of heat stress in the animal if not relieved by other strategies (West, 1994). This extra production of heat from the body and increasing rectal temperatures can be a

result of consuming an increased forage diet compared to an increased concentrate diet, a consequence believed to be associated with elevated acetate in the rumen when cows consume increased forage diets (Tyrell et al., 1979). Assisting the animal to maintain homeothermy occurs because when DMI decreases, there is a reduction in metabolic heat generated from ruminal fermentation and body metabolism (Collier and Beede, 1985; Sanchez et al., 1994). More recent data has shown that in times of heat stress DMI reductions can only be held accountable for ~40-50% of the total decrease in milk production, the remainder ~50-60% is due to other changes resulting from heat stress (Baumgard et al., 2006). Therefore, a portion of milk production losses as a consequence of heat stress may be recuperated through nutritional management. Past implementations of nutrition in order to alleviate heat stress have been minor compared to environmental modifications. However, some approaches have been somewhat successful, such as decreasing fiber intake in order to allow the rumen to function properly, adding fat supplementation mostly due to its high energy content and low heat increment, implementing increased concentrate diets with caution to avoid metabolic disorders, and more recently adding niacin supplementation (Beede and Collier, 1986; Knapp and Grummer, 1991; Morrison, 1983).

Decreasing fiber consumption can help the rumen function properly and thus help alleviate heat stress (Knapp and Grummer, 1991). Some of the depression of DMI reported during heat stress could be prevented when diets contain 14 to 17% acid detergent fiber (**ADF**) compared to 20% (Cummins, 1992). As a rule of thumb in order to avoid metabolic disorders and maintain proper rumen function ADF should not be

decreased below 18% and neutral detergent fiber (**NDF**) should not be decreased below 28% of the rations dry matter (West, 1994). Fat supplementation increases the energy content of the diet simultaneously lowering the heat increment of metabolism (Morrison, 1983; Beede and Collier, 1986; Knapp and Grummer, 1991). Research has shown when supplementing fats there has either been no affect or an increase in milk production, suggesting the animal's response to fat is not as responsive when fed under heat stressed conditions compared to unstressed animals (Chen et al., 1993; Huber et al., 1994). When conducting research evaluating supplementing fats during heat stress palatability should also be considered as animals tend to decrease DMI if palatability is an issue. Normally, the addition of supplemental fat is implemented during summer heat stressed months in order to provide the animal with extra energy to maintain or increase body condition scores.

Reports of feeding excess rumen degradable protein during heat stress have been shown to decrease DMI and milk production (Huber et al., 1994). This reduction is due to the increase in maintenance requirements and decrease in energy being consumed. In order to digest the excess protein extra energy is required to convert protein into urea for excretion (Huber et al., 1994). An option to prevent this is to improve the quality of the protein being fed so that it may support increased milk levels in cows during heat stress (Huber et al., 1994). Researchers have found supplementing with Lysine to increase milk yields by 11% when fed to animals undergoing heat stress (Chen et al., 1993). During periods of heat stress, the effects of decreased DMI and increased maintenance requirements result in the animal metabolizing more protein in order to meet energy

requirements compared to cows under moderate temperature environments (Beede and Collier, 1986).

Adding fat to the diet has been another alternative in alleviating heat stress. It is considered a method to alleviate heat production because of its low heat increment and its elevated energy content (Beede and Collier, 1986). Moody et al., (1967) evaluated the effects of feeding supplemental fat during times of heat stress in lactating dairy cows. Researchers fed concentrates containing no supplemental fat, 10% soy oil, and 10% hydrogenated vegetable fat and the animals were housed in a thermoneutral or heat stressed environment (Moody et al., 1967). These investigators reported did not detect a difference in environment by diet interactions for milk production, fat-corrected milk yields, or percentages of milk fat and/or protein (Moody et al., 1967). They concluded that there was no benefit to feeding supplemental fat during heat stress (Moody et al., 1967). Cows in this study were producing an average of ~20 kg/d therefore; the effect may have been detectable had they used higher producing cows. In contrast, other researchers have found feeding supplemental ruminally inert fat to high producing dairy cows (>35 kg/d) during summer months to be beneficial. Cows receiving 5% prilled long chain fatty acids during summer months produced 9 kg/d more milk than cows receiving the prilled long chain fatty acids during fall months (Skaar et al., 1989).

Niacin is a potential supplement for cows during heat stress because it induces vasodilation, potentially increasing body heat transfer to the periphery (Di Costanzo et la., 1997). The transfer of this body heat to the surface through peripheral and vasomotor function may assist in alleviating some of the reduction in DMI and therefore

consequently milk yields. Vasodilation caused by supplementation of niacin is thought to act through prostaglandin D production by the epidermal Langerhans cells (Benyo et al., 2006; Maciejewski et al., 2006) and prostaglandin $D_2$ receptors on the vascular endothelium (Cheng et al., 2006). During periods of mild to severe heat stress, skin temperatures are decreased when cows have been supplemented with raw niacin (Di Costanzo et al., 1997). Past observations have been inconclusive with the use of raw niacin due to the metabolism of raw niacin by rumen microbes (Campbell et al., 1994).

Skaar et al., (1989) found no difference in total milk yields when cows were supplemented with niacin, however these investigators did report that peak milk yields were lower than those cows not receiving niacin. They also found that cows supplemented with niacin in addition to supplemental fat returned to their two week postpartum BW faster (Skaar et al., 1989). In agreement, some researchers have found no benefit to supplementing nicotinic acid in addition to supplemental fat (Madison-Anderson et al., 1997; Jaster and Ward, 1990; Horner et al., 1988). Other researchers have found the opposite reporting slightly increased milk yields and/or an increase in lactation persistency (Muller et al., 1986; Dufva et al., 1983; Riddell et al., 1981; Kung et al., 1980). Drackley et al., (1998), reported that yields of milk, solid-corrected milk, and 4% fat-corrected milk were increased during early lactation when feed was supplemented with raw nicotinic acid at a rate of 12 g/cow/d. Di Costanzo et al. (1997) reported numerical increases in milk yield when cows were supplemented with nicotinic acid at 12, 24 or 36 g/cow/d during heat stress. Muller et al. (1986) found that first lactation cows had increased milk yields when supplemented with niacin at 6 g/cow/d during

summer months. They also reported that cows in their second lactation or greater averaged a 0.9 kg increase per d in milk yields when supplemented with niacin (Muller et al., 1986). Cows producing greater than 34 kg of milk per d regardless of parity at the beginning of the study averaged a 2.4 kg increase in milk yield per d (Muller et al., 1986) when supplemented with 12 g/d of niacin. When comparing all cows regardless of parity, supplementation of niacin increased milk and fat-corrected yields by 0.9 and 0.8 kg/d respectively (Muller et al., 1986). Jaster et al., (1983) reported primparous heifers supplemented with niacin appeared to produce greater amounts of milk yields and increased peak production compared to control cows, although no difference was found between the cows receiving 6 g/cow/d compared to the control group. Di Costanzo et al., (1997) and Muller et al., (1986) are the only studies evaluating some form of niacin during a period of heat stress, the rest of the studies were not conducted with heat stress as part of the experimental design.

Supplementing nicotinic acid alone decreased DMI however, when fed in the presence of supplemental fat increased DMI during early lactation (Drackley et al., 1998). Other studies have shown no affect on DMI when nicotinic acid was supplemented; however, during mid to late lactation cows being supplemented with 6g/cow/d of nicotinic acid had decreased DMI compared to cows not receiving any supplementation (Erickson et al., 1992; Martinez et al., 1991; Jaster and Ward, 1990; Horner et al., 1988; Skaar et al., 1989; Kung et al., 1980). During mild heat stress (THI 68-79) DMI was increased when cows were supplemented with nicotinic acid at 12

g/cow/d however, during times of no heat stress (THI 63-75) DMI was decreased when nicotinic acid was fed at 36 g/cow/d (Di Costanzo et al., 1997).

Few studies have researched niacin and its effects on thermal or respiratory responses. Di Costanzo et al. (1997) reported decreased ST for the tail and rump areas for cows fed nicotinic acid at 0800 and 1600 h. They also found a numerical decrease in RR at 0800, 1600 and 2200 h (Di Costanzo et al., 1997). Explanations for these reductions without observing a decrease in rectal or core temperature were due to heat transfer being decreased therefore a decrease in heat gain is anticipated if the core temperature were to remain constant (Di Costanzo et al., 1997). The second theory is an increase in EVHL, resulting in cooling of the skin, a lower temperature, and an increase in thermal gradients for heat loss processes (Di Costanzo et al., 1997). Cows fed 12 g/cow/d of nicotinic acid during early lactation lost less weight than cows fed a control diets (Skaar et al., 1989). Jaster and Ward (1990) reported significantly increase BW for cows supplemented with 6 g/cow/d of nicotinic acid during early lactation.

Niacin has been shown to be anti-lipolytic in adipose tissue consequently reduces plasma NEFA levels (Jaster et al., 1983; Dufva et al., 1983). Doses of 6.5 and 17 g of raw nicotinic acid have been shown to increase glucose and insulin concentrations in goats; they also found impairment in glucose tolerance tests in these animals (Thornton and Shultz, 1980). In dairy cows, some researchers have found no affect on glucose concentrations when niacin was supplemented at 12 g/cow/d (Skaar et al., 1989). Plasma concentrations of glucose were elevated for cows fed diets containing nicotinic acid during times of no heat stress (THI 63-75) compared to cows fed a control diet (Di

Costanzo et al., 1997). Jaster and Ward (1990) also found increases in glucose concentration in blood serum when cows were fed supplemental nicotinic acid.

**Summary and Objectives**

Identifying ways to alleviate heat stress remains to be a challenge in today's industry. The major factor contributing to the decisions made on farm is economic cost. Therefore, identifying concepts that can actually reduce the impacts of heat stress are key to continued progress in limiting effects of heat stress on dairy cattle. Technological advances have been fortunate to help alleviate some of the impacts however; new evidence (Rhoads et al., 2007; Zimbelman et al., 2007, Zimbelman et al., 2008) suggests that there are additional opportunities in nutritional management than originally estimated. As animals currently and in the future further their genetic potential it is important to re-define and re-evaluate the parameters that are used to evaluate heat stress. The purpose of this dissertation is to evaluate current methods used to estimate heat stress in cattle and to test effects of niacin feeding to improve thermal tolerance in dairy cows

Objective #1: Re-evaluate the temperature humidity index model and determine effects on minimum, maximum, and average THI and the hours THI exceeds 72 on milk yield of cows.

Objective #2: Identify if the encapsulation of niacin can reduce the impacts of acute heat stress.

Objective #3: Evaluate the effects of encapsulated niacin during heat stress on a large commercial dairy farm.

Objective #4: Identify the interaction between niacin and prostaglandin and its effects on heat shock proteins 27 and 70.

# CHAPTER TWO: A RE-EVALUATION OF THE IMPACT OF TEMPERATURE HUMIDITY INDEX AND BLACK GLOBE HUMIDITY INDEX ON MILK PRODUCTION IN HIGH PRODUCING DAIRY COWS

## ABSTRACT

The THI originally developed in 1958 and extended to cattle in 1964 is used to estimate cooling requirements of dairy cattle. Eight studies (100 multiparous Holstein cows) were used to determine the minimum, maximum, and average THI threshold for milk yield loss for high producing dairy cows. In addition, 48 lactating multiparous Holstein cows were used to evaluate the effects of BGHI to incorporate solar radiation. Physiological measures of heat strain included respiration rate (RR/min), infrared surface temperature (ST, ºC), rectal temperature (RT, ºC), heart rate (HR/min), and evaporative heat loss (EVHL, $g/m^2$). Mean body temperature (BT, ºC) was calculated using the formula BT = (0.33 x ST+0.67 x RT). Respiration rates, ST, RT, EVHL, and HR were routinely collected 2-4 times per d. Based on these results, we conclude that current THI guidelines are not adequate for high producing cows (>35kg/d). We conclude that THI threshold for high producing dairy cows (producing > 35kg/d) should be 68.

## INTRODUCTION

The THI was originally developed by Thom (1958) and extended to cattle by Berry et al (1964). It is currently used to estimate cooling requirements of dairy cattle in order to improve the efficiency of management strategies to alleviate heat stress. The Livestock Conservation Institute evaluated the biological responses to varying THI

values and categorized them into mild, moderate and severe stress levels for cattle (Whittier, 1993; Armstrong, 1994). However, as pointed out by Berman (2005) the supporting data for these designations are not published. In addition, the index is based on a retrospective analysis of studies carried out at The University of Missouri in the 1950's and early 1960's on a total of 56 cows averaging 15.5 kg/d, (range 2.7-31.8 kg/d). In contrast, average production per cow in the United States is presently over 30 kg/d with many cows producing above 50 kg/d at peak lactation. The sensitivity of cattle to thermal stress is increased when milk production is increased thus reducing the "threshold temperature" when milk loss begins to occur (Berman, 2005). This is due to the fact that metabolic heat output is increased as production levels of the animal increase. For example, the heat production of cows producing 18.5 and 31.6 kg/d of milk has been shown to be 27.3 and 48.5% higher than non-lactating cows (Purwanto et al., 1990). Research has shown that when milk production is increased to from 35 to 45 kg/d the threshold temperature for heat stress is reduced by 5°C (Berman, 2005). The physiological effects based on THI predictions on milk yield are currently underestimating the severity of heat stress on Holstein cattle. Radiant heat load and/or convection effects were not evaluated by Berry et al., (1964) and the majority of dairy cows are currently housed under a shade structure during heat stress months. Shade structures alleviate some of the radiant heat load however, there is still a conducive effect coming from the metal shade structure. In Israel, a typical shade structure is estimated to add 3°C to the effective ambient temperature surrounding the animals (Berman, 2005).

The use of fans for cooling management systems causes varying convection levels under shade structures as well.

An additional factor in utilizing THI values is the management time interval. In past research, the milk yield response to a given THI was the average yield in the second week at a given environmental heat load therefore milk yield measurements were not recorded until two weeks after experiencing the environment (Berry et al., 1964). In order to avoid economic production losses dairy producers need to be informed of the level of cooling to be implemented immediately when heat stress occurs. Research has indicated that the effects of a given temperature on milk production are maximal between 24 and 48 hours following heat stress (Collier et al., 1981; Spiers et al., 2004). It has also been reported that ambient weather conditions two days prior to milk yield measurement had the greatest correlation to decreases in milk production and dry matter intake (West et al., 2003). Research has shown that the total number of hours when THI is greater than 72 or 80 over a 4 d interval had the highest correlation with milk yield (Linville and Pardue, 1992). Collectively, these findings indicate that current THI values for lactating dairy cows underestimate the impact of a given thermal load on animal productivity and have an inappropriate time interval associated with a cooling management decisions. Avoiding a decline in milk production over a 48 hour period will automatically prevent a decrease in lactation persistency two weeks later. Utilizing the THI in order to reduce milk production losses has been effective; however the current THI is in need of updating on an appropriate time scale with data from higher producing animals. The pattern of stress application is a final component of the THI to be considered. In the research

conducted for the current THI, animals were exposed to given THI conditions continuously meaning, with no daily circadian environmental fluctuations, for the entire two week period (Berry et al., 1964). Under natural dairy management conditions temperatures are not kept constant rather they follow a circadian pattern which rises and falls during a normal 24 hour d. It is important to establish THI under conditions normally experienced by lactating dairy cows. In addition, the most appropriate parameters need to be identified. For example, average, minimum, maximum, and hours above a certain THI all need to be examined. Research has reported that minimum THI is more highly correlated with a reduction in feed intake compared to maximum THI (Holter et al., 1996). When evaluating test day yields results showed a decrease of 0.2 kg per unit of THI increase above 72 when THI was composed of maximum temperature and minimum humidity (Ravagnolo et al., 2000).

The effects of radiant heat load can be evaluated using the BGHI (BGHI = $t_{bg}$ + .36$t_{dp}$ + 41.5 where $t_{bg}$ = black globe temperature $^{\circ}$C and $t_{dp}$ = dew point temperature, $^{\circ}$C), developed by Buffington et al. (1981). Research has demonstrated that BGHI had increased correlations to rectal temperature increases and milk yield decreases compared to THI (Buffington et al., 1981). It has also been shown that the correlation of BGHI to milk yield is greater ($r^2$ = .36) under conditions of high solar radiation (no shade) than under a shade structure ($r^2$= .23; Buffington et al., 1981). However, milk production in this study was considered to be low (average 15 kg/cow). Therefore, correlations of BGHI to milk yield under shade structures might be higher with higher producing dairy cows (which are more sensitive to increased heat loads). It is also apparent a great deal

of variation is not explained by BGHI. This might be improved by determining the impact of an additional factor like skin temperature.

Another option in measuring radiant environmental temperature is by using infrared technology to measure skin surface temperature. In doing this we can account for differences in microenvironment around the animals and have a greater accuracy of measuring environmental heat load. Creating a skin temperature humidity index (STHI = $t_s + .36\ t_{dp} + 41.5$ where $t_s$ = infrared skin surface temperature $^0$C and $t_{dp}$ = dew point temperature, $^{\circ}$C), might allow for greater prediction of an animal's heat stress compared to BGHI or THI. Using infrared thermography guns it is possible obtain rapid and reliable skin surface temperatures. These parameters would be best to evaluated under controlled environmental conditions and confirmed under practical management conditions. Under commercial dairy conditions vaginal temperatures can be used to continuously record core body temperatures as other researchers have conducted (Araki et al., 1985; Ominski et al., 2002). Obtaining core body temperature in addition to simultaneous recording of black globe and dry bulb temperatures and humidity as well as milk yield will permit determining relationships between ambient heat load, core body temperature and subsequent milk yields.

Studies where temperature and humidity are controlled in a circadian manner, in order to mimic natural environmental conditions, have not been conducted. Feed intake and milk yield under natural conditions has resulted in mean THI two days prior to milk production to have the greatest effect on both intake and yield (Collier et al., 1981; West et al., 2003). Unfortunately, because these results were not obtained in controlled

environments researchers were unable to quantify the relationship between THI and milk yield.

The goal of this study was to utilize high producing dairy cows including radiant energy impacts on animal performance. Specific objectives were to determine the effects of minimum, maximum, average THI and the number of hours at a given THI on milk production of high producing dairy cows.

## MATERIALS AND METHODS

The data analyzed in this study was obtained from 8 different studies during the course of three years. One hundred multiparous Holstein cows were housed in individual tie stalls in one of two environmentally controlled chambers in the William Parker Agricultural Research Center at the University of Arizona. The University of Arizona's Institute of Animal Care and Use Committee approved all protocols and use of animals. Temperature humidity Index was calculated using dry bulb temperature ($\mathbf{T_{db}}$, °F) and relative humidity (**RH**), ($T_{db}$-(0.55-(0.55*RH/100)*($T_{db}$-58); Buffington et al., 1981). Black globe humidity index was calculated by using black globe temperature ($T_{bg}$, °C) and RH (Buffington et al., 1981).

### Groups 1-4

Forty-eight multiparous lactating Holstein cows were balanced for parity and stage of lactation and assigned to an incomplete crossover design involving two levels of radiant heat load, 2 levels of dry bulb temperature, and two levels of humidity. These parameters were then combined to produce eight experimental environments. Each of

these eight environments have a range of dry bulb temperature, radiant energy, relative humidity, and THI values mimicking a possible 24 hour period under shade structures during summer months in the southern part of the United States. Cows were housed at the University of Arizona, William J. Parker Research Complex, in two environmental chambers, with only one room capable of producing radiant heat load. Six cows were housed in each environmental chamber, each group consisted of 12 cows therefore, four groups of animals (n=12) were brought to the facilities at separate times in order to reproduce eight environments. Each group of animals experienced a minimum of two and a maximum of three environments over a 22 d period. Animals entering the facility were provided seven days to acclimate to the chambers in a thermal neutral environment (Environment #3). Followed by a four day experimental environment, then cow's switched environmental chambers and were provided seven days to acclimate to the new chamber in a thermal neutral environment (Environment #3). After seven days, cows experienced the opposite experimental environment for four days. Cows were milked and fed twice daily with orts measured once a day prior to the morning milking. During adjustment periods, respiration rates (RR), surface temperatures (ST), evaporative heat loss (EVHL), rectal temperatures (RT), and heart rate (HR) were measured. Skin temperatures and sweating rates were measured from the shoulder, ribs, and rump of the animal twice a day (0500 and 1700 h). These same heat parameters were measured four times a day (0500, 1000, 1400, and 1700 h) during the four day experimental periods. On the 3$^{rd}$ d of each 4 d period a 24 h recording of these same observations were made every hour on the hour. Skin temperatures were measured with an infrared temperature gun

(Raynger® MX™ model RayMX4PU Raytek C, Santa Cruz, CA). Rectal temperatures were measured using a digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA). Respiration rates were obtained by visually counting flank movements during a 15 sec interval and multiplying by 4 and evaporative heat loss was measured using an evapometer (Delfin Technologies, LTD., Finland). Heart rate was measured by cardiac auscultation. Environmental parameters recorded hourly and used for calculations of BGHI and THI are ambient temperature ($T_{db}$), relative humidity (RH), black globe temperature ($T_{bg}$), and radiant energy.

**Group 5**

Twelve multiparous mid lactation cows were assigned to one of two studies in January or June of 2004. Animals were balanced for parity and assigned to one of two environmental chambers at the University of Arizona, William J. Parker Research Complex. Environmental treatments consisted of one thermal neutral environment (8-15°C, 8-40% humidity) and two heat stressed environments 1) 30-40°C, 8-40% humidity and 2) the same heat stress conditions with the addition 4 h of solar radiation at 600 watts/h/$m^2$ from 1100 to 1500 h). Animals were provided seven days to adjust to the facilities and then experienced 3 14 d periods in an incomplete, crossover design. Solar lamps were only available in one of the environmental chamber rooms; cows had to switch rooms prior to the third period so that all animals experienced all environments. Once animals switched rooms they were provided 7 d to re-adjust to their new environmental chamber prior to period 3. Cows were milked two times a day (0600 and 1800 h) and milk weights recorded at each milking. Animals were fed a total mixed

ration two times a day (0700 and 1700 h) and orts were weighed and recorded prior to the morning feeding.

Heat parameters were measured bihourly on d 6 of each period. Skin temperatures were measured with an infrared temperature gun on the right and left side of the animal on the middle of the rump and loin (Raynger® MX™ model RayMX4PU Raytek C, Santa Cruz, CA). Rectal temperatures were measured using a digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA). Respiration rates were obtained by visually counting flank movements during a 15 sec interval and multiplying by 4 and evaporative heat loss was measured using an evapometer (Delfin Technologies, LTD., Finland). Heart rate was measured by cardiac auscultation.

**Group 6**

A total of twelve lactating multiparous Holstein cows averaging 140 ± 13 DIM were assigned randomly to one of two environmental chambers at the William J. Parker Agricultural Research Complex at The University of Arizona. Cows were milked twice a day and recorded for daily milk yield. A total mixed ration was fed twice daily and weigh backs were measured once a day prior to the morning feeding. Dairy Nutrition Services (Chandler, AZ) formulated the TMR to meet or exceed energy requirements according to NRC, 2001. All studies were approved by the University of Arizona Institutional Animal Care and Use Committee. Cows were given seven days to acclimate in the environmental chambers and both groups regardless of treatment were exposed to thermal neutral conditions (20°C, 20% humidity, THI = 64). Following acclimation, cows continued to experience the same thermal neutral conditions for an additional 9 d

and allowed to eat ad libitum (Period 1; P1). Period 1 and period 2 (P2) were separated by a 7 d where cows remained in the same thermal neutral condition. During P2, cows in group 1 remained in the same thermal neutral condition while cows in group 2 experienced heat stress (HS) and were fed ad libitum. In order to mimic daily variations, during HS cyclical temperatures ranged from 29.4 to 38.9°C with humidity held constant at 20%, THI ranged from 73 to 82 daily. Respiration rate (RR), skin temperatures (ST) and rectal temperatures (RT) were measured and recorded four times daily (0600, 1000, 1400, and 1800 h). Measuring RR was done by counting flank movements for 60 sec. On a shaved section on the shoulder of the cow skin temperatures were measured with an infrared temperature gun (Raynger® MX™ model RayMX4PU Raytek C, Santa Cruz, CA). Rectal temperatures were measured using a digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA).

**Group 7**

Ten lactating multiparous Holstein cows averaging 99.8 ± 20.2 DIM were randomly assigned to one of two environmental treatments over the course of three experimental periods. All animals were housed at The University of Arizona, William J. Parker Research Complex in tie-stall stanchions in the environmental chambers. Animals were exposed to three experimental periods, period 1 (P1), 7 d of thermal neutral conditions, period 2 (P2), 7 d of heat stress, and period 3 (P3), 7 d of heat stress; totaling 21 d to complete the entire study. Period 1 consisted of thermal neutral conditions (20°C, with humidity held constant at 20% with a 12 and 12 h light and dark cycle). Period 2 and P3 environments consisted of heat stress with cyclical daily temperatures in order to

mimic daily variations (ranging from 29.4 to 38.9°C with humidity being held at 20%, THI = 72.4 to 82.2, with a 12 and 12 h light and dark cycle). All cows were fed a total mixed ration three times a day (0500, 1200, and 1700 h) and orts were recorded once a day prior to the morning feeding. All cows were allowed to eat ad libitum. All cows were milked two times a day (0500 and 1700h) and recorded at each milking.

Heat parameters such as respiration rate, surface temperature (from the shoulder, rump, and tail head), and rectal temperatures were measured four times a day (0600, 1000, 1400, and 1800 h). Surface temperatures were measured using an infrared temperature gun (Raynger®MX™ model RayMX4PU Raytek C, Santa Cruz, CA). Rectal temperatures were obtained using a standard digital thermometer (GLA 525/550 Hi-Performance Digital Thermometer, San Luis Obispo, CA).

**Group 8**

Eighteen second lactation Holstein cows averaging 89.2 (± 8.1) DIM were randomly assigned to an environmental chamber room into individual tie stalls located at the University of Arizona William J. Parker Research Complex. The chamber rooms only house six cows at a time therefore the study was replicated three times and one cow was removed from the study due to temperament issues in the facilities. All cows were milked two times a day and milk weights were recorded at each milking (0500, 1700 h). All cows were fed a totally mixed ration (TMR) two times day at milking times and orts were recorded prior to the morning feeding. Cows were housed at the University of Arizona dairy for 19 days prior to entering the environmental chambers. While at the dairy, cows received one of two dietary treatments 1) Control diet with 0 g/ton Rumensin

or 2) the control diet top dressed with Rumensin at 450 mg/cow/d. Once they entered the facility they were provided 3 d to adjust however, continued on their dietary treatment. All cows regardless of dietary treatment experienced a constant thermal neutral environment (20% humidity, THI = 64, with 14 h light and 10 h dark cycles) and allowed to eat ad libitum for 9 d (experimental period [**P**] 1). They were then given 2 d in the same thermal neutral environment prior to experiencing P2 (experimental period [**P**] 2) which consisted of cyclical temperatures (29.4 to 38.9°C with constant 20% humidity, THI ≥73, ≤ 82 and 14 h light and 10 h dark cycles) and were fed ad libitum. This environment was made to replicate daily variations in temperatures throughout the day. Heat parameters were collected three times a day (0600, 1500 and 1800 h). Respiration rates were obtained by counting flank movements for 15 sec and multiplied by 4 for a total of breaths per minute. Surface temperatures were measured on a shaved patch (~5 $cm^2$) of skin on the shoulder of the animal using an infrared temperature gun (Raynger®MX™ model RayMX4PU Raytek C, Santa Cruz, CA). Using a standard digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA) rectal temperatures were measured. Environmental parameters recorded hourly and used for calculations of THI are ambient temperature ($T_{db}$) and relative humidity (RH).

***Statistics***

Data was analyzed using ANOVA and REGRESSION procedures of SAS (SAS, 1999). Milk yields were recorded during the acclimation periods and prior to environment initiation and were included as a covariate in the analysis. The dependent variable

analyzed was milk yield, RR, ST, RT, HR, and EVHL. The independent variables included daily THI, ST, STHI and BGHI. The level of significance was set at $P < 0.05$ for all main effects and interactions and the LSMEANS test was conducted when significance was detected.

## RESULTS

Over the entire data set there were some general observations made regarding physiological responses to increasing environmental temperature regardless of method of heat index utilized. As rectal temperatures increased, respiration rates increased, ($P < 0.001$; $r^2 = 0.5658$; Figure 2). As rectal temperatures increased heart rate increased as well ($P < 0.001$; $r^2 = 0.0803$; Figure 3). Evaporative heat loss was also found to increase as rectal temperatures were increased ($P < 0.001$; $r^2 = 0.0556$; Figure 4). When mean body temperature increased there were concomitant increases in rectal temperature ($P < 0.001$; $r^2 = 0.6636$; Figure 5). Increasing surface temperature of the skin was associated with increased rectal temperatures ($P < 0.001$; $r^2 = 0.4367$; Figure 6). As rectal temperatures increased, milk yields were shown to decrease linearly ($P < 0.001$; $r^2 = 0.0494$; Figure 7).

Respiration rates were increased as the surface of the skin increased ($P < 0.001$; $r^2 = 0.6026$; Figure 8). Heart rate was also elevated as skin surface temperature increased ($P < 0.001$; $r^2 = 0.0445$; Figure 9). As evaporative heat loss increased, skin surface temperature was also elevated ($P < 0.001$; $r^2 = 0.2563$; Figure 10). Mean body temperature increases also resulted in increased skin surface temperature ($P < 0.001$; $r^2 =$

0.5425; Figure 11). Milk yield decreased as skin surface temperature increased ($P < 0.001$; $r^2 = 0.0190$; Figure 12).

Respiration rates increased by 2.0065 breaths per minute per increase in THI unit ($P < 0.001$; $r^2 = 0.4343$; Figure 13). Rectal temperatures were also increased as THI increased (0.0587°C; $P < 0.001$; $r^2 = 0.2691$; Figure 14). Heart rate increases by 0.1363 beats per unit as THI increases per unit ($P < 0.005$; $r^2 = 0.0099$; Figure 15). Evaporative heat loss increased as THI increased ($P < 0.001$; $r^2 = 0.2298$; Figure 16). Mean body temperature was shown to increase by 0.1518°C per unit of THI increase ($P < 0.001$; $r^2 = 0.1929$; Figure 17). Skin surface temperature were also shown to increase per unit of THI (0.381°C; $P < 0.001$; $r^2 = 0.4771$; Figure 18). As THI increased, decreases in milk yield were observed ($P < 0.001$; $r^2 = 0.1929$; Figure 19). When the average daily THI was equal to 68 over a 24 h period milk yield were reduced by -0.2831 kg per hour ($P < 0.001$; $r^2 = 0.1405$). When the average daily THI was equal to 71 over a 24 h period production declined -0.3033 kg per hour ($P < 0.001$; $r^2 = 0.1468$). When the average daily THI was equal to 72 over a 24 hour period milk yields were reduced by -0.3217 kg per hour ($P < 0.001$; $r^2 = 0.1622$).

Respiration rates were also increased when compared to BGHI at a rate of 0.501 breaths per minute as BGHI increases per unit ($P < 0.001$; $r^2 = 0.2543$; Figure 20). As BGHI increases per unit, rectal temperatures increase by 0.0092°C ($P < 0.001$; $r^2 = 0.0636$; Figure 21). In comparison, when heart rate was compared to BGHI there was no significant increase ($P = 0.8302$; Figure 22). When EVHL was correlated to BGHI it was also increased per unit of THI (0.1295 $g/m^2/h$; $P < 0.001$; $r^2 = 0.0628$; Figure 23).

Compared to BGHI unit increase mean body temperature was increased by 0.03532°C ((*P* < 0.001; $r^2$ = 0.0732; Figure 24). When skin ST was compared to BGHI it was increased by 0.095°C (*P* < 0.001; $r^2$ = 0.2342) per unit of BGHI increase (Figure 25). As BGHI increased, milk loss was seen to decrease but this decrease was not significant (*P* = 0.1881; $r^2$ = 0.0062; Figure 26).

## DISCUSSION

Researchers have reported that minimum relative humidity and maximum temperature are the most critical variable in order to quantify heat stress (Ravagnolo et al., 2000). As discussed previously, the current THI appears to be underestimating the effects of heat stress on milk production and based on our results we can confirm this hypothesis. Previously, work by Armstrong (1994) indicated that when the THI exceeds 72, high producing dairy cows are affected adversely (Armstrong, 1994). Although current cooling standards utilize on this threshold, our research indicates that adverse affects can be shown as early as a minimum THI of 65 and an average daily THI of 68. During this study we found that physiological parameters and milk yields were affected at THI values well below 72 (Figures 1, 13-17). When analyzing the data it was observed that between a THI of 64 and 72 there were large reduction in milk yields therefore chose to analyze hours above a given THI between 65 and 72 to get a more precise estimate of the threshold (Figure 1). When other researchers analyzed data of on farm studies they have also concluded that at an average THI of 68 milk production begins to decline however, based on entire analysis of the data they still summarize that a THI of ≥ 72 is

when adverse affects are seen (Ravagnolo et al., 2000). Our results indicate that a daily THI equal to 68 results in a milk loss of 0.28 kg for each 24 hours. Milk yield reductions estimates become larger when milk production was measured two days later (-0.34 kg). Over the course of 24 hours when daily THI was equal to 71 there was a decrease in milk production of 0.30 kg and this value increased when THI was equal to 72 (-0.32 kg). A daily THI equal to 70 showed to decrease milk yields of 0.37 kg two day after THI initiation ($P < 0.001$; $r^2 = 0.2311$). When daily THI is equal to 71 milk yields decrease by 0.37 kg two days after THI initiation ($P < 0.001$; $r^2 = 0.2389$). When daily THI was equal to 72 milk production declined by 0.39 kg two days after THI initiation ($P < 0.001$; $r^2 = 0.2543$). Milk yield losses per unit of THI increase were reported to be 0.32 kg per d (Ingraham, 1979). Another study reported milk yield decreases by 0.2 kg per d as THI increased above 72 (Ravagnolo et al., 2000). In the current study milk yield decreases averaging 2.2375 kg were observed when animals experienced a minimum THI of 65 or greater ($P < 0.05$). Johnson et al., (1962) summarized that DMI and milk yield were shown to decrease significantly when maximum THI reached 77, this was later re-assessed and values of 64, 72, and 76 for minimum, average, and maximum THI were given respectively (Igono et al., 1992).

It has been reported that the ambient temperature alone has an effect on body temperature rises beginning at 25-26°C however; the impact of relative humidity and solar radiation should be taken into account as well (Berman et al., 1985; West, 2003). Increasing relative humidity within a temperature range of 24 to 38°C resulted in increased RR and RT in dairy cows (Kibler and Brody, 1944). Currently, this study

observed as THI increases per unit, RT increase linearly by 0.06°C. Respiration rate was also seen to increase as THI increased per unit by 2.01 breaths per minute. Respiration rate would be expected to increase as THI increases due to the rising heat stress, in addition with increasing relative humidity conditions an animal's ability to dissipate heat through evaporative strategies are reduced therefore respiratory mechanisms are only available. The rise in RT with increasing thermal load was also expected. The increase in THI and the inability to dissipate heat cause the animal to store more body heat. Results also showed an increase in heart rate (0.14 beats per minute) while THI increased. Again, as discussed previously, the increase in RR would simultaneously increase heart rate. Evaporative heat loss increased as THI increased per unit (2.24 $g/m^2/h$). Evaporative heat loss is also increased with increasing THI; however, previous studies have shown large variance between animals in EVHL (Collier et al. 2006). The animals' skin surface temperature was also found to increase as THI increased per unit (0.38°C). This relationship is also associated with increasing radiant heat load from the solar lamps or from other objects in the room as ambient temperatures rises.

The black globe humidity index may perhaps be a more ideal measurement of heat stress due to the fact that solar radiation is incorporated. When calculating the black globe humidity index from these studies only four out of the eight studies actually recorded the appropriate values in order to produce the BGHI. Therefore, small numbers of observations are attributed to the lower values and correlations observed. Skin surface temperature was increased as BGHI was increase per unit by 0.095°C. Respiration rates and RT were also shown to increase linearly by 0.50 breaths per minute and 0.009°C,

respectively as BGHI increased. Evaporative heat loss was increased as BGHI increased (0.1295 g/m$^2$/h). Heart rate however was not significantly increased when BGHI increased.

After obtaining the results discussed above, it appeared that perhaps arithmetic means should be evaluated in order to decide which index correlated better with these physiological parameters. Respiration rate were found to have a greater correlation to THI ($r^2$ = 0.8152; Figure 27) compared to BGHI ($r^2$ = 0.6235; Figure 30) however, both were significant ($P < 0.001$). Skin surface temperatures had greater correlation to THI ($r^2$ = 0.8487; Figure 28) then when compared to BGHI ($r^2$ = 0.7917; Figure 31). Evaporative heat loss appeared to have greater correlation to BGHI ($r^2$ = 0.4140; Figure 32) than to THI ($r^2$ = 0.0558; Figure 29).

## CONCLUSION

Re-evaluation of the temperature humidity index provides the industry with solutions for tomorrow. Results of this study are in agreement with other studies confirming that current THI threshold is too high for current high producing dairy cows. Results show that THI beginning at 68 affect dairy cows adversely during heat stress. Therefore, cooling methods on commercial dairy farms should be implemented earlier to prevent these effects. Parameters indicative of heat stress were also shown to be correlated with THI and therefore are measurements that can be obtained to evaluate the degree of heat stress in the animal. Further research should be conducted to evaluate the relationship between BGHI and physiological parameters as with the addition of solar radiation effects perhaps the correlations would be greater; especially after arithmetic

means demonstrate strong correlations between BGHI and THI to skin surface temperature and respiration rate.

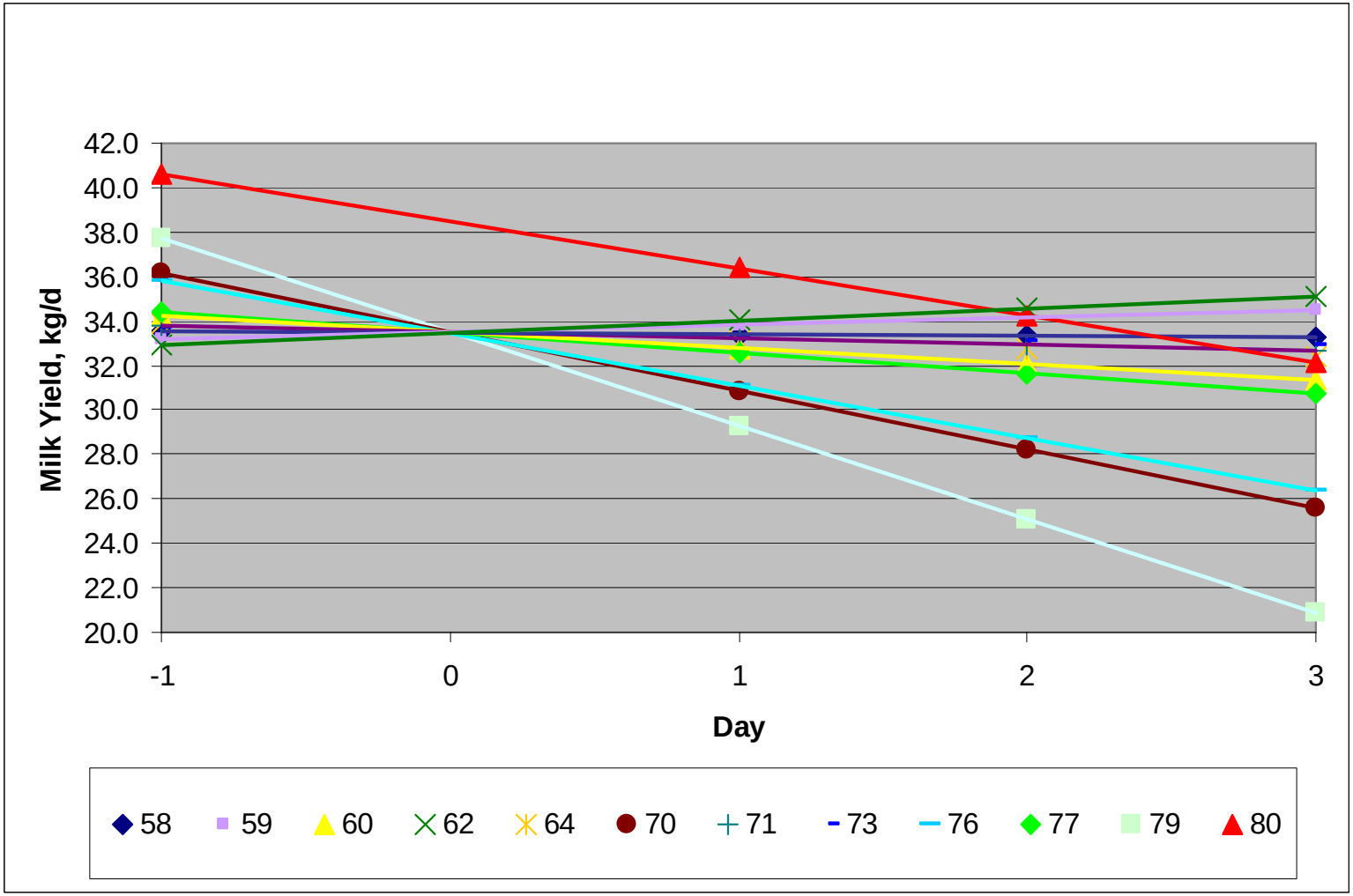


**Figure 2.1.** Effect of increasing temperature humidity index levels on varying milk production levels in lactating Holstein cows.

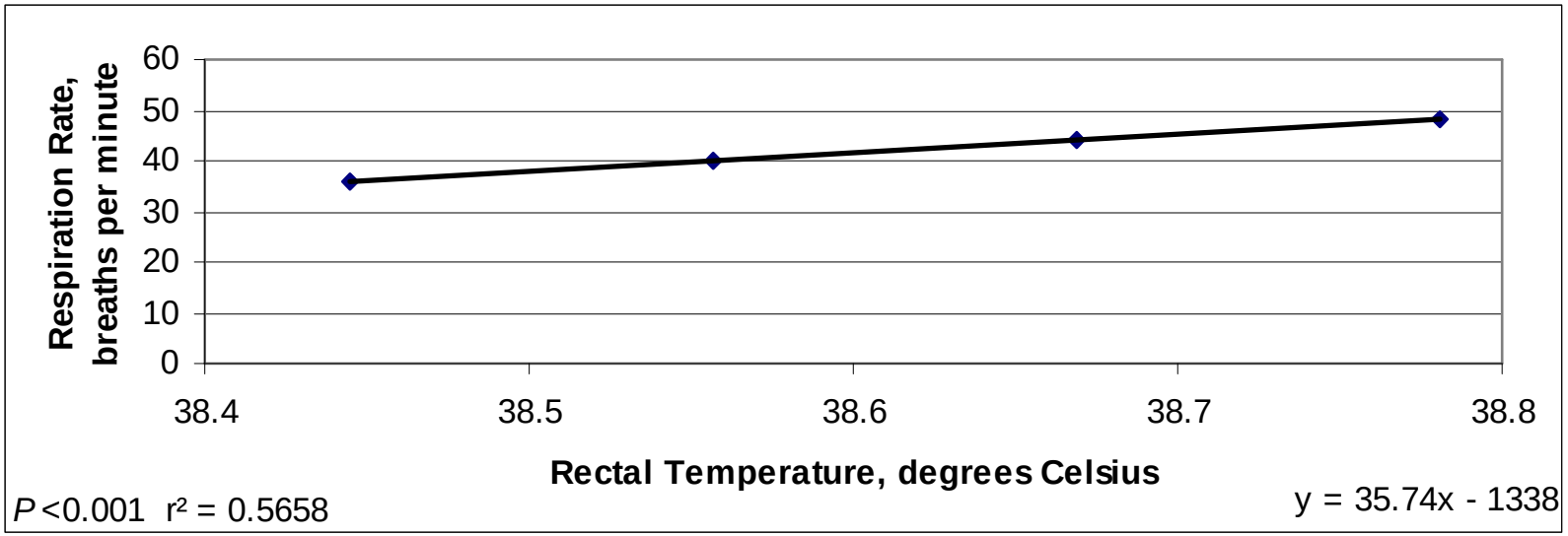


**Figure 2.2.** Effect of increasing rectal temperature on respiration rates in lactating Holstein cows.

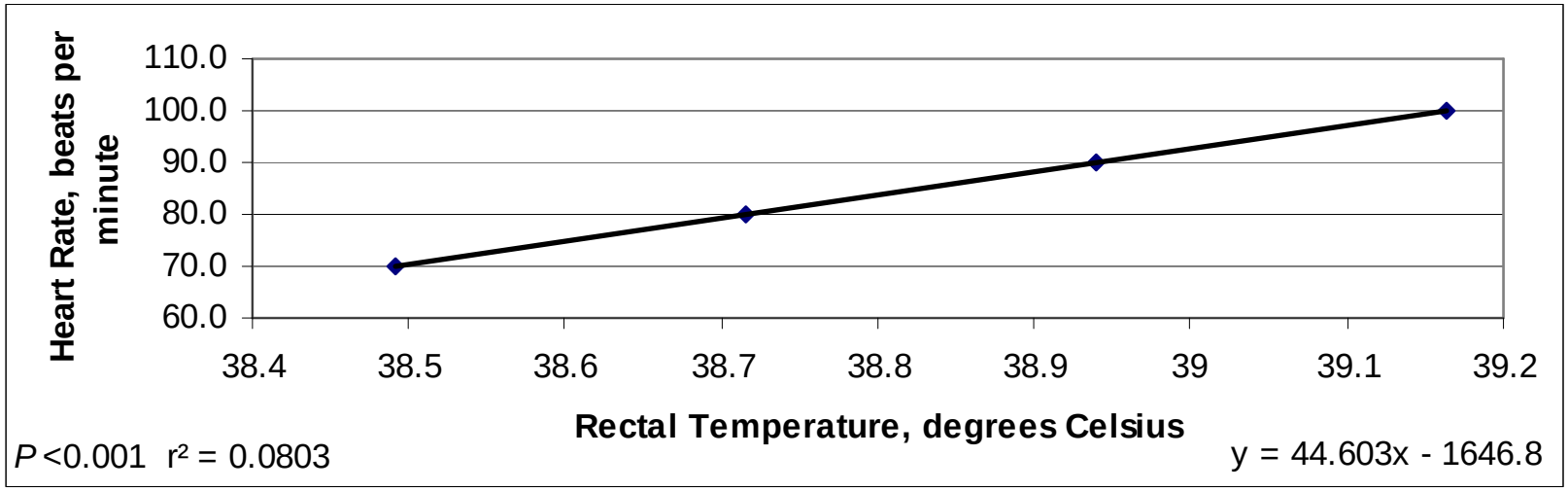


**Figure 2.3.** Effect of increasing rectal temperature on heart rate in lactating Holstein cows.

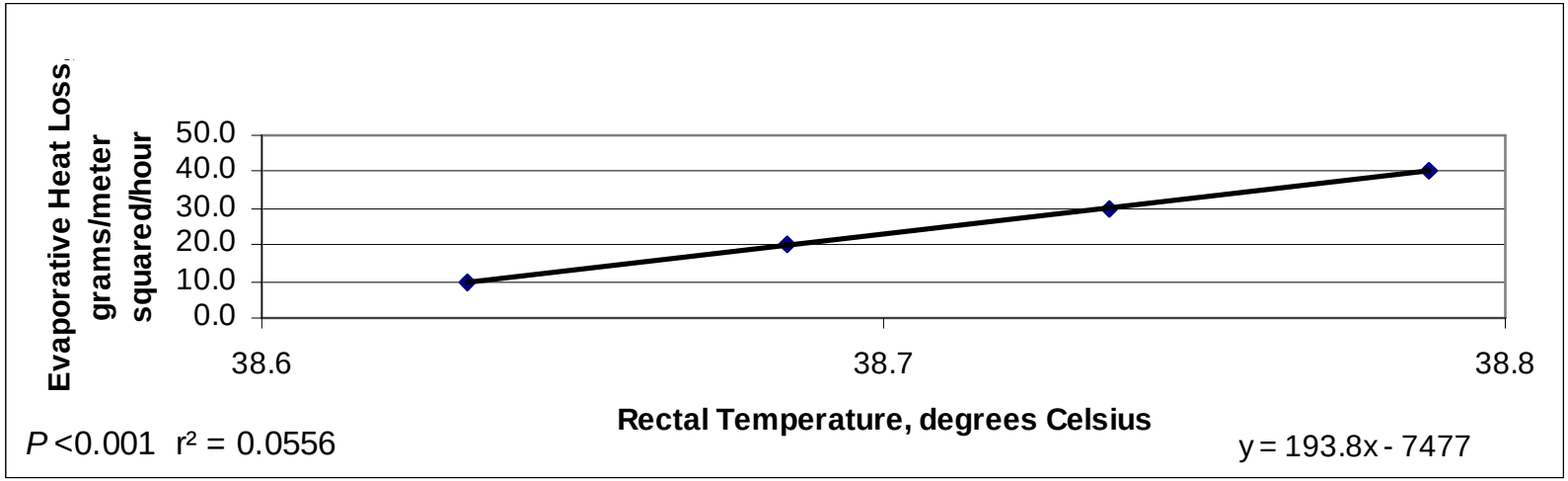


**Figure 2.4.** Effect of increasing rectal temperature on evaporative heat loss in lactating Holstein cows.

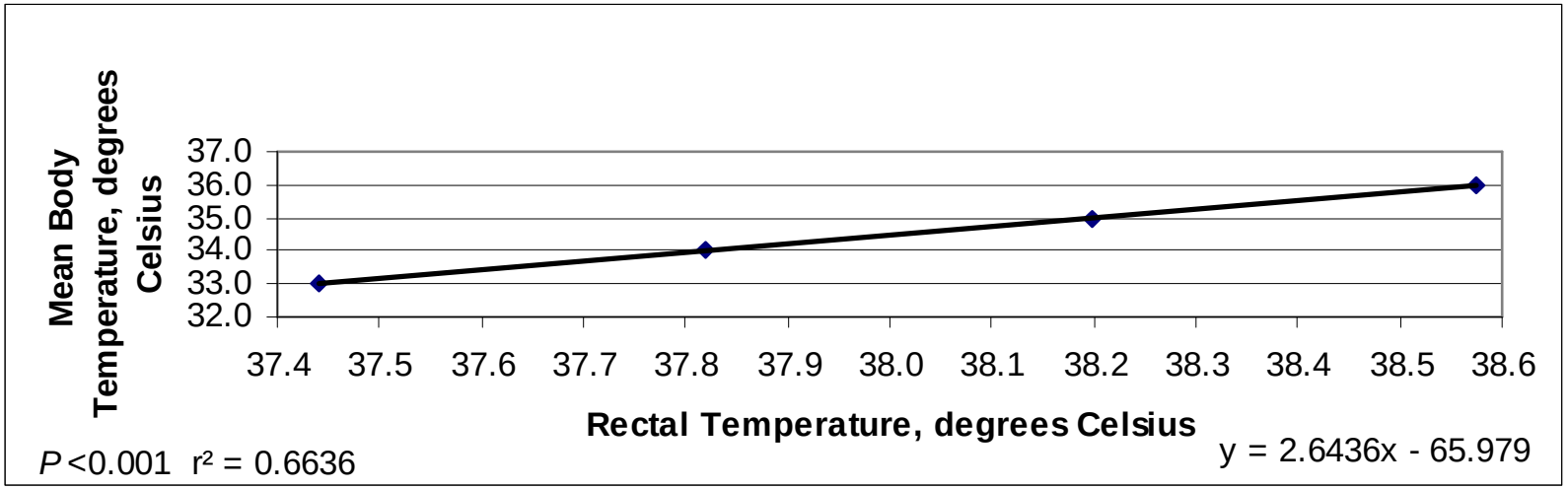


**Figure 2.5.** Effect of increasing rectal temperature on mean body temperature in lactating Holstein cows.

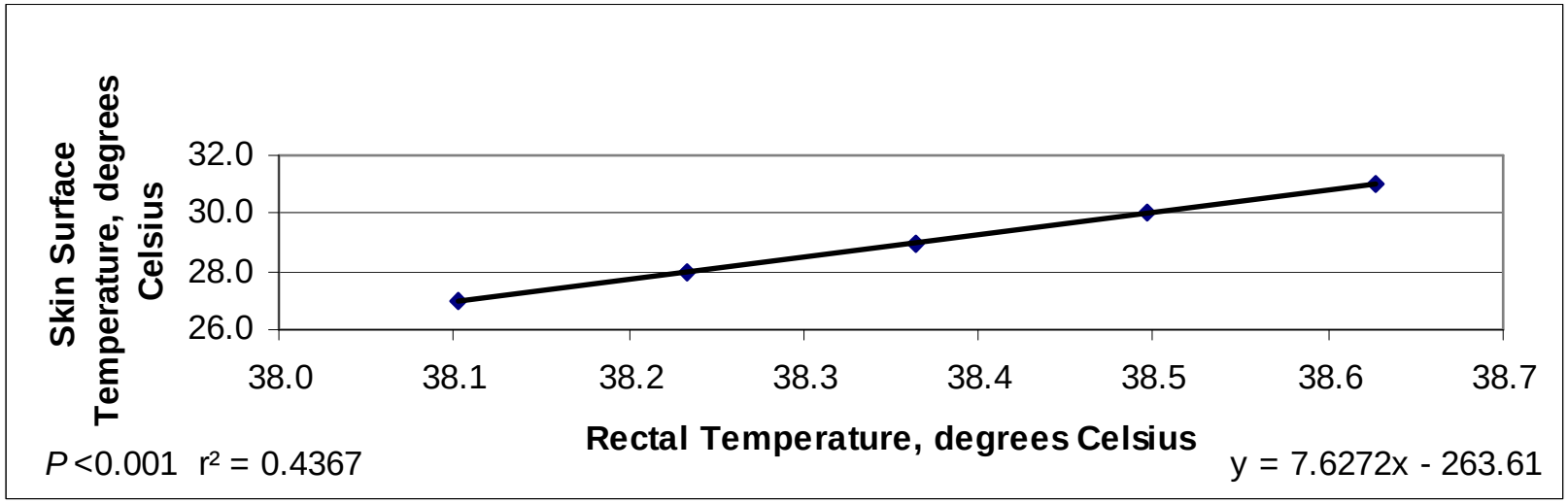


**Figure 2.6.** Effect of increasing rectal temperature on skin surface temperature in lactating Holstein cows.

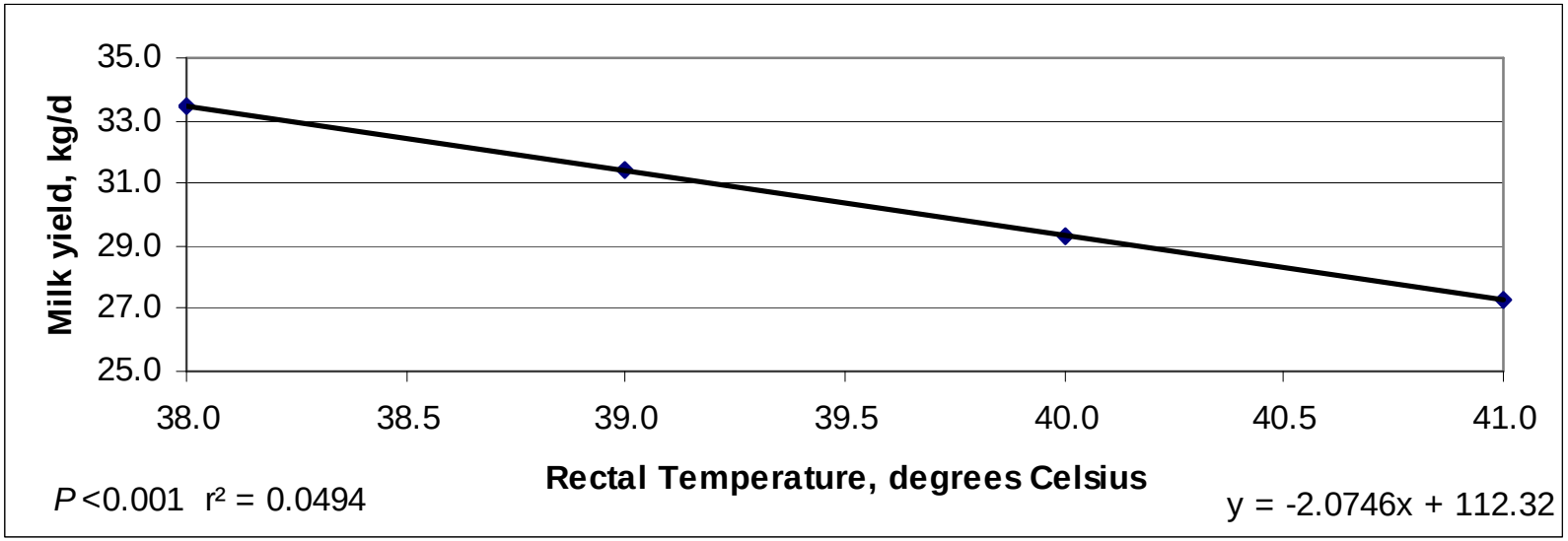


**Figure 2.7.** Effect of increasing rectal temperature on milk yield in lactating Holstein cows.

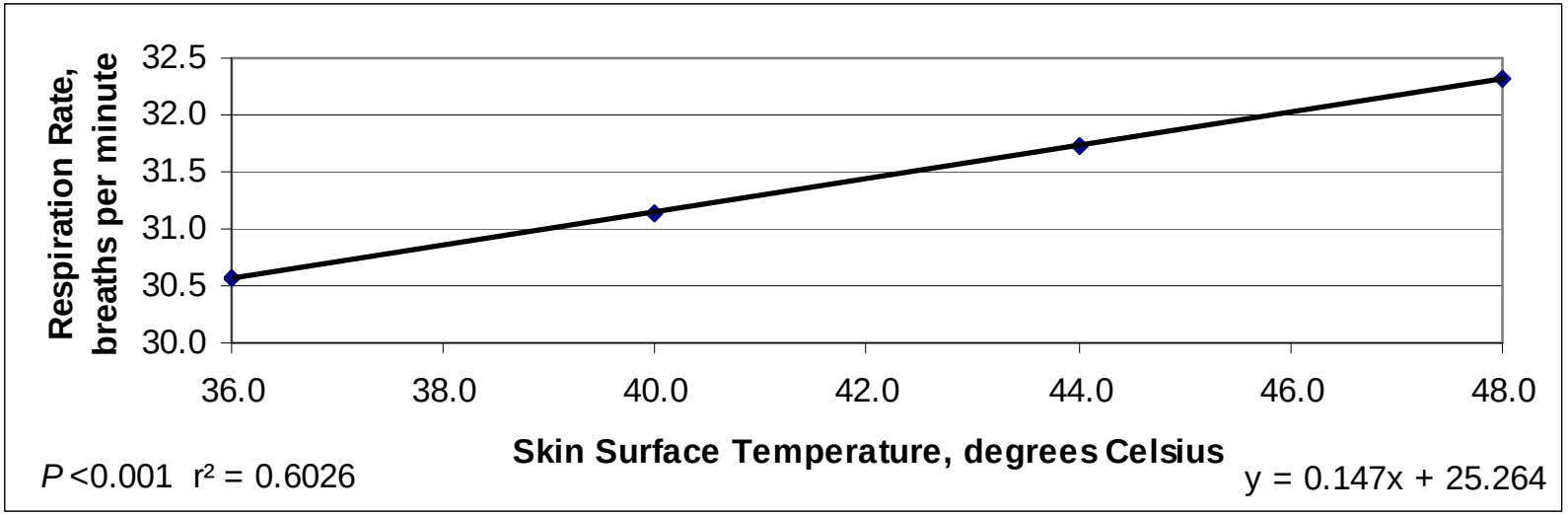


**Figure 2.8.** Effect of increasing skin surface temperature on respiration rate in lactating Holstein cows.

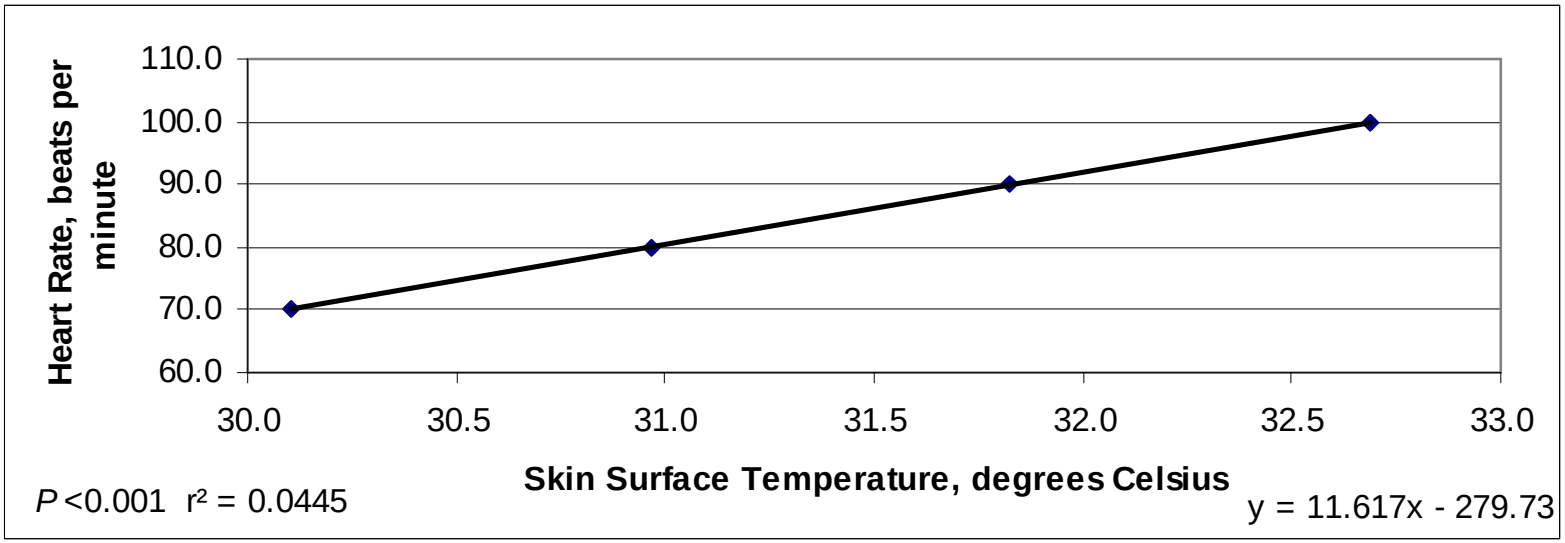


**Figure 2.9.** Effect of increasing skin surface temperature on heart rate in lactating Holstein cows.

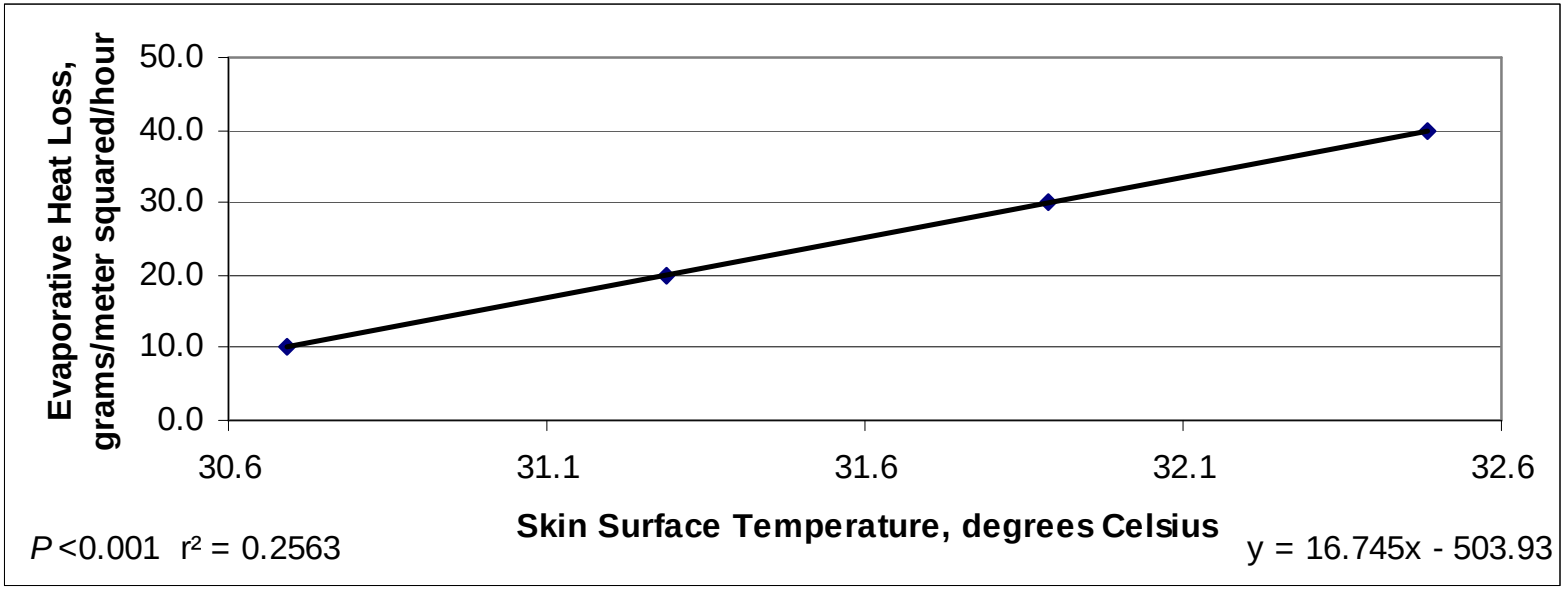


**Figure 2.10.** Effect of increasing skin surface temperature on evaporative heat loss in lactating Holstien cows.

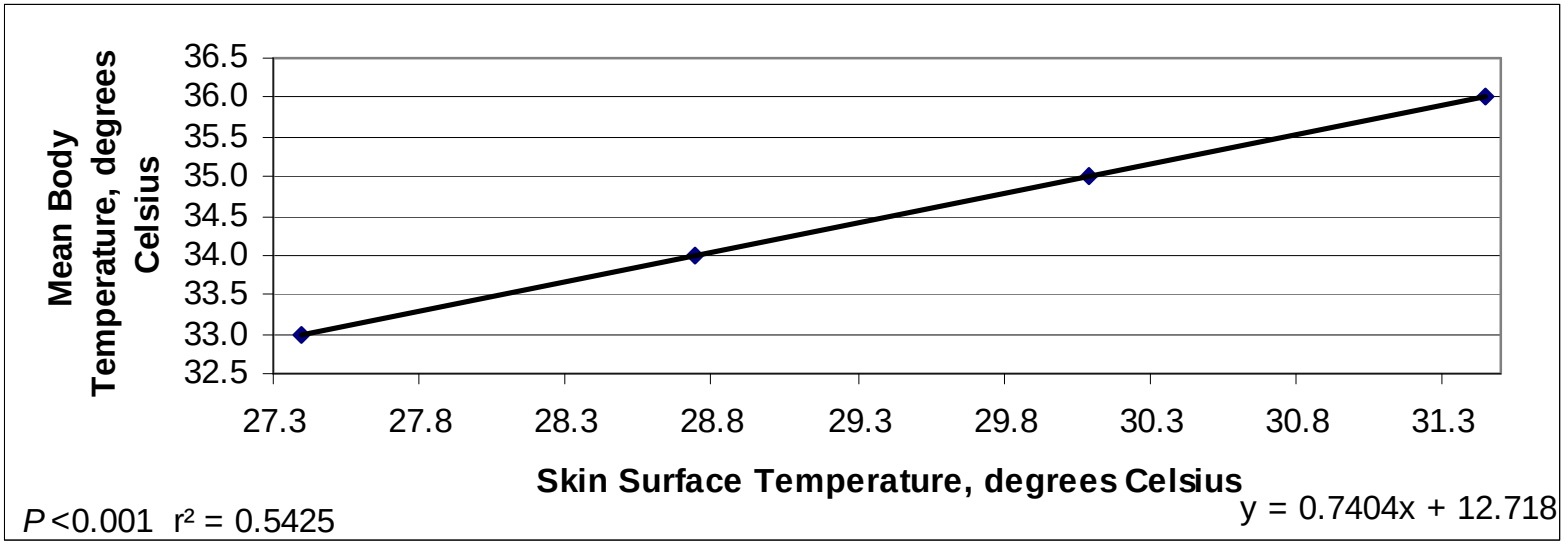


**Figure 2.11.** Effect of skin surface temperature on mean body temperature in lactating Holstein cows.

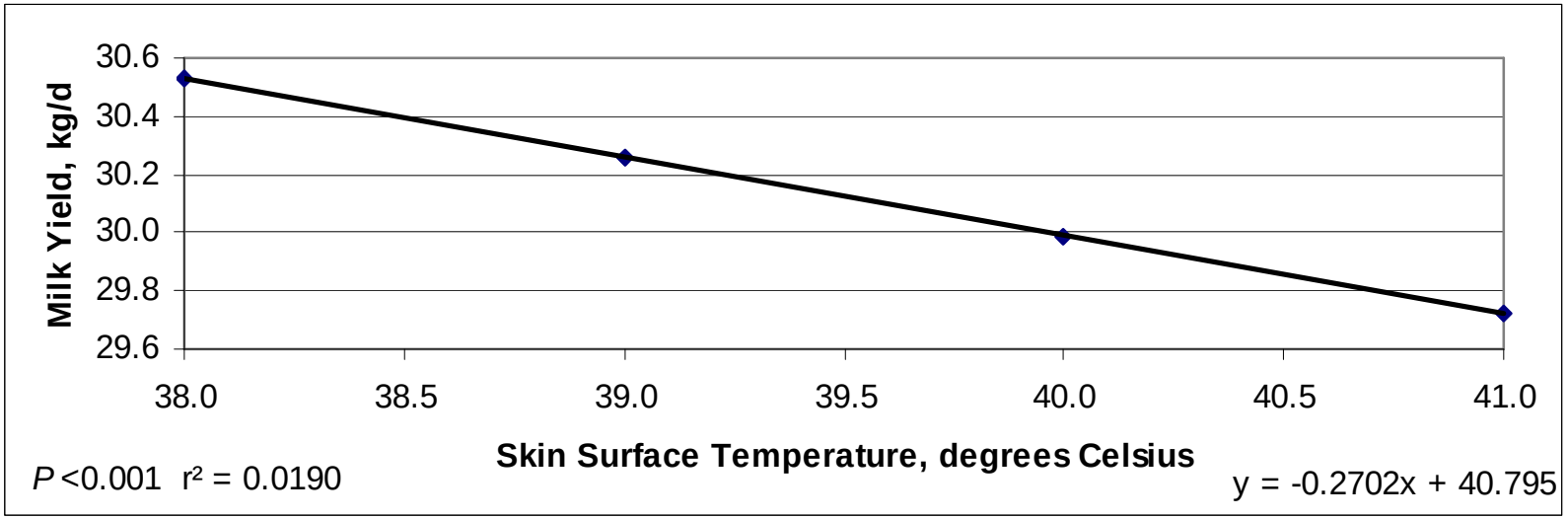


**Figure 2.12.** Effect of skin surface temperature on milk yield in lactating Holstein cows**.**

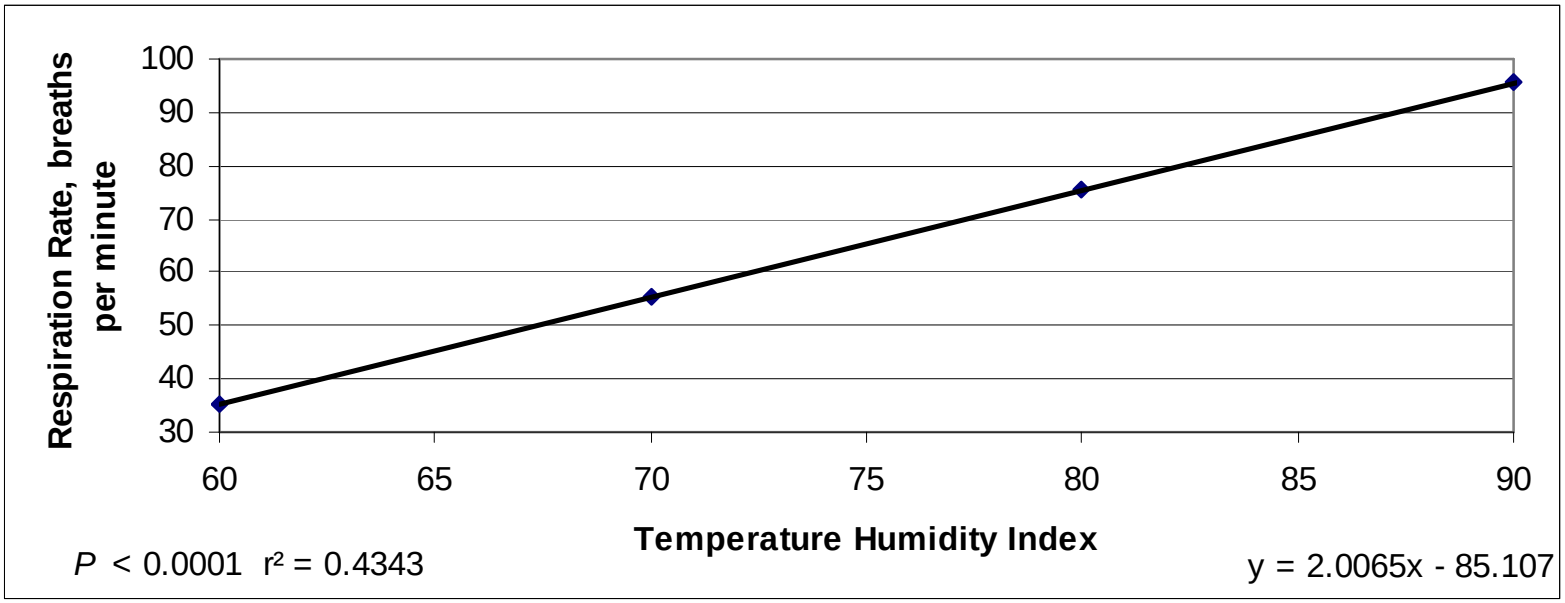


**Figure 2.13.** Effect of increasing temperature humidity index on respiration rate in lactating Holstein cows.

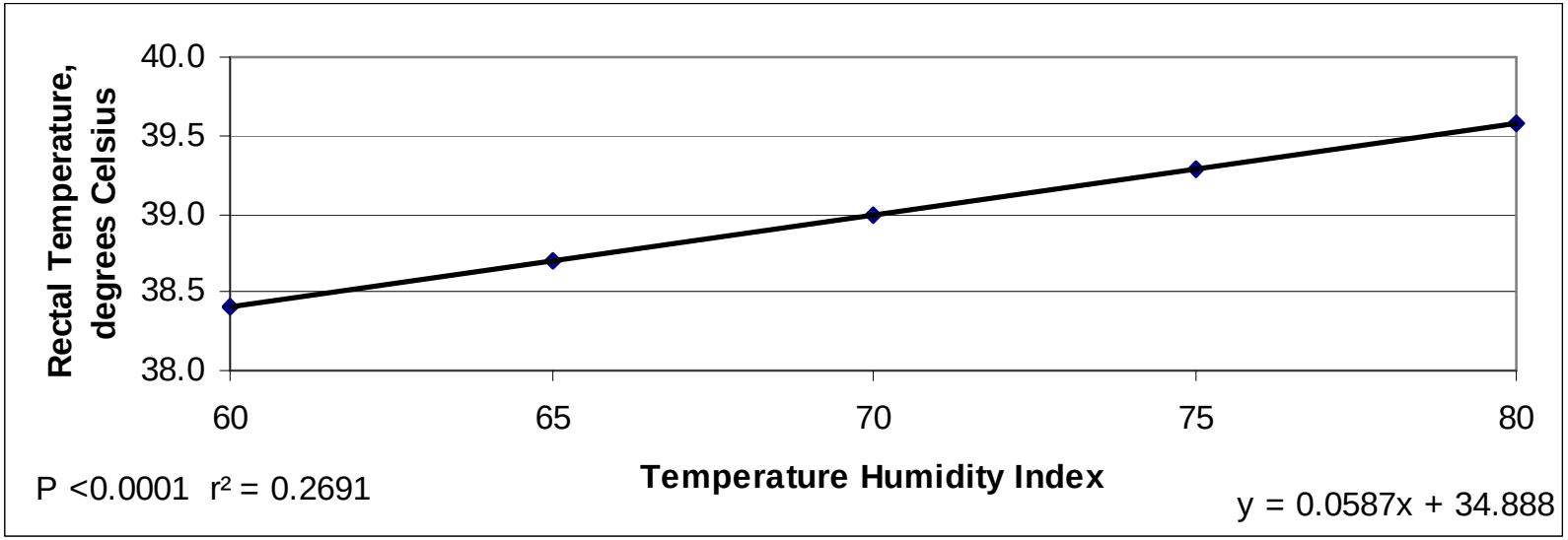


**Figure 2.14.** Effect of increasing temperature humidity index on rectal temperatures in lactating Holstein cows.

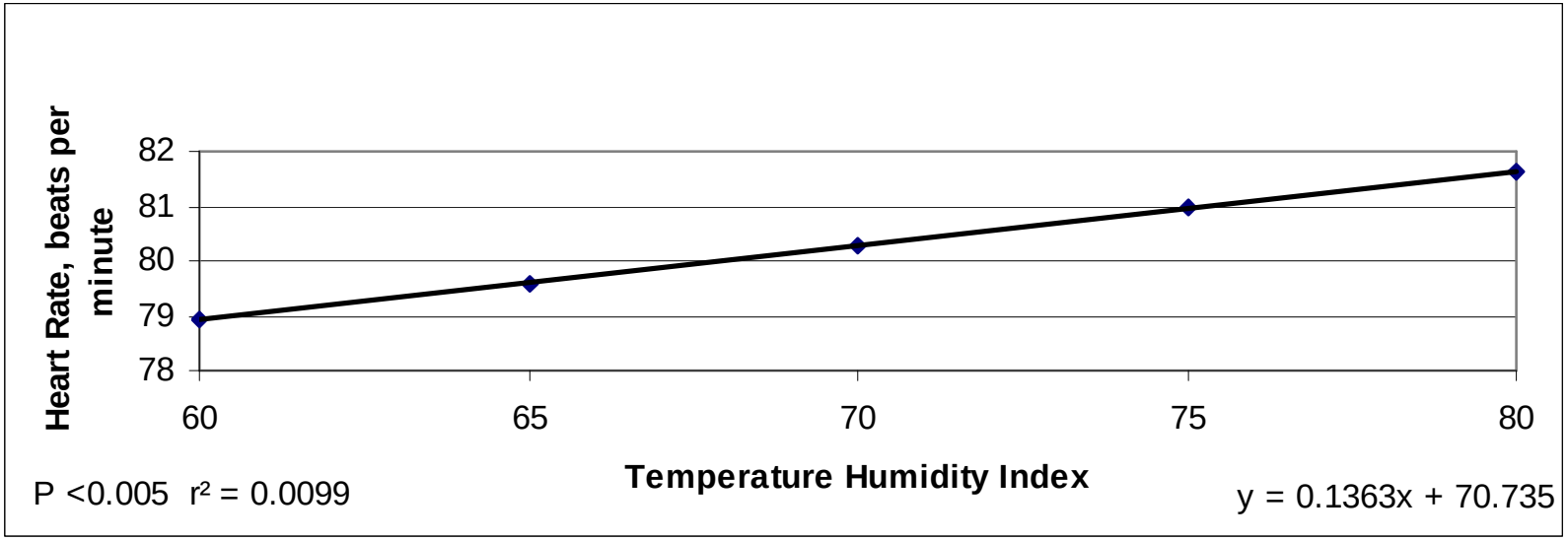


**Figure 2.15.** Effect of increasing temperature humidity index on heart rate in lactating Holstein cows.

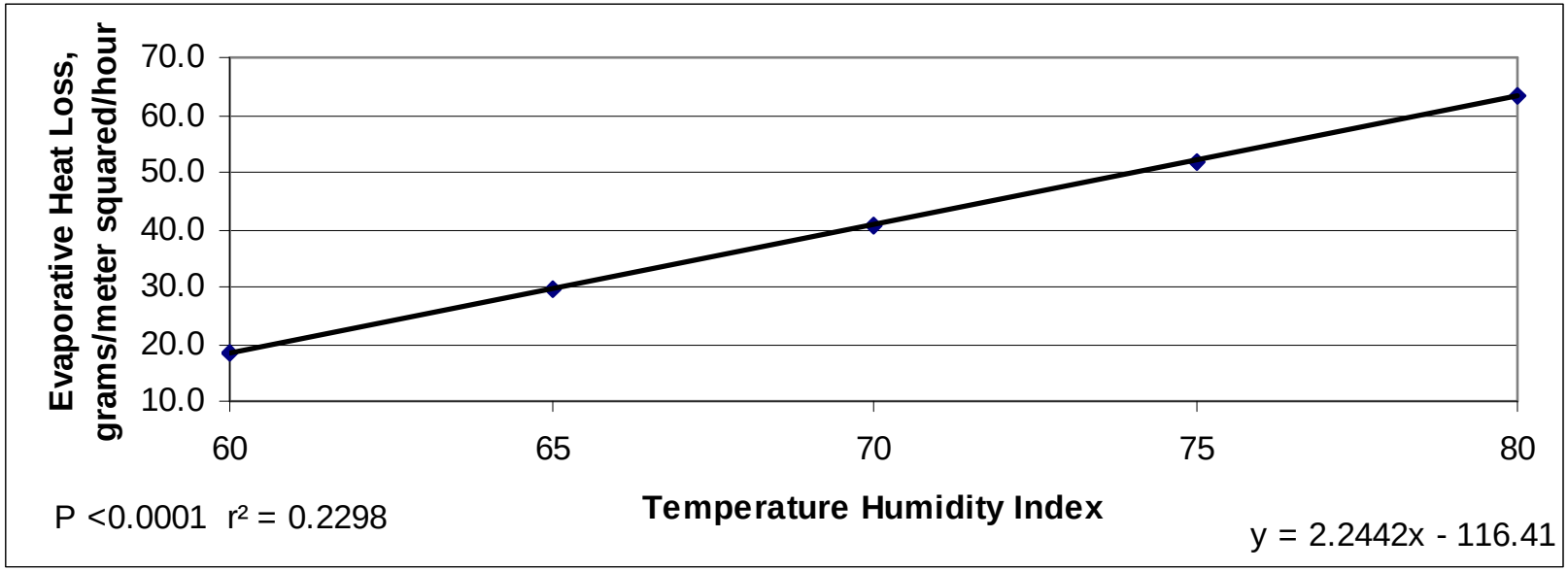


**Figure 2.16.** Effect of increasing temperature humidity index on evaporative heat loss in lactating Holstein cows.

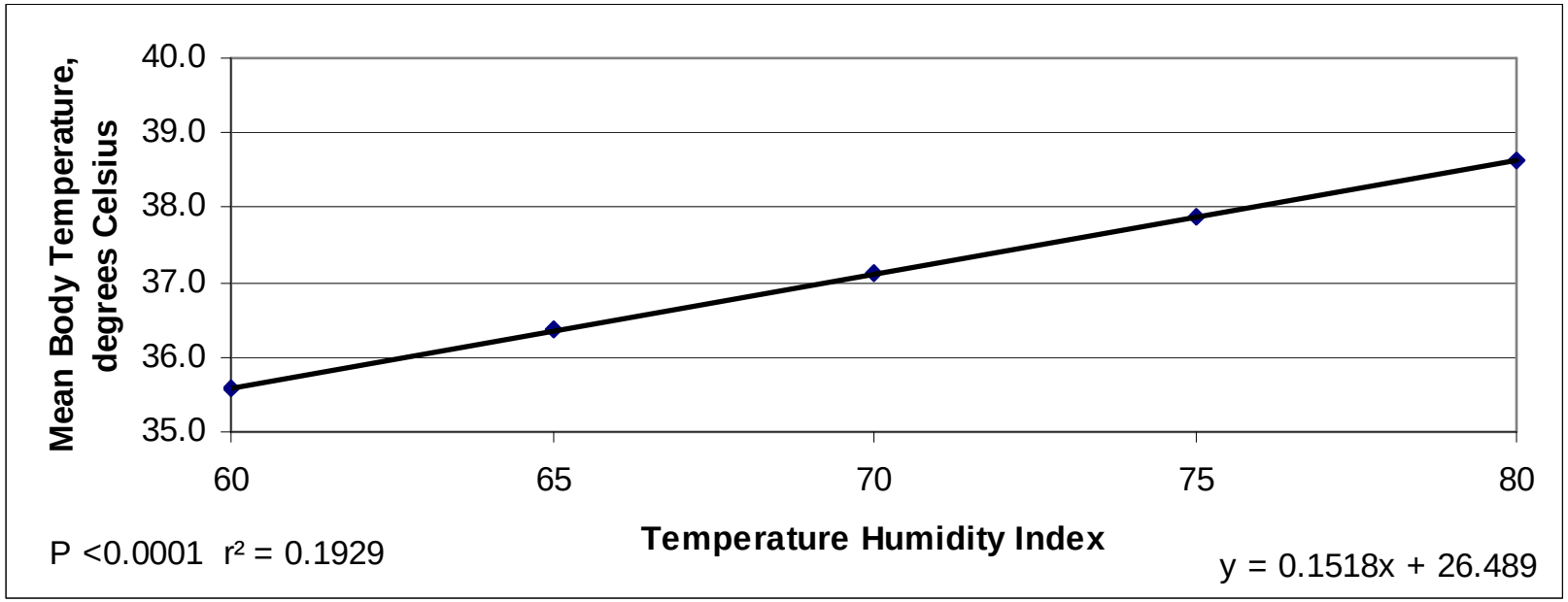


**Figure 2.17.** Effect of increasing temperature humidity index on mean body temperatures in lactating Holstein cows.

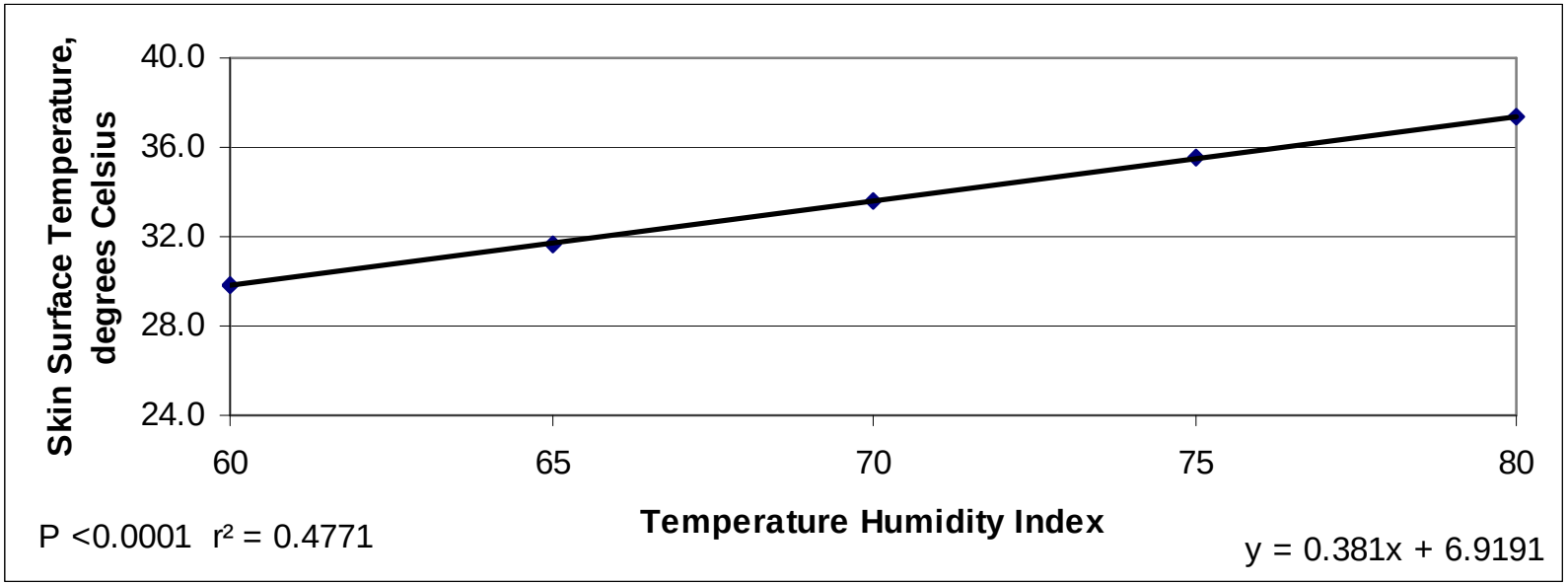


**Figure 2.18.** Effect of increasing temperature humidity index on skin surface temperatures in lactating Holstein cows.

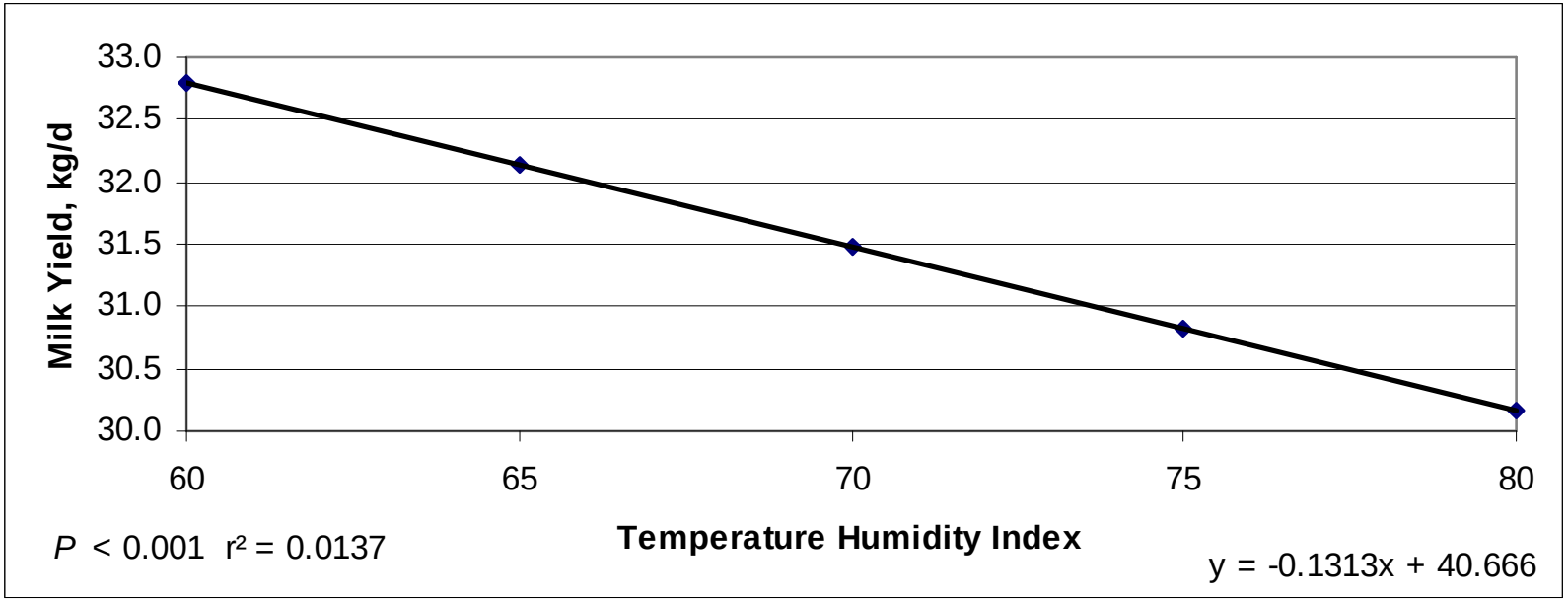


**Figure 2.19.** Effect of increasing temperature humidity index on milk yield in lactating Holstein cows.

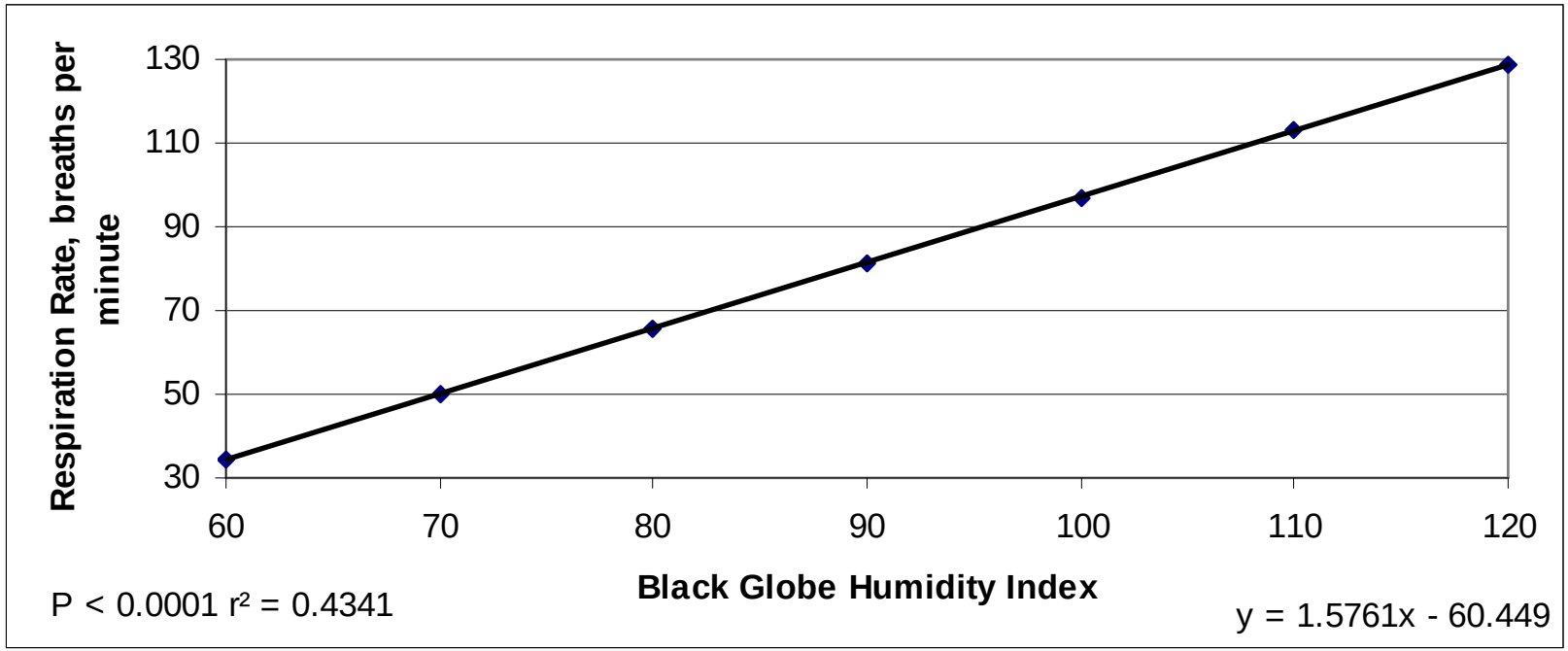


**Figure 2.20.** Effect of increasing black globe humidity index on respiration rate in lactating Holstein cows.

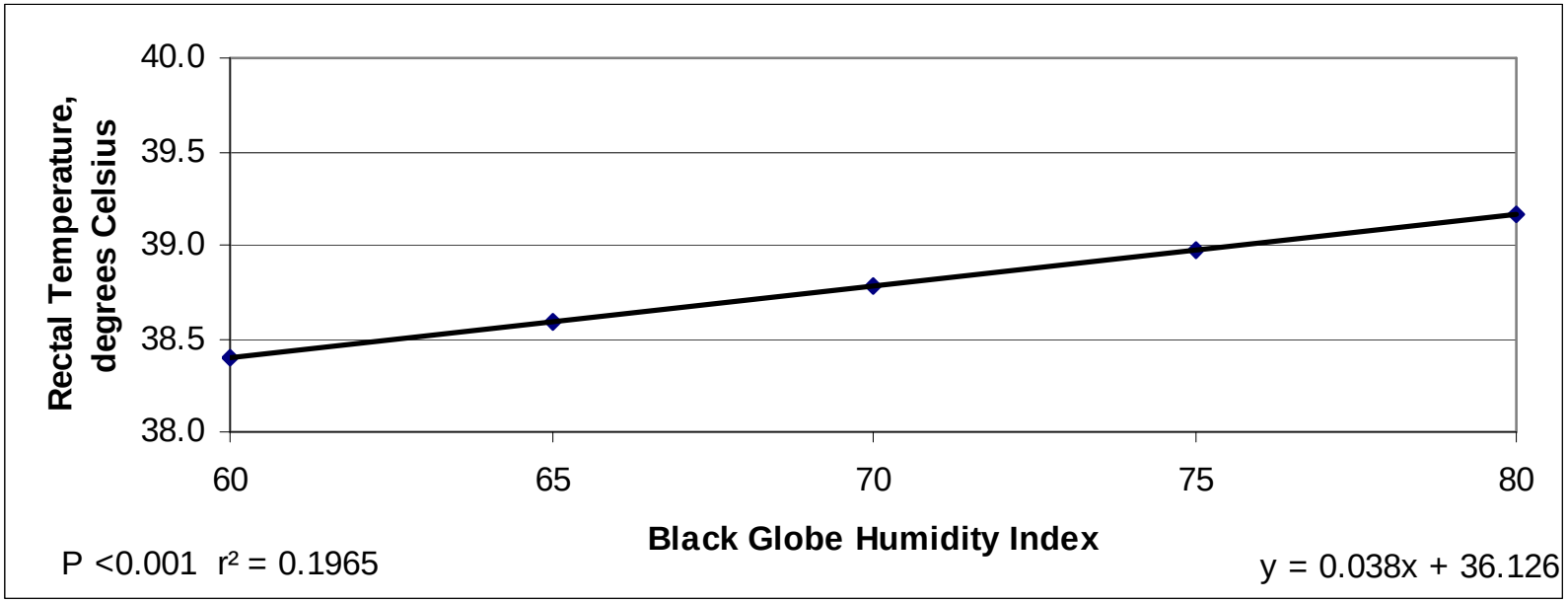


**Figure 2.21.** Effect of increasing black globe humidity index on rectal temperature in lactating Holstein cows.

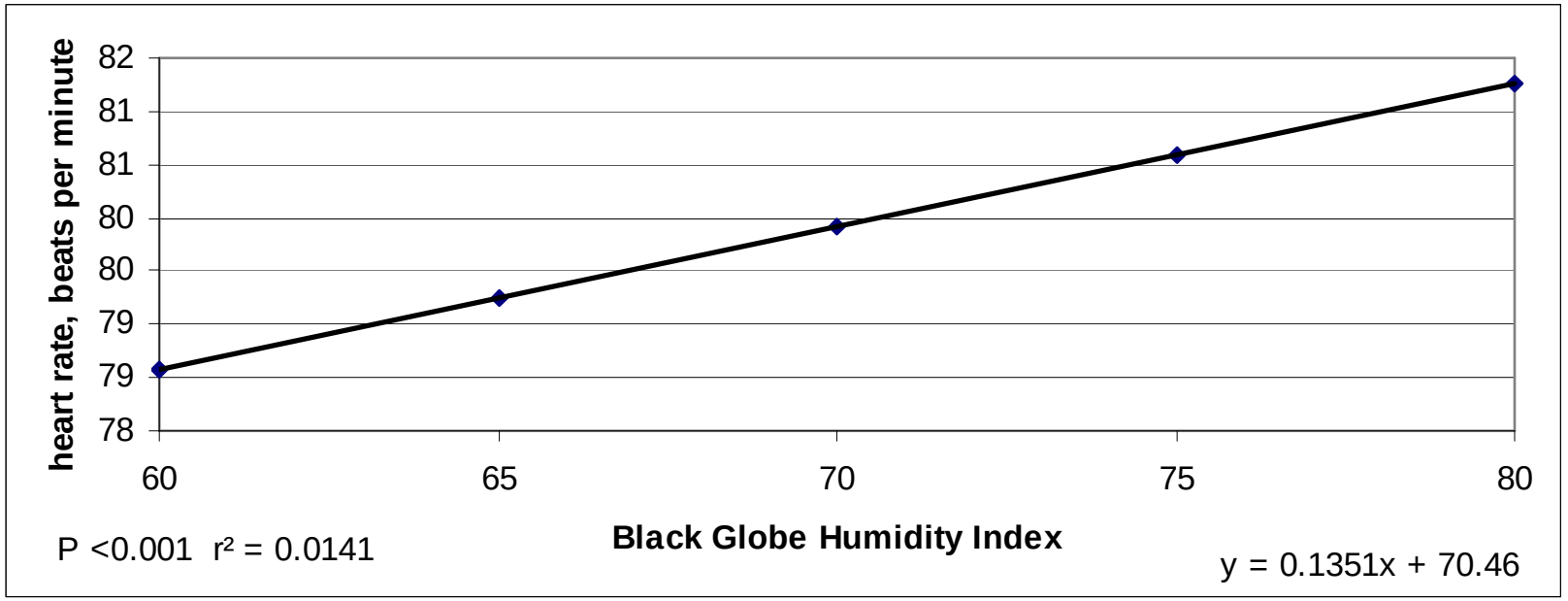


**Figure 2.22.** Effect of increasing black globe humidity index on heart rate in lactating Holstein cows.

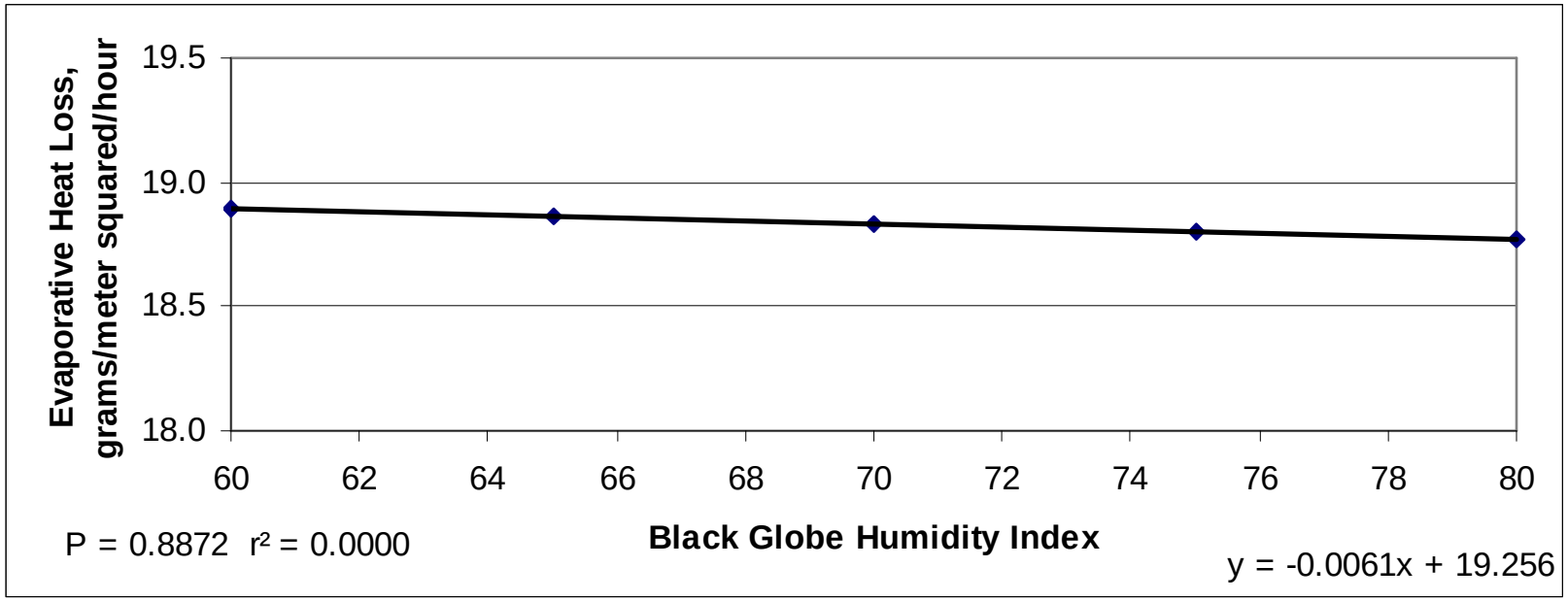


**Figure 2.23.** Effect of increasing black globe humidity index on evaporative heat loss in lactating Holstein cows.

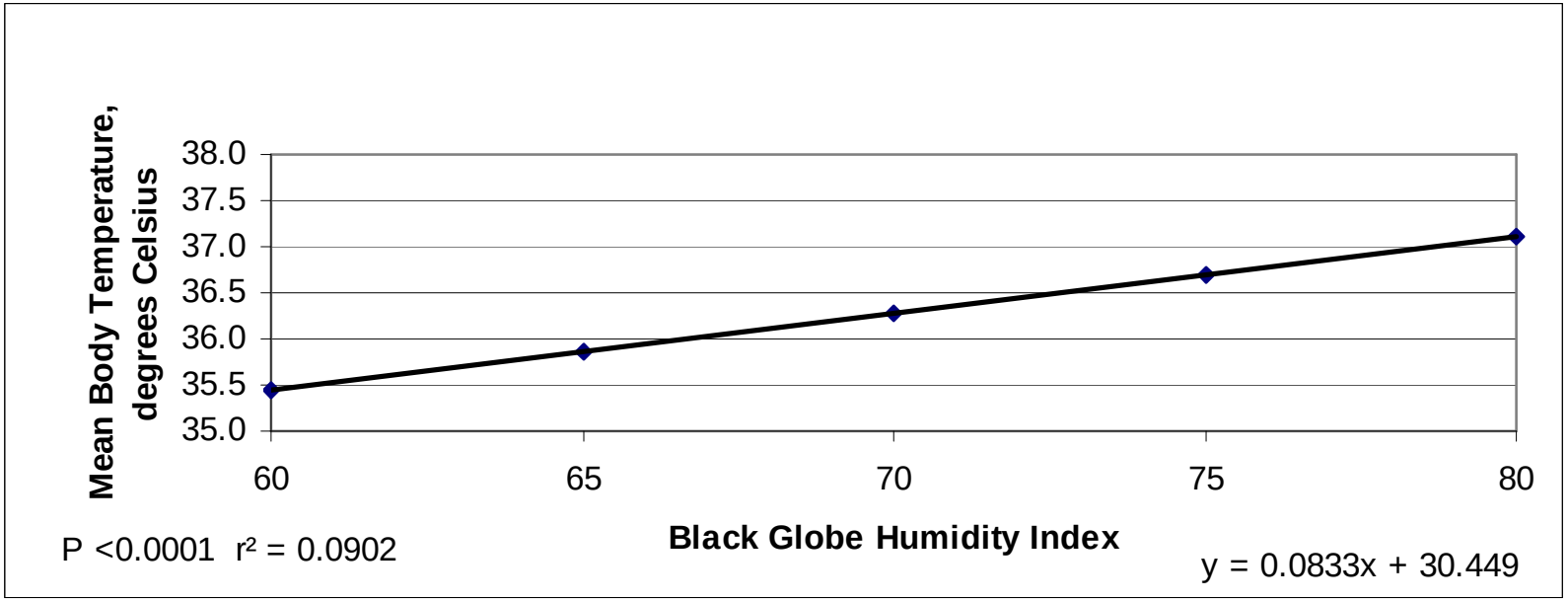


**Figure 2.24.** Effect of increasing black globe humidity index on mean body temperature in lactating Holstein cows.

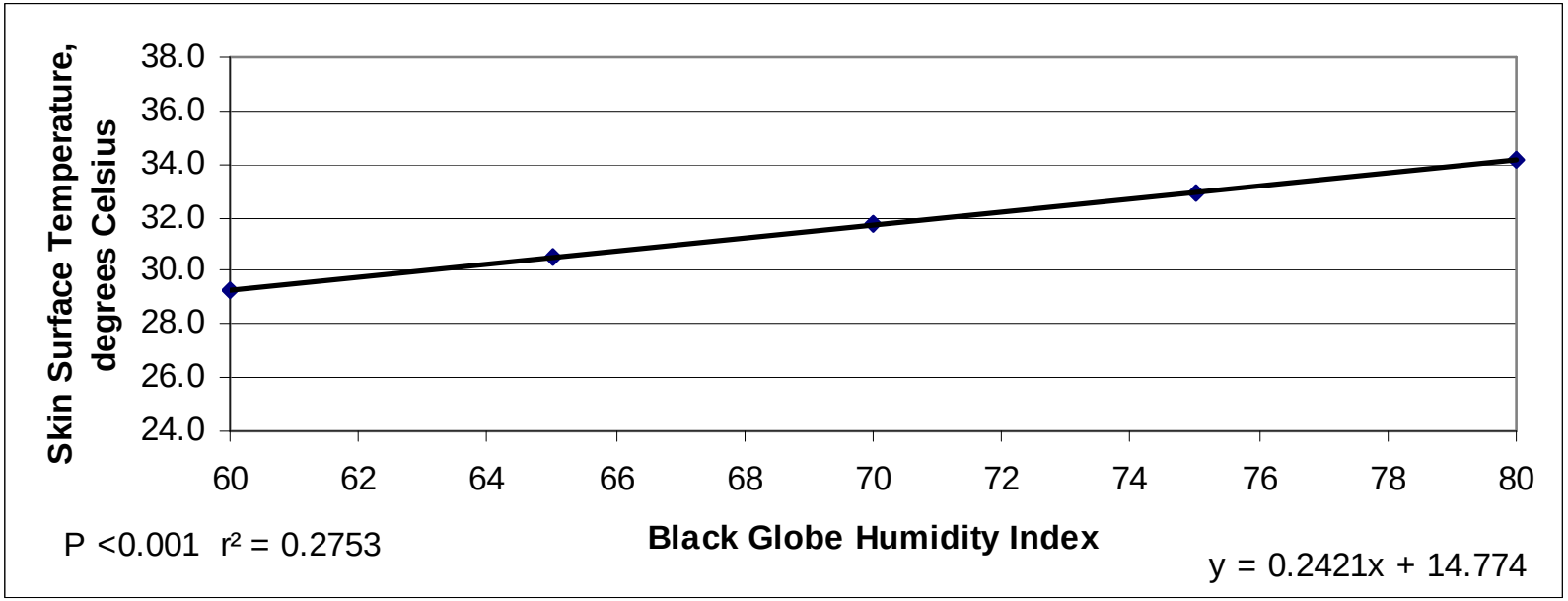


**Figure 2.25.** Effect of increasing black globe humidity index on skin surface temperature in lactating Holstein cows.

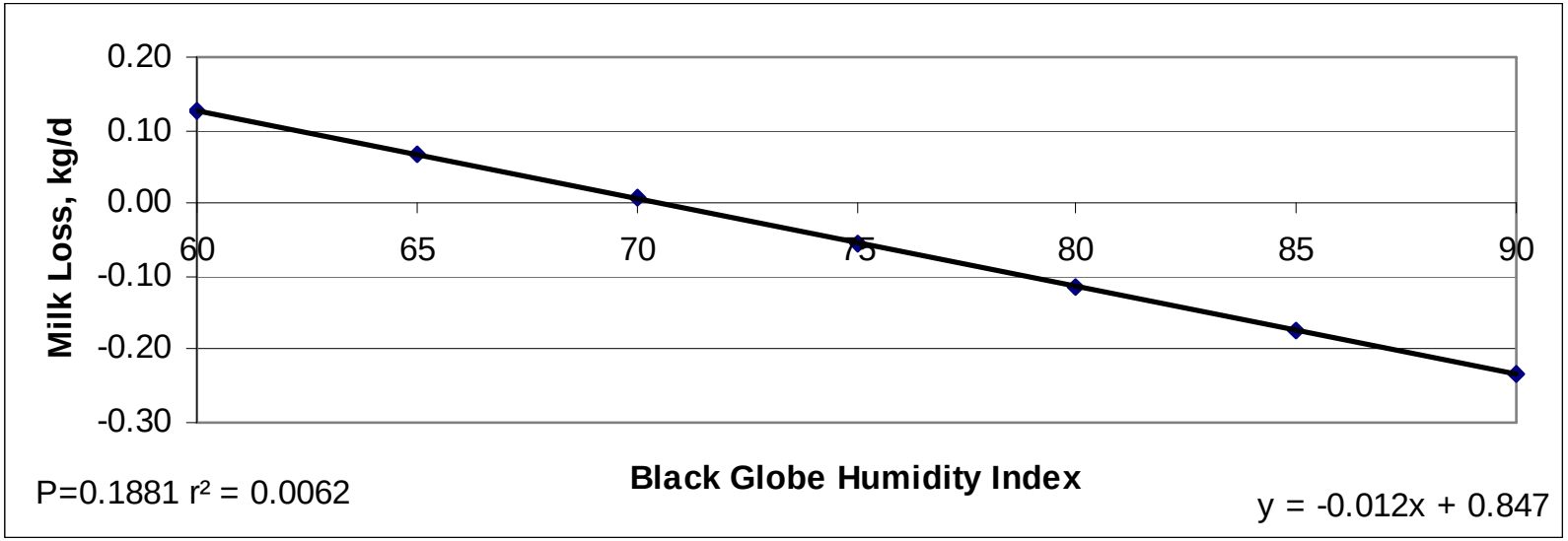


**Figure 2.26.** Effect of increasing black globe humidity index on milk loss per day in lactating Holstein cows.

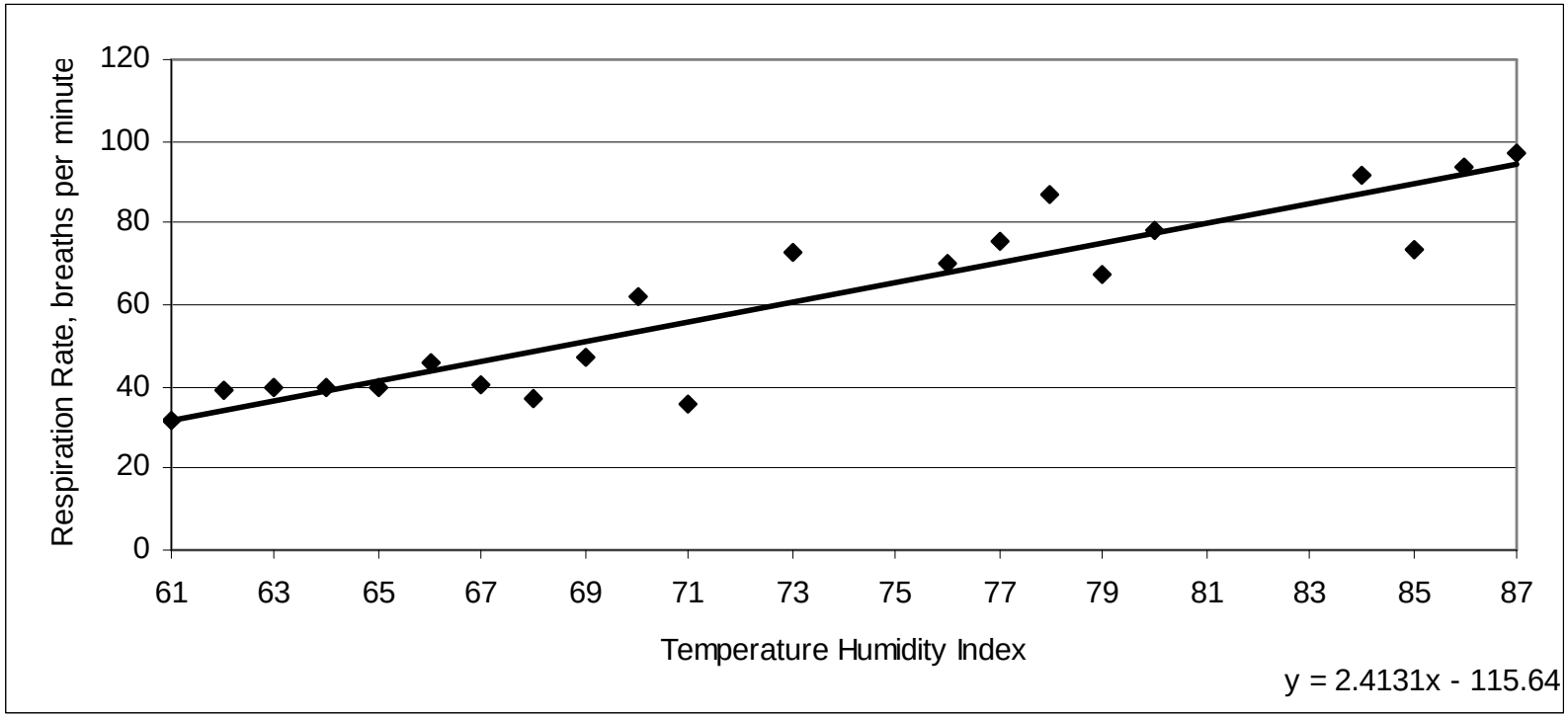


**Figure 2.27.** Effects of increasing temperature humidity index on respiration rate based on hourly arithmetic means from the data set of lactating Holstein cows.

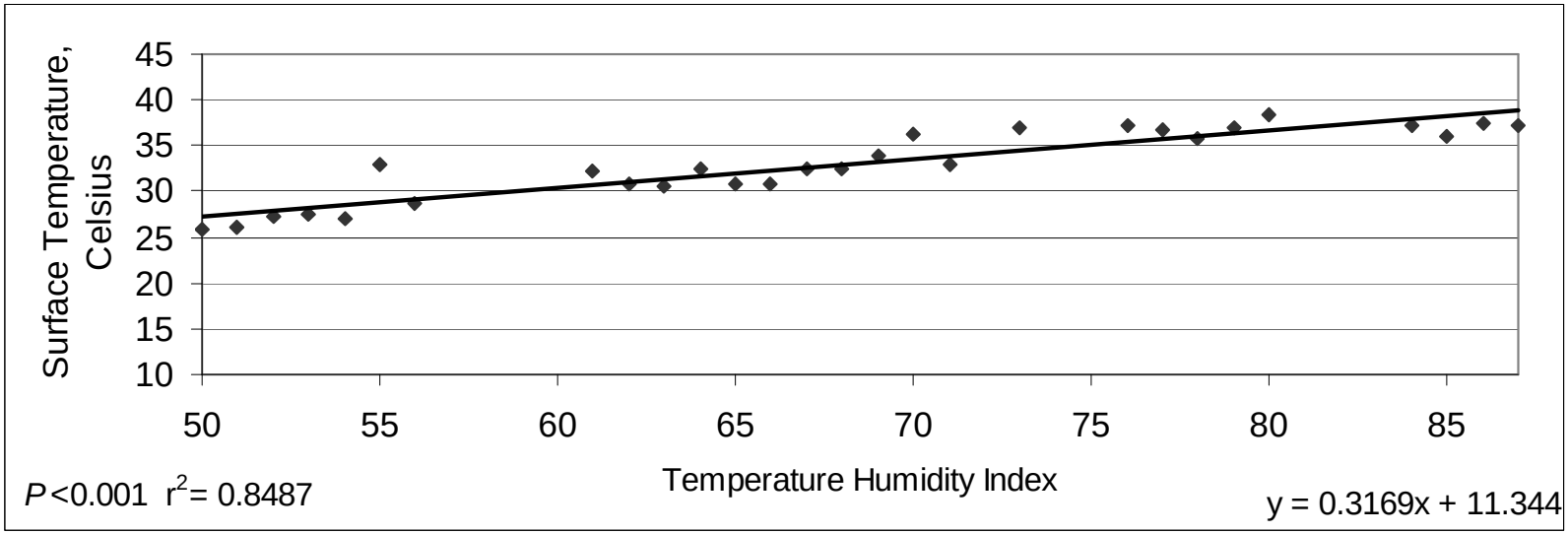


**Figure 2.28.** Effect of increasing temperature humidity index on skin surface temperature based on hourly arithmetic means from the data set of lactating Holstein cows.

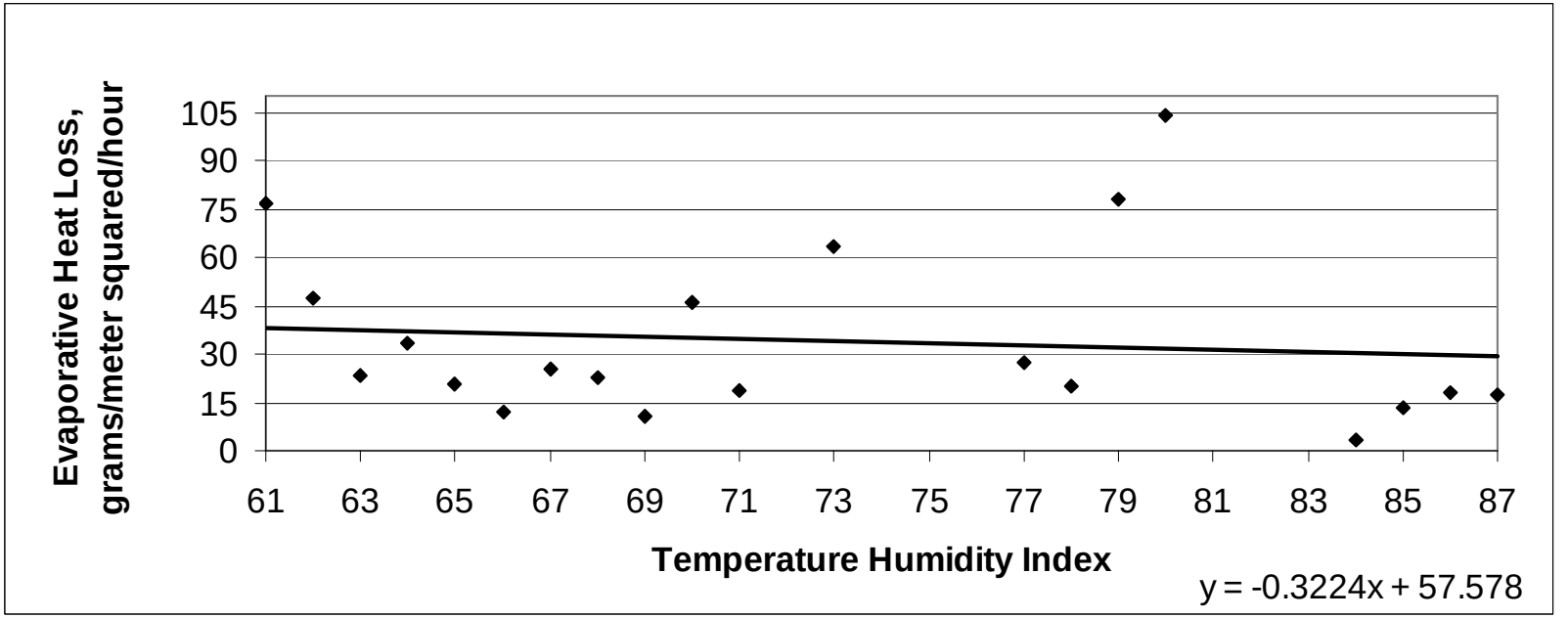


**Figure 2.29.** Effect of increasing temperature humidity index on evaporative heat loss based on hourly arithmetic means from the data set of lactating Holstein cows.

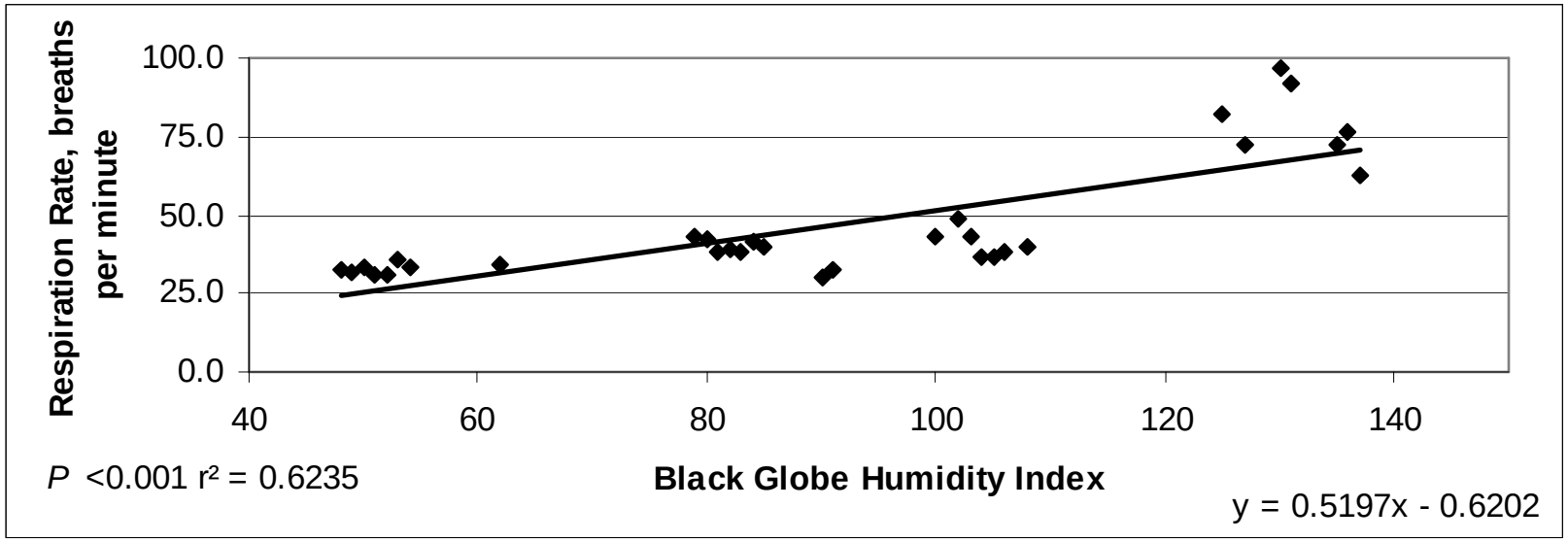


**Figure 2.30.** Effect of increasing black globe humidity index on respiration rate based on hourly arithmetic means from the data set of lactating Holstein cows.

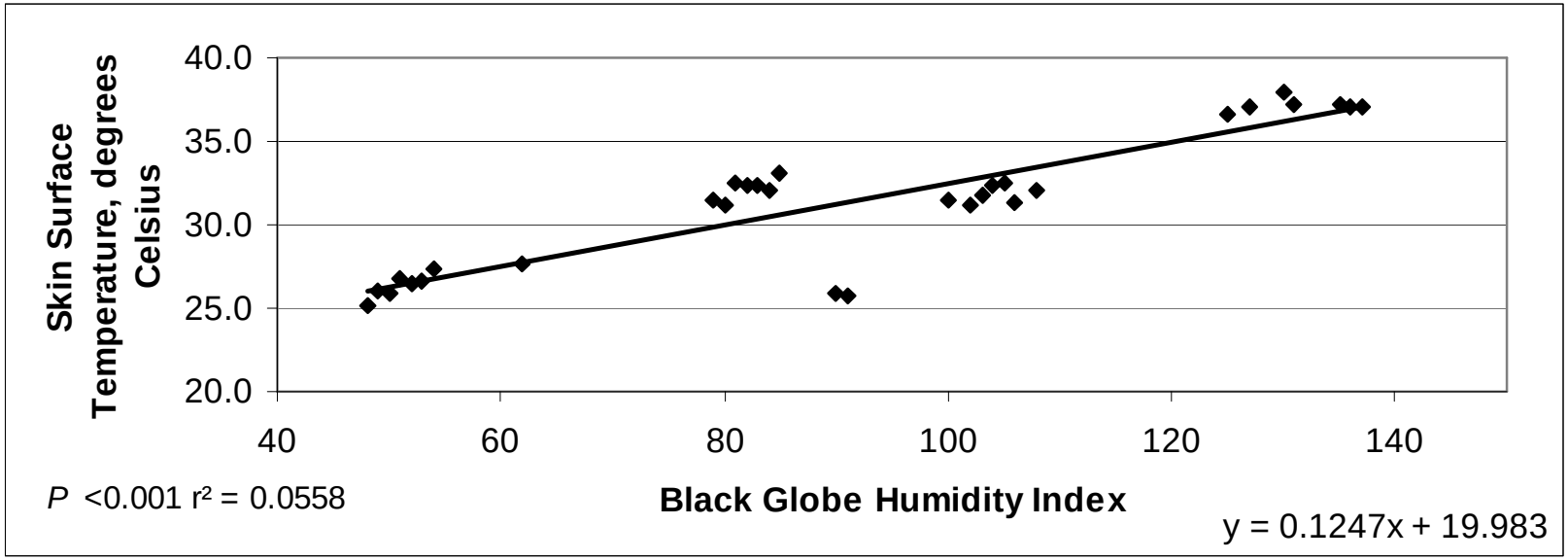


**Figure 2.31.** Effect of increasing black globe humidity index on skin surface temperature based on hourly arithmetic means from the data set of lactating Holstein cows.

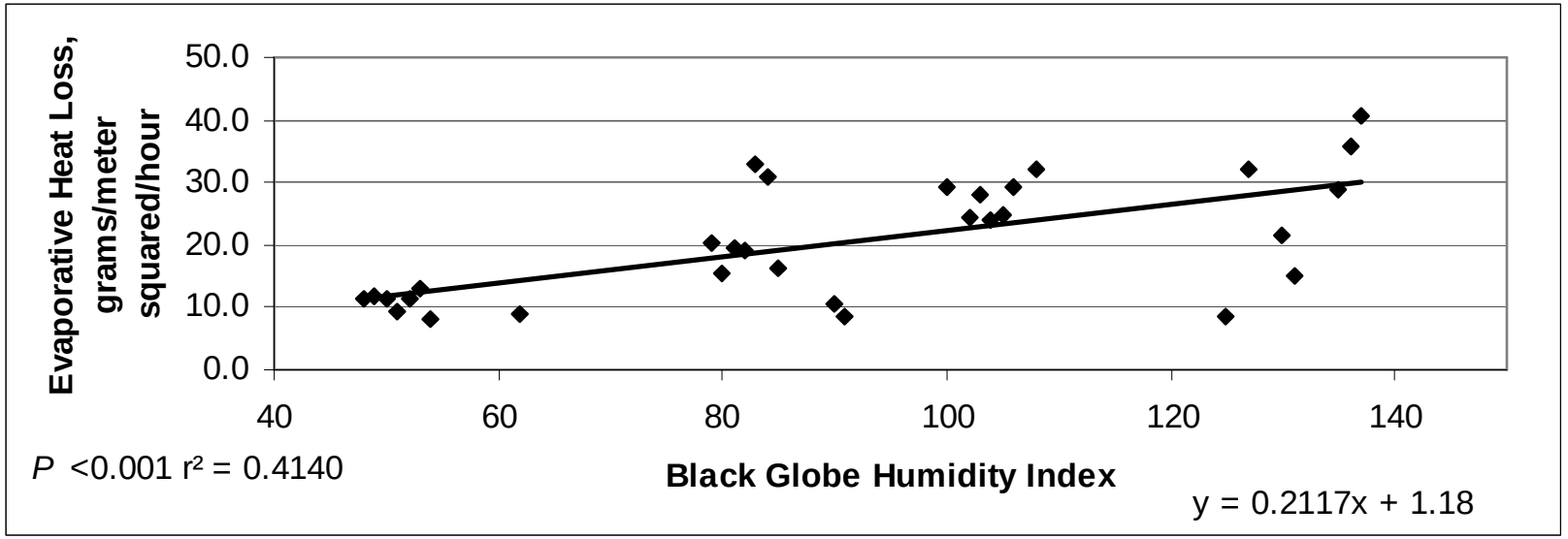


**Figure 2.32.** Effect of increasing black globe humidity index on evaporative heat loss based on hourly arithmetic means from the data set of lactating Holstein cows.

# CHAPTER THREE: EFFECTS OF ENCAPSULATED NIACIN ON EVAPORATIVE HEAT LOSS AND BODY TEMPERATURE IN MODERATELY HEAT-STRESSED LACTATING HOLSTEIN COWS

## ABSTRACT

Twelve multiparous Holstein cows producing an average of 31 kg/d and balanced for parity and stage of lactation (145 ± 9 days in milk) were randomly assigned to receive either 0 g (**C**) or 12 g supplemental encapsulated niacin/d (**SU**) and exposed to thermoneutral (**TN, period 1**) or heat stress (**HS, period 2**) conditions in climate controlled chambers. The THI range during TN never exceeded 72 while HS consisted of a circadian temperature range where THI exceeded 72 for 12 h/d. Body temperature indices obtained 4 times/d included: respiration rates (**RR**), surface temperatures (**ST** of both shaved [**SH**] and unshaved [**USH**] areas) at the rump, shoulder, and tail head, and evaporative heat loss (**EVHL**) of the shoulder shaved (**EVHL-SH**) and unshaved (**EVHL-US**) areas. Milk yield did not differ between dietary groups or periods. Dry matter intake was not affected by SU, but decreased in P2. Surface temperatures were unaffected by SH but were higher in SH than US areas (32.6 vs. 31.4°C). Cows fed SU had higher EVHL (66.3 vs. 57.8 g/m²/h, EVHL-SH) throughout the trial and (57.4 vs. 52.7 g/m²/h, EVHL-US) over the 24 h measurement period during HS and these differences grew larger during periods of peak thermal stress. Between 1100 to 1600 h during the 24 h measurement period, EVHL for SU cows was higher than C (81.1 vs. 68.2 g/m²/h, EVHL-SH and 70.6 vs. 62.3 g/$m^2$/h, EVHL-US). Cows fed SU had lower RT during period 2 (HS) compared to C (38.17 vs. 38.34°C) and lower vaginal temperatures (38.0 vs. 38.4°C). Calculated metabolic rate was not different between C

and SU, but increased numerically during HS regardless of diet (Period 1: 49.16 and Period 2: 50.99 kcal/kg of body weight/d). Heat storage was lower in SU cows during the entire trial (30.49 vs. 30.54 kcal/kg of BW; $P<0.05$). These differences were greater during Period 2 (30.68 vs. 30.52 kcal/kg of BW; $P<0.01$). Supplemented cows had elevated plasma niacin concentrations throughout both TN and HS periods compared to the C ($P<0.05$). We conclude that feeding encapsulated niacin increases plasma niacin levels, EVHL and lowers core body temperatures in cows experiencing a mild thermal load.

## INTRODUCTION

During warm summer months milk production can decrease between 10-35% and this is a costly issue in the global dairy industry (St. Pierre et al., 2003). The reduced milk yield is a result of increased body temperature induced-decline in feed intake as well as alterations in endocrine profiles, energy metabolism (Baumgard and Rhoads, 2007) and other unidentified factors (Collier et al., 2008). Increasing heat dissipation (the transfer of body heat from the core to the surface) via enhanced peripheral vasomotor function and evaporative heat loss may alleviate some of the decrease in dry matter intake and thus milk production.

Niacin, nicotinic acid, or vitamin $B_3$ induces vasodilatation at the skin and this increases heat loss at the periphery (Di Constanzo et al., 1997). The vasodilatory effects of niacin act through prostaglandin D production by epidermal Langherhans cells (Benyo et al., 2006; Maciejewski et al., 2006) and vascular endothelial prostaglandin D2 receptors (Cheng et al., 2006). Indeed, skin temperatures are decreased during periods of

mild to severe heat stress in cows supplemented with 12, 24, or 36 g of raw niacin (Di Constanza et al., 1997). Past research evaluating niacin supplementation during heat stress has utilized raw niacin which presumably would largely be metabolized by rumen microbes (Campbell et al., 1994). Previous research (Miller et al., 1986; Zinn et al., 1987; Santschi et al., 2005) has demonstrated that very little (3-10%) niacin or nicotinamide escape ruminal degradation. Lipid encapsulation has been used for many years to coat and 'protect' bioactive substances from rumen degradation, with advances in the technology used more recently to protect choline. Kung et al. (2003) reported a high level of in vitro rumen protection with lipid encapsulated choline (> 70%). Deuchler et al. (1998) observed increased milk choline when supplementing lipid encapsulated choline to lactating dairy cows, indicating extensive rumen bypass and intestinal choline release. Using the same coating technology, a niacin product has been developed that allows niacin to be protected from rumen degradation (> 90%) and released in the small intestine (Balchem Corporation, New Hampton, NY). Encapsulation technology can dramatically increase the bioavailability of compounds like niacin to the small intestine (Deuschler et al., 1998). The effects of feeding encapsulated niacin during thermal stress have not been evaluated, but we hypothesized that if niacin is "rumen protected" then more would be bioavailable and thus produce a greater vasodilatory response. We hypothesize that this would then lead to improved heat loss in cattle fed encapsulated niacin.

Study objectives were to: 1) determine if supplementing encapsulated niacin to lactating dairy cows increased free plasma niacin concentrations and 2) to determine if

niacin supplementation altered EVHL and core body temperature indices during moderate thermal stress.

## MATERIALS AND METHODS

### *Animals*

Twelve multiparous Holstein cows producing an average of 31 kg/d (± 4.75 kg) and balanced for parity (2 +1) and stage of lactation (DIM 145 ± 9) were housed in individual tie stalls in one of two environmentally controlled chambers in the William Parker Agricultural Research Center at the University of Arizona. After 4 d of adjusting to the chambers, cows entered period (**P**) one of the experiment (P1), which lasted 7 d and consisted of thermal neutral conditions (**TN**; THI<72 for 24 h/d). After P1, cows entered P2 which lasted for 7 d and consisted of a moderate thermal stressful environment (THI >72 for 12 out of 24 h/d). At the beginning of P1, six cows (3 in each chamber) were randomly assigned to receive either 0 g encapsulated niacin per cow/d (C) or 12 g encapsulated niacin per cow/d (**SU**; NIASHURE®; Balchem Corp, New Hampton, NY) and all cows remained on dietary treatment until the end of P2. The form in which niacin was supplemented was encapsulated and therefore only 68% pure niacin resulting in an actual dosage of 8.2 g of raw niacin per day. Encapsulated niacin was top dressed by suspending it in molasses (50 mL) and pouring on individual feed buckets and mixed by hand. During P1, the THI pattern never exceeded 72 while during P2 the circadian temperature induced moderate heat stress (THI exceeded 72 for 12 h/d; Figure 1). Relative humidity was held constant at 18% during both periods and THI changes were achieved through ambient temperature alterations. Milk yields were measured

twice daily and sampled once a day in the morning for composition analysis conducted at Arizona DHIA Tempe, AZ. Milk fat, protein, and lactose were analyzed using AOAC approved infrared analysis (AOAC, 2000); SCC was analyzed using AOAC approved cell-staining techniques (AOAC, 2000). The International Dairy Federation and Food and Drug Administration (FDA) certified all equipment used in the analyses. Milk yield was recorded at each milking and combined for daily milk yield data. Cumulative water intake was recorded daily. Cows were fed twice/d and refusal was measured once/d.

***Ambient temperature (AT), THI, RR, ST, EVHL, and BW***

Data loggers continuously recorded ambient temperature; relative humidity and black globe temperature at 15 min intervals each day in both environmental chambers using a computer based program (PARC Control Coding™, Copy write 2003-2008, John R. Bauer, LLC.).

***Animal Measurements***

Core body temperatures indices were recorded 4 times/d at the following locations: **ST** of both **SH** and **US** areas were obtained at the rump, **ST-R-SH, ST-R-US** shoulder, (**ST-S-SH, ST-S-US**, and tail head **ST-T-SH, ST-T-US** using an infrared temperature gun (Raynger®MX™ model Ray MX4PU Raytek C, Santa Cruz, CA). Rectal temperatures were obtained 4 times/d using a YSI rectal thermometer (Yellow Springs Instruments, Yellow Springs, Ohio). Temperature loggers (ibutton thermochrons, Maxim Dallas Semiconductor, TX) were used as a means to record core body temperature circadian patterns. The manufacturer reports a measurable range of +15

to +46°C measuring 1/8°C increments with ±1°C accuracy. The temperature loggers were also calibrated in our laboratory -ibutton thermochrons which were used in the study were placed in one of two incubators set at 38.5 or 42°C for 24 h allowed to sit at room temperature for 24 h and then placed in the opposite incubator for 24 h. During those times the ibuttons were measuring and recording temperatures inside the incubator. The data was then extracted into excel files and analyzed for variance. Based on these analyses we found the variability to be 38.5 ± 0.70°C and 42 ± 0.40°C. The mean offset for each thermochron button was found to be consistent from one calibration to the next and was subsequently used as a co-variate when vaginal temperature measurements were analyzed. The calibrated ibuttons were then attached to blank continuous intravaginal drug release devices (CIDR's, Pfizer Inc., New York, NY) and inserted in to the vagina of the animal on the d 4 of and removed on d 7 of P1 and P2. To measure insensible heat loss, respiration rates (**RR**) were obtained by visually counting flank movements during a 15 s interval and multiplying by 4 and evaporative heat loss (**EVHL**) of the shoulder shaved (**EVHL-SH**) and unshaved (**EVHL-US**) areas were also measured four times/d using an evapometer (Delfin Technologies, LTD., Finland). Total stored heat was calculated using the formula: body temperature, °C x specific heat of tissue, (0.8°C) x body weight, kg) (Sawka and Castellani, 2007; Silanikove, 2000). These measures were then summed across days and cows and divided by total animal numbers and days in a period to obtain average total stored heat per group. Average metabolic rate (basal metabolism plus milk energy) was calculated using the formula: 70.5 x (body weight, kg) $^{0.734}$ + (milk yield, kg x 750 kcal/kg) (Kibler and Brody, 1944). Average total metabolic

rate/kg was then calculated by summing daily averages of all cows for each period (TN and HS) and dividing by number of cows, cow weights and days.

Blood samples were collected into heparanized tubes using coccygeal venipuncture from individual cows at 1200 h one day prior to start of SU, and d 1 and 7 of P1 and P2. Plasma was then harvested after centrifugation and then stored at -20 ºC until analysis. Cows were weighed 3 times at the beginning of the study, middle of the study, and end of the study.

***Free serum niacin concentrations***

Plasma harvested from blood samples obtained at 1200 h was split into two aliquots and frozen at -20°C for later analysis of niacin concentrations using the VitaFast® (R-Biopharm, Dharmstadt, Germany) Niacin microbiological assay. Preparation of the plasma samples for assay involved pipetting 1.0 mL plasma into a sterile 50 mL conical centrifuge tube (Greiner Bio-one, 227261, Monroe, NC) followed by the addition of 20 mL sterile 20 mM sodium citrate buffer pH 4.5 (Sigma Aldrich, C0909 , St. Louis, MO) to the plasma which was then manually shaken. A total of 300 mg of taka diastase (Sigma Aldrich, Fluka, 86247, St. Louis, MO) was then added to the tube and shaken vigorously and then incubated for 1 h in an incubator without light at 37°C. The samples were then removed from the incubator and sterile dd$H_2O$ was added to the 40 mL mark on each tube. The tubes were then heated for 30 min in a water bath at 95°C, and shaken well every 5 min. Following heating, tubes were then chilled quickly to below 30°C using an ice bath. The tubes were then centrifuged and the supernatant was decanted into sterile tubes. Three separate 150 uL samples were then pipetted into

individual wells of a 96 well microtitre plate. The well of each plate was also coated with Lactobacillus plantarum. The plate is then incubated in the dark for 44-48 h at 37 ºC. Growth of the Lactobacillus is enhanced as niacin concentration in the media is increased. The assay is read spectrophometrically for turbidity at 610-630 nm. The sensitivity limit of the assay is 160 ng/mL. The standard curve ranged from 160 ng/mL – 1.60 μg/mL. All samples were run in a single assay with an intraassay coefficient of variation of <1.5%.

***Statistics***

Data was analyzed using ANOVA procedures of SAS (SAS, 1999). Milk yields and DMI (recorded during the acclimation period and prior to treatment or environment initiation) were included as a covariate in the analysis. Dependent variables tested were milk yield, DMI, ST (rump, shoulder, tail head, shaved and unshaved areas), EVHL, RR, core body temperature, SNF, lactose, fat, protein, SCC, and water intake. The independent variables included treatment, day, parity, time of day, room, period, and the respective interactions. The level of significance was set at $P < 0.05$ for all main effects and interactions and the LSMEANS test was conducted when significance was detected.

## RESULTS

Plasma niacin concentrations were measured through out the study. Prior to experiment initiation plasma niacin levels did not differ between treatments (1.32 vs. 1.38 μg/mL, for C and SU, respectively). By the end of P1, plasma niacin concentrations are higher in animals supplemented with encapsulated niacin compared to the C (1.75 vs.

1.50 µg/mL, $P<0.03$). Plasma niacin remained elevated in SU cows compared to the controls throughout period 2 (1.65 vs. 1.44 µg/mL, $P<0.03$).

Environmental conditions controlled during this study were thermoneutral (P1) and mild to moderate heat stress (P2; Figure 1). During P1 the minimum THI was 52 and the maximum was 66 (Figure 1). During P2 the minimum THI was 67 while the maximum was 79 which resulted in an environment where the THI was greater than 72 over 14 h/d (Figure 1). Relative humidity was kept constant at 18% for both periods throughout each day (Figure 1).

Surface temperatures obtained from the shoulder, rump and tail head were unaffected by SU but were altered by shaving (32.5 SH vs. 31.4 US °C, Table 3). All ST in both groups were higher in P2 compared to P1 (Table 3). Cows fed SU had higher EVHL (57.4 vs. 52.7 g/m²/h, EVHL-U, $P<0.05$, Table 3) or were numerically higher, (66.3 vs. 57.8 g/m²/h, EVHL-SH, $P=0.11$) over the entire 24 h period. Furthermore, these differences became larger during peak thermal stress. The EVHL for SU fed cows were higher than C (81.1 vs. 68.2 g/m²/h, $P<0.0001$) during P2 between 1100 and 1600 h. The SU cows had lower average RT during P2 compared to C fed cows (38.17 vs. 38.34°C; Table 1) and lower mean vaginal temperatures for the 72 h data collection, from d 4 thru d 7,(38.0 vs. 38.4°C; $P<0.001$). Respiration rates tended to be higher for SU cows compared to C fed cows during both P1 and P2 ($P = 0.14$; Table 1). There was also a SU by period interaction when C and SU groups had higher respiration rates than C cows during P2, (30.6 vs. 50.8 and 32.5 vs. 54.5 bpm; $P<0.0001$; Table 1). Average RT tended ($P = 0.07$) to be lower for cows fed SU compared to C during P2 (38.17 vs.

38.34°C; Table 1). Also, during P1, cows in the SU group had higher average RT than C (38.06 vs. 38.01°C; $P = 0.05$; Table 1). Average stored body heat was greater for cows fed SU compared to the C during both P1 and P2 (Table 4). This was an artifact produced by the coincidental greater body weight of SU cows. When stored heat was calculated per kg of body weight total stored heat was lower throughout the entire study when cows were in the SU group (30.49 vs. 30.54 kcal/kg of BW; $P<0.05$). Overall, all cows displayed greater heat storage during P2 compared to P1 regardless of diet as expected when cows are heat stressed (30.59 vs. 30.44 kcal/kg of body weight; $P<0.01$). Cows fed SU tended to have less average heat storage than C during P2 (30.53 vs. 30.66 kcal/kg of BW; $P<0.06$). In P2 during the hottest part of the day (1400 to 1700 h), cows in the SU group had reduced heat storage compared to C (30.54 vs. 30.71 kcal/kg of BW; $P <0.05$).The metabolic rates of the two groups did not differ by treatment or period. Although there were clear signs of increased heat load in period 2 such as increased body temperature and respiration rate although there was no change in milk yield in the two groups.

Milk yield did not differ between dietary groups or periods (environments; $P = 0.17$; Table 1). Dry matter intake was not affected by diet, however DMI decreased during P2 (38.9 vs. 37.7 kg/d; $P < 0.05$, Table 1). Water intake tended to be higher for SU animals ($P = 0.11$) regardless of environment; however during P2 C and SU fed cows had higher water intakes, respectively (40.4 vs. 52.7 and 48.6 vs. 57.7 L/d; $P<0.01$, Table 1). Milk fat percentages did not differ between dietary groups ($P = 0.55$; Table 2) however during P2 SU group had a tendency for lower percentages (3.51 vs. 3.77 %; $P =$

0.06; Table 2). Milk protein percentages were lower for animals in the SU group (2.84 vs. 2.93 %; $P<0.001$; Table 2) however, during P2 protein percentages were higher for both groups (C: 2.86 vs. 2.99 and SU: 2.76 vs. 2.91 %; $P < 0.001$; Table 2). Lactose was not affected by treatment or period. Solids-not-fat was lower for cows fed encapsulated niacin (8.52 vs. 8.61 %; $P < 0.01$; Table 2). During P2 both groups also had increased solids-not-fat percentages (C: 8.67 vs. 8.55 and SU: 8.59 vs. 8.44 %; $P < 0.001$). Somatic cell counts were lower for cows in the SU group (175 vs. 980 cells/mL x 1000; $P < 0.001$; Table 2).

## DISCUSSION

Studies have shown that both nicotinamide and nicotinic acid are synthesized in the rumen (Santschi et al., 2005). Researchers feeding three diets found that duodenal niacin concentrations were greater than the amount in their intakes (Miller et al., 1986). Other researchers have not found the same results on ruminal escape of niacin (Campbell et al., 2004). When free nicotinamide was supplemented to cattle its disappearance was almost complete prior to the duodenal cannula (Santschi et al., 2005; Zinn et al., 1987). Absorption through the ruminal wall is also a plausible as when nicotinamide is supplemented the levels of free niacin were increased but the extent was minimal (Santschi et al., 2005). However, these investigators reported that minimal niacin escaped ruminal degradation therefore the benefits reported when niacin has been fed are likely due to its effects in the rumen or the diffusion across the gastrointestinal wall prior to the duodenal cannula (Santschi et al., 2005). Researchers have also found that nicotinamide is absorbed through the rumen wall at a rate of 0.98 g/h and nicotinic acid

was not absorbed during a 1-hour period (Erickson et al., 1992). It has also been reported that when large quantities of niacin in their free form are present absorption through the rumen wall can occur (Rérat et al., 1958b). When nicotinamide was infused post-ruminally duodenal flows of nicotinamide were not increased however, duodenal nicotinic acid concentration was increased (Santschi et al., 2005). This implies there is rapid conversion of nicotinamide to nicotinic acid however; this might be occurring in the abomasums where it is more an acidic environment (Santschi et al., 2005). The plasma niacin data from this study supports the concept that encapsulation of niacin would improve rumen bypass and lead to increased blood niacin concentrations. Since the majority of niacin leaving the intestine and entering the vascular system is rapidly taken up by red blood cells (Klein et al. 1942) it is interesting that we were able to detect a significant increase in plasma niacin. Niacin in blood is stored as pyridine nucleotides (Levitas et al., 1947). We did not measure blood pyridine nucleotide content in this study which may have resulted in greater nicotinamide differences between supplemented and control animals. Future studies will need to address this question.

The NRC (2001) estimated the synthesis and absorption of niacin from the small intestine and reported that 1,804 mg/d are synthesized in the rumen with an escape of 6%. Santschi et al., (2005) reported the disappearance of niacin as nicotinamide to be 98.5% prior to the small intestine. Although the percent of actual niacin found in the encapsulated product is only 65% compared to 100% with raw niacin; the rumen stability is 80% more and the bioavailability of niacin is 35% more than raw niacin (Santschi et al., 2005; Deuschler et al., 1998).

No previous studies have evaluated use of supplementary dietary encapsulated niacin on evaporative heat loss and heat storage in lactating dairy cows. Previous studies in heat stress models have involved supplementing niacin in a raw form that is not encapsulated and have resulted in inconclusive results. Additionally, very few studies have reported plasma serum concentrations or whole blood concentrations of niacin in cattle supplemented with niacin (Jaster et al. 1983, Campbell et al.., 1994). Data from the analysis of plasma free nicotinic acid and nicotinamide in this study indicate that feeding encapsulated niacin increased plasma concentrations of niacin while cows were being supplemented.

The majority of niacin absorbed across the gut wall in cattle is rapidly incorporated and stored in red blood cells (Campbell et al., 1994). However, there is measurable free niacin in blood (Campbell et al., 1994). At the start of the study SU and C fed cows free plasma niacin levels did not differ. Plasma niacin concentrations in this study were in agreement with previously reported values for lactating dairy cattle (Jaster et al., 1983; Campbell et al. 1994). Supplementing encapsulated niacin increased plasma levels of free niacin during both TN and HS periods. Serum niacin concentration levels in SU fed cows returned to pre-supplementation values by 3 d post supplementation (1.51 vs. 1.50 µg/ml). Thus, feeding encapsulated niacin at a dose of 12 g/cow/d increased free plasma niacin concentration. It is possible that measuring total blood niacin would provide greater differences in niacin concentrations between SU and C animals since the majority of niacin in blood is stored in red blood cells (Campbell et al. 1994).

Environments implemented during this study were considered thermoneutral and mild to moderate HS. The HS environment caused a 0.5° C rise in vaginal temperature in C fed cows but no change in vaginal temperature in SU fed cows. Average RT during HS were also reduced by 0.17°C in TRT compared to C fed cows. Although the HS environment altered RT and RR there was no apparent effect on milk yield of the cows. This study was conducted in September and the cows utilized were well adapted to the summer heat in Arizona. It is possible that winter adapted cattle would have been more severely stressed.

It is also apparent that removal of the hair coat increased EVHL in both C and SU groups. This is likely due to increased airflow over the skin surface in shaved areas due to hair removal (Gebremedhin and Wu, 2001). The increases in EVHL in SU cows appears to be associated with increased EVHL, via increased sweating rate since it was most apparent in unshaved areas of the hair coat. Vaginal probes were inserted during days 4-7 of each period to record circadian patterns of core body temperatures. During P1 there were no treatment differences in core body temperature patterns however during P2 SU cows had significantly lower core body temperatures than C fed cows (Figure 2). This is further supported by the increased EVHL in SU cows. The vasodilatory effects of niacin have been shown by others to act through prostaglandin D production by epidermal Langherhans cells (Benyo et al., 2006; Maciejewski et al., 2006) and vascular endothelial prostaglandin D2 receptors (Cheng et al., 2006). Niacin has been known to induce vasodilation of the skin in humans (Gille et al., 2008); this mechanism appears to

be associated with the reduction in core body temperature and increases in EVHL during P2 in this study

During both periods, water intake was greater for the SU cows (Table 1). This is possibly related to a numerically higher milk yield and increased insensible heat loss through increased respiratory and EVHL in the SU cows experiencing HS during P2. However, since urinary water loss was not measured it is not possible to be definitive regarding the reason for increased water intake other than to state that it is associated with higher EVHL in TRT cows.

During summer months environmental conditions (such as those implemented in our experiment), can cause heat stress resulting in enhanced heat storage in dairy cows. This results in a reduction in dry matter intake which contributes to a decrease in milk yield. However, others have recently estimated that only 50% of the loss in milk yield during thermal stress is related to a reduced dry matter intake (Wheelock et al., 2006; Rhoads et al., 2007). Thus, increased heat storage must also be associated with changes in whole body and mammary metabolism (Rhoads et al., 2007; Wheelock et al., 2006). Therefore, alleviating some or all of the increased heat storage may reduce the effects of heat stress on lactating dairy cows.

There were no differences in DMI between dietary treatments which agrees with previous nicotinic acid or nicotinamide research (Kung et al., 1980; Jaster and Ward, 1990). Heat stress tended to decrease DMI in both C and SU cows. Milk yield was numerically higher in SU compared to C however; these differences existed prior to study initiation and were unexpected since the groups were balanced for parity and yield during

treatment assignment. Again, supplementing with raw niacin has resulted in inconclusive results where some have found increases (Muller et al., 1986; Drackley et al., 1998) in milk yield others have reported no difference (Di Constanzo et al., 1997; Madison-Anderson et al., 1997). Milk component differences found in this study were most likely attributed to the existing milk yield difference prior to the start of the study or small number of animals (n=12) used in the study rather than actual dietary effects.

A previous study did not detect a difference in rectal temperature, or respiration rates in cattle fed raw niacin during thermal stress (Di Constanzo et al., 1997). However, the niacin used in the study was not encapsulated, the time of day temperatures were recorded varied and no other study has reported continuous circadian patterns of core body temperature measurement in cattle fed encapsulated niacin. Some studies have reported a decrease in ST at different times throughout the day and have yet to be consistent throughout all studies (Di Constanza et al., 1997). Also, previous studies were conducted using raw or non-encapsulated products of niacin, nicotinic acid, or nicotinamide (Jaster and Ward, 1990; Kung et al., 1980; Di Constanzo et al, 1997; Madison-Anderson et al., 1997) Therefore, the results from our trial are unique and suggest more research should be conducted on evaluating encapsulated niacin affects on alleviating heat stress.

## CONCLUSION

The supplementation of encapsulated niacin to thermally stressed lactating dairy cows increased EVHL and this was associated with increased water intake, decreased RT, vaginal temperatures and RR The number of cows (12) utilized in this study precluded

any serious analysis of milk production responses such as Mueller et al. (1986) which is the only large scale study with 240 animals reported to date. Further studies evaluating the impact of encapsulated niacin on lactating dairy cows during thermal stress are warranted.

## AKNOWLEDGMENTS

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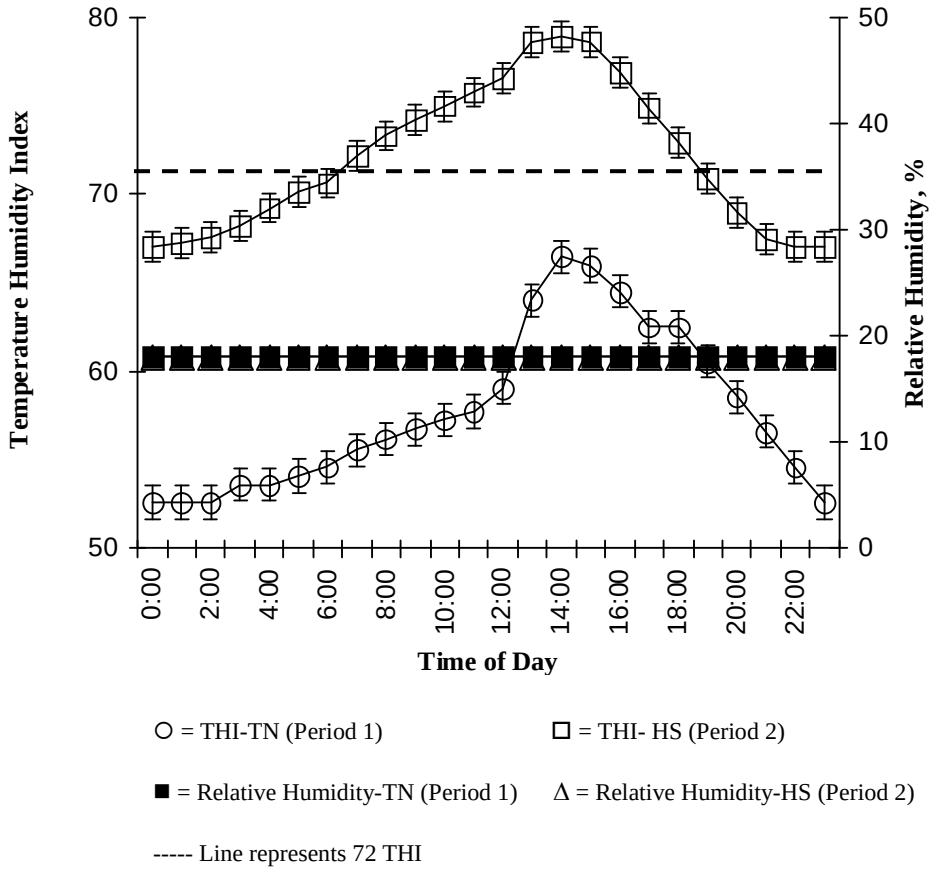


**Figure 3.1.** 24 hour circadian patterns for thermoneutral (TN, Period 1) and heat stressed (HS, Period 2) periods.

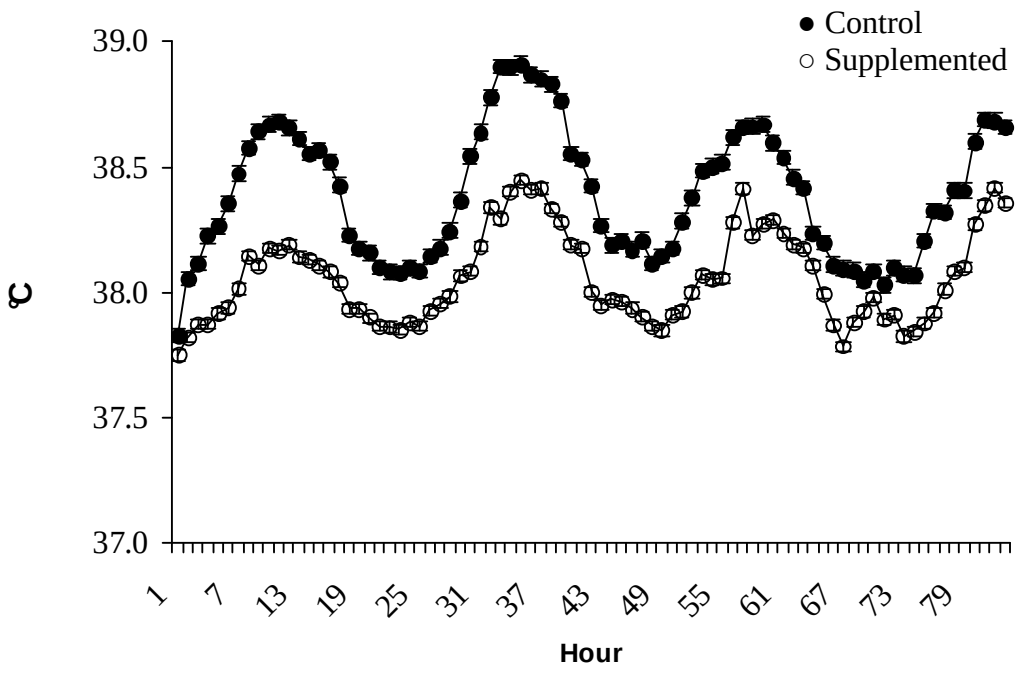


**Figure 3.2.** Core body temperatures during period 2 (heat stress) from day 4 to day 7 period. Treatment: $P < 0.001$; SEM = 0.04.

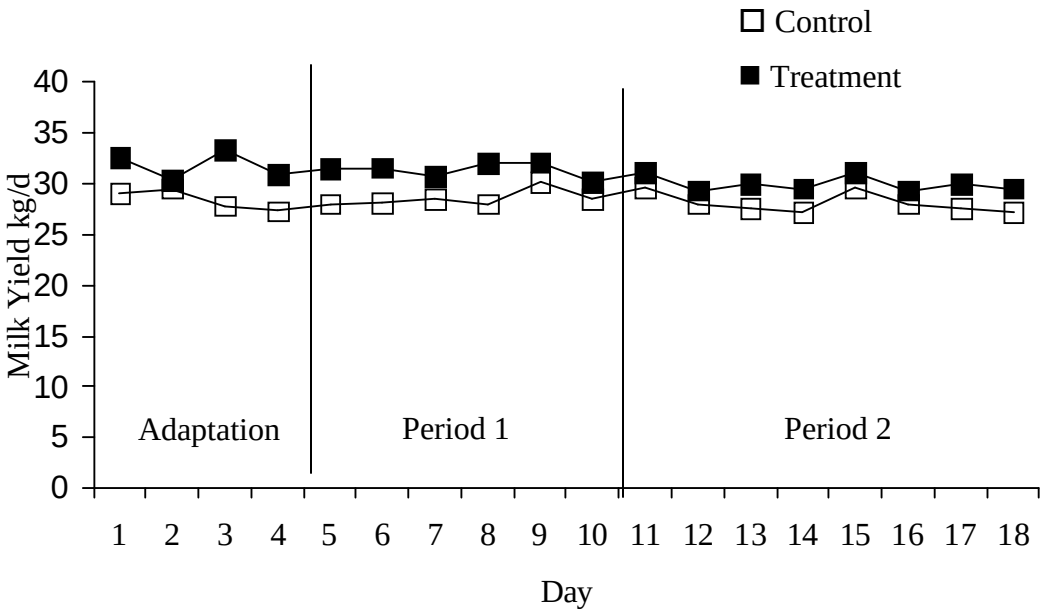


**Figure 3.3.** Temporal pattern of milk yield during adaptation, period 1 (thermoneutral) and 2 (heat stress). *P* = 0.14; SEM = 0.34

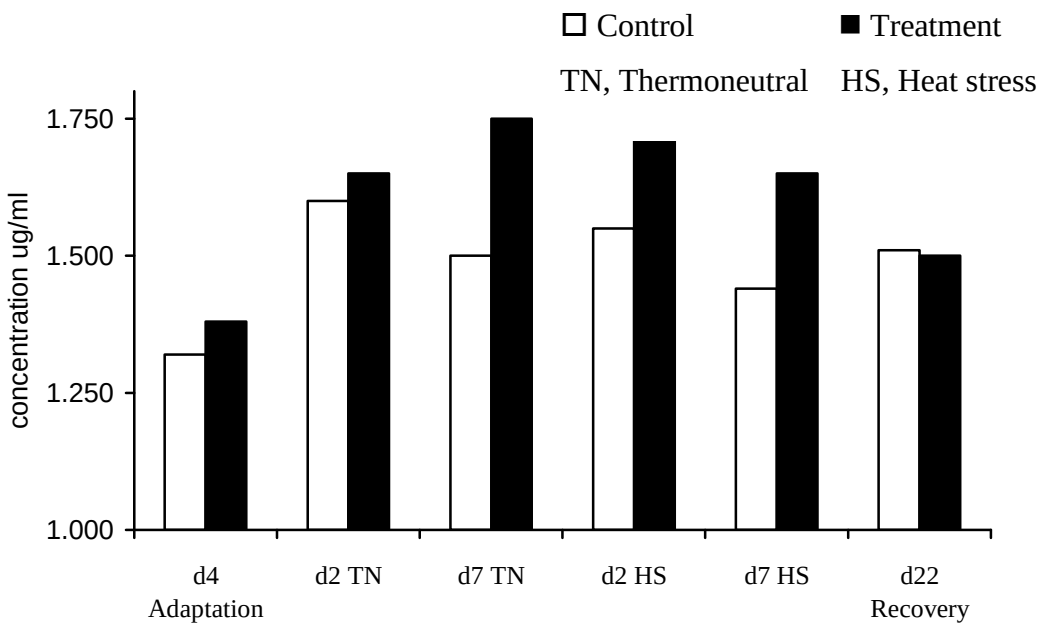


**Figure 3.4.** Plasma serum concentrations of free niacin of animals supplemented with 0 g (Control) or 12 g of encapsulated niacin (Treatment).

Table 3.1. Summary of the dry matter intake, water intake, respiration rate, and milk yield.

| | Period 1 | | Period 2 | | Diet | | Period | | Diet*Period | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | C[1] | SU[2] | C | SU | SEM | *P* | SEM | *P* | SEM | *P* |
| Dry matter intake, kg/d | 39.1 | 38.7 | 38.8 | 36.7 | 1.69 | 0.69 | 1.74 | 0.05 | 1.75 | 0.14 |
| Water intake, L/d | 40.4 | 52.7 | 48.6 | 57.7 | 1.01 | 0.11 | 0.68 | <0.01 | 0.64 | 0.45 |
| Respiration rate, bpm | 30.6 | 32.5 | 50.8 | 54.5 | 1.78 | 0.14 | 2.12 | <0.001 | 2.12 | 0.59 |
| Rectal temperatures, °C | 38.01 | 38.06 | 38.34 | 38.17 | 0.06 | 0.05 | 0.07 | <0.001 | 0.08 | 0.07 |
| Milk yield, kg/d | 29.3 | 30.5 | 29.4 | 29.6 | 0.35 | 0.17 | 0.32 | 0.35 | 0.47 | 0.25 |

[1] C= Control (0g Niashure™)
[2] SU = Treatment (12g Niashure™)

**Table 3.2**. Summary milk composition analysis.

| | Period 1 | | Period 2 | | Diet | | Period | | Diet*Period | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | C[1] | SU[2] | C | SU | SEM | *P* | SEM | *P* | SEM | *P* |
| Fat, % | 3.94 | 4.00 | 3.77 | 3.51 | 0.16 | 0.55 | 0.16 | 0.06 | 0.20 | 0.36 |
| Protein, % | 2.86 | 2.76 | 2.99 | 2.91 | 0.02 | <0.001 | 0.02 | <0.001 | 0.03 | 0.77 |
| Lactose, % | 4.68 | 4.66 | 4.69 | 4.69 | 0.03 | 0.66 | 0.03 | 0.62 | 0.04 | 0.75 |
| Solids-not-fat, % | 8.55 | 8.44 | 8.67 | 8.59 | 0.02 | <0.01 | 0.02 | <0.001 | 0.03 | 0.71 |
| Somatic cell count, cells/mL x 1000 | 1210 | 249 | 749 | 101 | 149 | <0.001 | 149 | 0.15 | 210 | 0.46 |

[1] C= Control (0g Niashure™)
[2] SU = Treatment (12g Niashure™)

**Table 3.3**. Surface temperatures and evaporative heat loss (EVHL) for shaved and unshaved areas.

| | Period 1 | | Period 2 | | Diet | | Period | | Diet*Period | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | C[1] | SU[2] | C | SU | SEM | *P* | SEM | *P* | SEM | *P* |
| Surface temperature, °C | | | | | | | | | | |
| Shoulder, shaved | 31.3 | 30.9 | 34.3 | 34.1 | 0.18 | 0.62 | 0.18 | <0.01 | 0.24 | 0.88 |
| Shoulder, non-shaved | 29.9 | 29.6 | 33.4 | 33.6 | 0.21 | 0.32 | 0.20 | <0.001 | 0.26 | 0.23 |
| Rump, shaved | 31.4 | 31.3 | 34.5 | 34.5 | 0.16 | 0.85 | 0.17 | 0.51 | 0.23 | 0.74 |
| Rump, non-shaved | 30.4 | 30.3 | 33.8 | 33.7 | 0.24 | 0.92 | 0.21 | <0.01 | 0.28 | 0.97 |
| Tail head, shaved | 30.5 | 30.7 | 33.4 | 33.7 | 0.19 | 0.18 | 0.19 | <0.05 | 0.26 | 0.89 |
| Tail head, non-shaved | 28.4 | 28.5 | 32.8 | 32.6 | 0.28 | 0.93 | 0.26 | <0.001 | 0.34 | 0.65 |
| EVHL[3] | | | | | | | | | | |
| Shaved, $g/m^2/h$ | 23.2 | 18.3 | 92.4 | 114.4 | 5.30 | 0.11 | 5.80 | <0.001 | 7.53 | 0.07 |
| Non-shaved[4], $g/m^2/h$ | 18.2 | 13.1 | 87.2 | 101.7 | 4.93 | <0.05 | 4.79 | <0.001 | 6.34 | 0.08 |

[1] C= Control (0g Niashure™)
[2] SU = Treatment (12g Niashure™)
[3] Closed chamber evapometer
[4] EVHL average for all 4 measurement times

**Table 3.4**. Effects of encapsulated niacin on physiological and metabolic parameters.

| | Period 1 | | Period 2 | | Diet | | Period | | Diet*Period | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | C[1] | SU[2] | C | SU | SEM | *P* | SEM | *P* | SEM | *P* |
| Average weight[3], kg | 594 | 603 | ——— | ——— | ——— | ——— | ——— | ——— | ——— | ——— |
| Average surface area[3],m[2] | 5.54 | 5.58 | ——— | ——— | ——— | ——— | ——— | ——— | ——— | ——— |
| Metabolic rate[4], kcal/d | 28325 | 30540 | 29544 | 31517 | 177.59 | 0.05 | 282.54 | <0.05 | 399.57 | 0.81 |
| Metabolic rate, kcal/kg of body weight | 47.68 | 50.65 | 49.73 | 52.27 | 0.30 | 0.05 | 0.47 | <0.05 | 0.67 | 0.81 |
| Total stored heat[5], kcal/d | 18073 | 18361 | 18223 | 18405 | 507.47 | 0.92 | 359.24 | <0.0001 | 507.88 | <0.10 |
| Total stored heat, kcal/kg of body weight | 30.42 | 30.45 | 30.68 | 30.52 | 0.85 | 0.92 | 0.60 | <0.0001 | 0.85 | <0.10 |
| Mean body temperature[6], °C | 35.70 | 35.70 | 36.90 | 36.80 | 0.07 | 0.45 | 0.07 | <0.0001 | 0.09 | 0.43 |
| EVHL kcal/d | | | | | | | | | | |
| Shaved | 74931 | 60216 | 297170 | 370975 | 19843 | 0.11 | 20037 | <0.001 | 26298 | <0.10 |
| Non-shaved[7] | 58843 | 42965 | 279974 | 330847 | 20728 | 0.05 | 18106 | <0.001 | 24375 | <0.10 |

[1] C= Control (0g Niashure™)
[2] SU = Treatment (12g Niashure™)
[3] over entire trial, not separated by period
[4] $=70.5(\text{BW, kg})^{0.734}$ + (MY, kg*750 kcal/kg)
[5] =Body temperature,°C*Specific heat of tissue(0.8)*Body weight, kg
[6] =0.33*Temperature of skin, °C +0.67*rectal temperature, °C
[7] EVHL average for all 4 measurement times

# CHAPTER FOUR: EFFECT OF NIACIN AND PROSTAGLANDIN A, D, AND E ON HEAT SHOCK PROTEIN GENE EXPRESSION IN BOVINE MAMMARY EPITHELIAL CELLS IN VITRO[1]

## ABSTRACT

Niacin induces peripheral vasodilatation via prostaglandins D (PGD), and E (PGE) release by Langerhans cells in skin. We evaluated if niacin alone or in combination with, PGD and PGE alters expression of heat shock proteins (Hsp) 27 and 70. Bovine mammary epithelial cells (BMEC) were cast in collagen in 24-well plates containing growth media (GM) composed of DMEM/F-12, insulin, EGF, IGF-I, BSA and antibiotics at 37°C, 5% $CO_2$. Cultures grew into ductal structures with media changes at 48 h intervals. On d 8 cultures were divided into controls (C) receiving GM, GM with niacin (0.5, 1.0, or 10.0 mM), $PGD_2$ (10 or 24 uM), $PGD_2$ with $PGE_1$ (both at 24 uM) alone or in combination with niacin. Half were placed into incubators at 37°C (TN) and the remainder at 42°C (HS) for 8 h. At 0 h and 8 h, replicates were pooled, placed in TRIzol and stored at –80°C until extracted for RNA. Expression of HSP's-27 and 70 was measured by q- PCR. Addition of PGD at both individual 10 and 24 uM doses and in combination with niacin increased Hsp-27 and 70 gene expression in HS, ($P<.0001$). Peak fold increases in Hsp-70 expression at 8 h over time zero differed between C and PGD, (–2.4 vs. 9.3, $P<.0001$) and were greater for Hsp-27 (–115 vs. +10, $P<.0001$). Addition of PGE increased Hsp-27 and 70 expression compared to C and PGD alone ($P<.05$). We conclude that niacin with PGD or PGD+PGE alters Hsp-27 and Hsp-70 gene expression in BMEC during HS

## INTRODUCTION

Niacin, nicotinic acid, is a potential nutritional management supplement for heat stressed cattle because it induces vasodilation and sweating rate in cattle, therefore transferring more body heat to the periphery (Di Constanza et al., 1997, Zimbelman et al., 2007). The vasodilatory effects of niacin have been shown to act through prostaglandin D (PGD) and E (PGE) production by epidermal Langerhans cells ( Maciejewski et al., 2006 and Benyo et al., 2006) acting on vascular endothelial prostaglandin $D_2$ receptors, (Cheng et al., 2006). The general scheme for prostaglandin synthesis is shown in Figure 1 below.

Current data indicates that niacin induces increased Prostaglandin D and E synthesis through activation of cyclooxygenase 1 (PGH synthase) and Prostaglandin D and E synthases in immune cells such as Langerhans cells and macrophages (Benyo et al.,2005, Meyers et al., 2006, Kamanna and Kashyap, 2008). The prostaglandins then act on their specific receptors in vascular endothelial cells to induce vasodilation (Benyo et al., 2006, Meyers et al., 2006).

In addition, these prostaglandins (cyclopentenones) such as PGA, PGD or its immediate metabolite $15dPGJ_2$ activate heat shock factor I and induce the synthesis of Hsp's in a variety of mammalian cells (Amici et al., 1992, Santoro, 2000, Kozawa et al., 2001, Ianoro et al., 2003).

It has further been demonstrated that the cyclopentenone ring structure itself, 2-cyclopenten-1-one, specifically induces the expression of Hsp 70 through activation of HSF-1 in human erythrocytes (Rossi et al., 1996).

Previous studies using bovine mammary epithelial cells (BMEC) have shown that exposure of BMEC to heat stress (42°C) over a 24 h period resulted in regression of ductal branches and a decrease in cell growth; along with a reduction in gene expression for genes that are known to be associated with protein synthesis and metabolism of the cell (Collier et al., 2006). In this study, they reported an elevation of Hsp 70 gene expression in BMEC for 4 h at 42°C prior to returning to basal levels 8 h later, Figure 2, (Steining, 2005). Thermotolerance at the cellular level is defined as the ability of a cell to maintain elevated Hsp production in the face of a thermal stress. When this ability is lost the cell is described as having lost thermotolerance and gene expression is shifted to proteins associated with apoptosis (Collier et al. 2006). The results of Stiening (2005) are in agreement with this statement. After 8 h of heat shock when expression of the gene coding for Hsp 70 had dropped back to baseline values there was a dramatic up-regulation in genes associated with apoptosis or cell death and a down-regulation of genes associated with cellular growth and metabolism. Therefore, it was of interest to determine if niacin, alone or in combination of prostaglandins A and E could up-regulate Hsp gene expression and improve thermotolerance of BMEC exposed to thermal shock. A prior study had shown that the addition of $PGA_1$ another member of the cyclopentenone prostaglandin family to cell culture media increased thermotolerance of

BMEC in part by increasing Hsp 70 gene expression 150-fold over controls during an 8 h period at 42°C (Collier et al., 2007).

The objective of this study was to determine if PGD and niacin alone or in combination increases Hsp 70 or Hsp 27 gene expression in bovine mammary epithelial cells in vitro.

## MATERIALS AND METHODS

### *Cell Culture and Sampling*

Tissue dissociation, BMEC isolation, and preparation of Type I collagen were performed according to Collier et al., (2006). Cell isolates were thawed, resuspended in DMEM/F-12, mixed with neutralized collagen and cultured in 24-well plates as described (Collier et al. 2006). Stock collagen was neutralized on ice using 0.75 *N* NaOH. A base layer (300µL/well) of neutralized collagen was added to a 24-well tissue culture plate (Falcon, BD Biosciences, San Jose, CA) and allowed to gel at room temperature for 5 minutes. The resuspended cells were then added to the remaining neutralized collagen and a 500-µL collagen-cell suspension was seeded directly onto the base layer of each well. Collagen was then allowed to gel for 20 to 30 minutes at 37°C; then appropriate medium was added to each well; and plates were then placed in an incubator at 37°C, 5% $CO_2$ in air.

Cultures initially received a serum-free basal medium consisting of DMEM/F-12, 0.1% BSA, antibiotic-antimycotic (100 U/ml penicillin, 100 ug/ml streptomycin, and 0.25 ug amphotericin B; 15240, Invitrogen Corp, Carlsbad, CA). The growth factors

(recombinant human **IGF-1**, 100 ng/ml; A.F. Parlow, NIDDK, Torrance, CA) and recombinant-human epidermal growth factor (**EGF**, 25 ng/ml; 13247-051, Invitrogen Corp.) were included to induce proliferation and ductal development. Media was exchanged every 48 h for 7 d. A total of six plates were used with each sample in six wells. Two wells were pooled to provide one RNA sample; therefore triplicates were formed for RNA extraction. Triplicates were then pooled to give one value for analysis. On d 8 treatment groups were divided into: Controls (C) receiving GM, GM with niacin (0.5, 1.0, or 10.0 mM), and $PGD_2$ (10 or 24 uM), $PGD_2$ with $PGE_1$ (both at 24uM) alone or in combination with niacin. Half of the cultures were incubated at 37°C (TN) and the remainder at 42°C (HS) for 8 h.

At 0 h and 8 h, 3 replicates were pooled, placed in TRIzol and stored at -80°C until extracted for RNA. Isolated RNA was reverse transcribed into cDNA. Expression of Hsp's-70 and 27 was measured by real time Polymerase Chain Reaction (q-PCR)**.**

***Sample Preparation***

Total RNA was isolated from cell culture samples using TRIzol Reagent (Invitrogen). For precipitation, half of the recommended volume of isopropanol was used, with the other half being replaced with a salt solution (0.8 *M* sodium citrate, 1.2 *M* NaCl). The RNA concentration, purity and integrity was confirmed on the 2100 BioAnalyzer (Agilent Technologies, Palo Alto, CA), as well as spectrophotometrically using a Nano-Drop (ND-1000; Nanodrop Technologies, Wilmington, DE).

***Real-Time Quantitative PCR***

One microgram of total RNA was DNase-treated at room temperature for 15 minutes in 10-µL reaction containing 0.5 U of DNase I (amplification grade, Invitrogen). The EDTA was added to a final concentration of 2.5 m*M*, and the DNase was inactivated at 65°C for 15 minutes. Resulting RNA was used for cDNA synthesis (20- µL reactions) using iScript cDNA synthesis Kit (BioRad, Hercules, CA). Analysis was conducted using the iCycler IQ Real-Time PCR Detection System (BioRad). Hypoxanthine phosphoriboyltransferase I (HPRT1) was used as the internal control gene following standard curve analysis across all treatment group samples [ribosomal protein (S18) and GAPDH were also evaluated]. Resulting gene expression data were calculated and analyzed based on the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

***Statistical Analyses***

Quantitative real-time reverse-transcription-PCR data using gene expression relative to the control was using PROC MIXED procedure of SAS for analysis (SAS, 9.1, 2001; SAS Institute, Cary, NC). Representation of the data is shown in graphs and is represented by expression of treatments relative to the control ($2^{-\Delta\Delta CT}$). The ΔΔCT was calculated as ΔCT of a respective treatment minus ΔCT of the control.

## RESULTS & DISCUSSION

Expression of the gene coding for Hsp 70 in BMEC was unchanged at 37°C from 0 to 8 hours (Figure 3). When BMEC in control media was switched to an environment of 42 ºC for 8 h there was no detectable change in Hsp-70 gene expression. This is misleading because as Stiening (2005) has demonstrated (Figure 2) the pattern of Hsp 70

gene expression at 42 °C is a rapid up-regulation2 and 4 h and a return to baseline values by 8 h. Thus, data from this study are in agreement with Stiening 2005. However, when niacin alone or in combination with PGD and PGE are added to the culture media there is a fold increase that ranges from 2 to 20 depending on the combination, Figure 3. The largest fold increase occurred when both PGD and PGE are added to the culture media along with niacin at a dose of 1 mM. The decrease in down regulation of Hsp 70 seen at 8 h is also associated with a down regulation of Hsp 27 and regression of ductal branching, cell growth and metabolism (Collier et al., 2006). When 0.5 mM or 1 mM niacin alone was added to the culture media containing BMECs at 37°C for 8 h here was a slight numerical decrease in Hsp 70 gene expression (-1.85 fold and -0.39 fold) . Significant increases in Hsp 70 expression were seen when 0.5 mM (2.73 fold) and 1 mM niacin (9.59 fold) was added to BMEC's at 42°C indicating that there may be a direct effect of niacin on Hsp 70 gene expression during HS. There were additive affects of 0.5 mM and 1.0 mM niacin and PGD on Hsp 70 gene expression by BMEC, Figure 3. The addition of 0.5 mM PGD alone to BMECs at both 37°C (2.70 fold) and 42°C (11.89 fold) increased up regulation of Hsp 70. Administering 1 mM PGD alone to pBMECs resulted in up regulation of Hsp 70 regardless of environment however; there was a larger response in fold expression during heat shock (42°C; 9.42 fold vs. 0.62 fold). During heat shock (42°C), the combination of 1mM niacin and PGD (14.75 fold) increased Hsp 70 expression drastically compared to 0.5 mM niacin (2.73 fold) and PGD (9.82 fold) alone or in combination (5.82 fold). The addition of PGD and PGE in combination to BMEC resulted in both up regulation of Hsp 70 regardless of environment; however,

there was a larger increase during heat shock (42°C; 20.22 fold vs. 0.30 fold). Combining both PGD and PGE with 1mM niacin (2.23 fold) resulted in up regulation of Hsp 70 at 37°C for 8 h however, when 0.5 mM niacin was added down regulation was observed (-0.19 fold). The addition of 0.5 mM niacin with PGD and PGE to BMECs at 42°C increased Hsp 70 up regulation (17.06 fold). When 1 mM niacin was added with PGD and PGE the increase in Hsp 70 expression was greater than all the other treatments during heat shock (42°C; 21.59 fold).

Heat shock protein 27 is heavily involved in development and maintenance of ductal branching and one very obvious effect of heat stress is the collapse of the cytoskeleton of BMEC and loss of ductal branching (Collier et al. 2006). Therefore, it was of interest to determine what effect niacin alone or in combination with PGD and PGE had on ductal structure. In figure 4, it is apparent that Hsp 27 is unchanged between 0 and 8 h at 37°C. However after 8 h at a temperature of 42°C for 8 h it is drastically down regulated in BMEC by -52.86 fold, Figure 4. When 0.5 mM niacin was added to the media after 8 h at 42 °C the expression of Hsp 27 is severely down regulated -174.72 fold; emphasizing the low concentration of niacin is not effective in activating Hsp 27 during heat stress, Figure 4. The addition of 24 µM PGD alone at 37°C for 8 h is as effective as adding 0.5 mM niacin at 37°C for 8 h resulting in the down regulation of Hsp 27 by -1.71 versus -1.65 fold, respectively, Figure 4. Adding 24 µM PGD along with 0.5 mM niacin at 37°C for 8 h causes the expression of Hsp 27 to less down regulated (-0.13 fold) compared to adding 0.5 mM niacin alone, Figure 4. At 42°C for 8 h Hsp 27 expression is up regulated by 7.02 fold relative to time zero. Addition of 1 mM niacin at

42°C for 8 h the expression of Hsp 27 is up regulated by 18.66 fold. When 1 mM niacin is added in combination with 24 µM PGD at 37°C for 8 h the expression of Hsp 27 is down regulated (-1.61 fold) at 42°C for 8 h results in an up regulation of Hsp 27 expression (11.08 fold). Combining 24µM PGD and 24µM PGE at 37°C for 8 h Hsp 27 expression is down regulated (-0.76 fold) and at 42°C for 8 h the expression is up regulated (9.11 fold), Figure 4. The combination of prostaglandins (9.11 fold) resulted in a response of Hsp 27 expression similar to the combination of 1 mM niacin and PGD combined however the expression was greater for the niacin and prostaglandin combination (11.08 fold), Figure 4. Thus when PGD and niacin are combined there appears to be greater synthesis and activation of Hsp 27. When 24 µM PGD, 24 µM PGE, and 0.5 mM niacin are combined at 37°C for 8 h the down regulation of Hsp 27 expression is continued to be down regulated however, at 42°C for 8 h the expression of Hsp 27 is up regulated by 17.39 fold. Raising the concentration to 1 mM niacin also promotes an up regulation (15.32 fold) of Hsp 27 expression at 42°C for 8 h however, the response is not as increased compared to 1 mM niacin alone (18.66 fold) or the combination of PGD, PGE, and 0.5 mM niacin (15.32 fold).

The next question is whether or not the increase in Hsp 27 at 42 °C in response to niacin and prostaglandins D and E results in any change in the morphology of BMEC. The histology data, Figure 5 demonstrates that as previously reported, HS causes collapse of the cytoskeleton in BMEC (Collier et al., 2006). However, addition of niacin and prostaglandins D and E preserves the cytoskeleton of BMEC, Figure 6 which is in

agreement with the increased expression of the gene coding for Hsp 27 leading to increased cytoprotection and improved cellular integrity.

The addition of PGD and PGE alone or in combination with niacin concentration appears to increase the expression of Hsp 70 and 27. Past research evaluating the effects of PGD, PGE, and niacin alone or in combination have not been reported, therefore this is the first study to evaluate their possible interactions with Hsp's. Researching the effects of PGD and PGE with niacin to Hsp 70 and 27 were justified by the vasodilation affects shown to act through the mechanisms behind these prostaglandins (Maciejewski et al., 2006; Benyo et al., 2006). If PGD and PGE are involved as shown recently in research then the up regulation of heat shock proteins seen with these prostaglandins alone or in combination is logical. The addition of niacin with PGD and PGE allows for increased release of the prostaglandins therefore activating greater synthesis of Hsp's and protecting the viability and cytoskeleton of the cell. These data demonstrate for the first time that niacin has a protective role during heat stress at both the systemic and cellular level in the bovine. The improved evaporative heat dissipation leading to lower body core temperatures is the systemic effect while at the cellular level, niacin up-regulated the thermal tolerance by up-regulating the heat shock family of proteins. Others have already shown that the cyclopentenone prostaglandins up-regulate Hsp gene expression by activating HSF-1 (Amici et al., 1994, Santoro, 2000, Kozawa et al., 2001, Ianoro et al., 2003). This is the first report that niacin acts additively with the cyclopentenone prostaglandins (D and E) to improve the gene expression of Hsp 70 and 27. It is

currently not known if niacin acts through further activation of HSF-1 or by some other route.

## CONCLUSION

Adding PGD increased Hsp-70 and 27 gene expression in during heat stress at 42°C. Peak fold increases in Hsp-70 expression over time zero differed between C and PGD, (-2.4 vs. 9.3, $P<.0001$). Addition of PGE increased Hsp-70 expression compared to C and PGD alone (-2.4 vs. 9.3 vs.18.0, $P<.05$). We conclude that niacin with PGD or PGD+PGE alters Hsp-70 and Hsp-27 expression in BMEC. Therefore, niacin has both systemic and cellular impacts on resistance to thermal stress by increasing heat dissipation through improved evaporative heat loss and increased protection at the cellular level by up-regulation of the heat shock family of proteins leading to improved cellular tolerance of heat stress. This suggests that niacin is playing a major role in improving resistance of the bovine to thermal stress. Further research is required to delineate the systemic and cellular mechanisms of this improved resistance and to identify nutritional management programs to utilize protected niacin to enhance performance and survival of cattle during periods of thermal stress

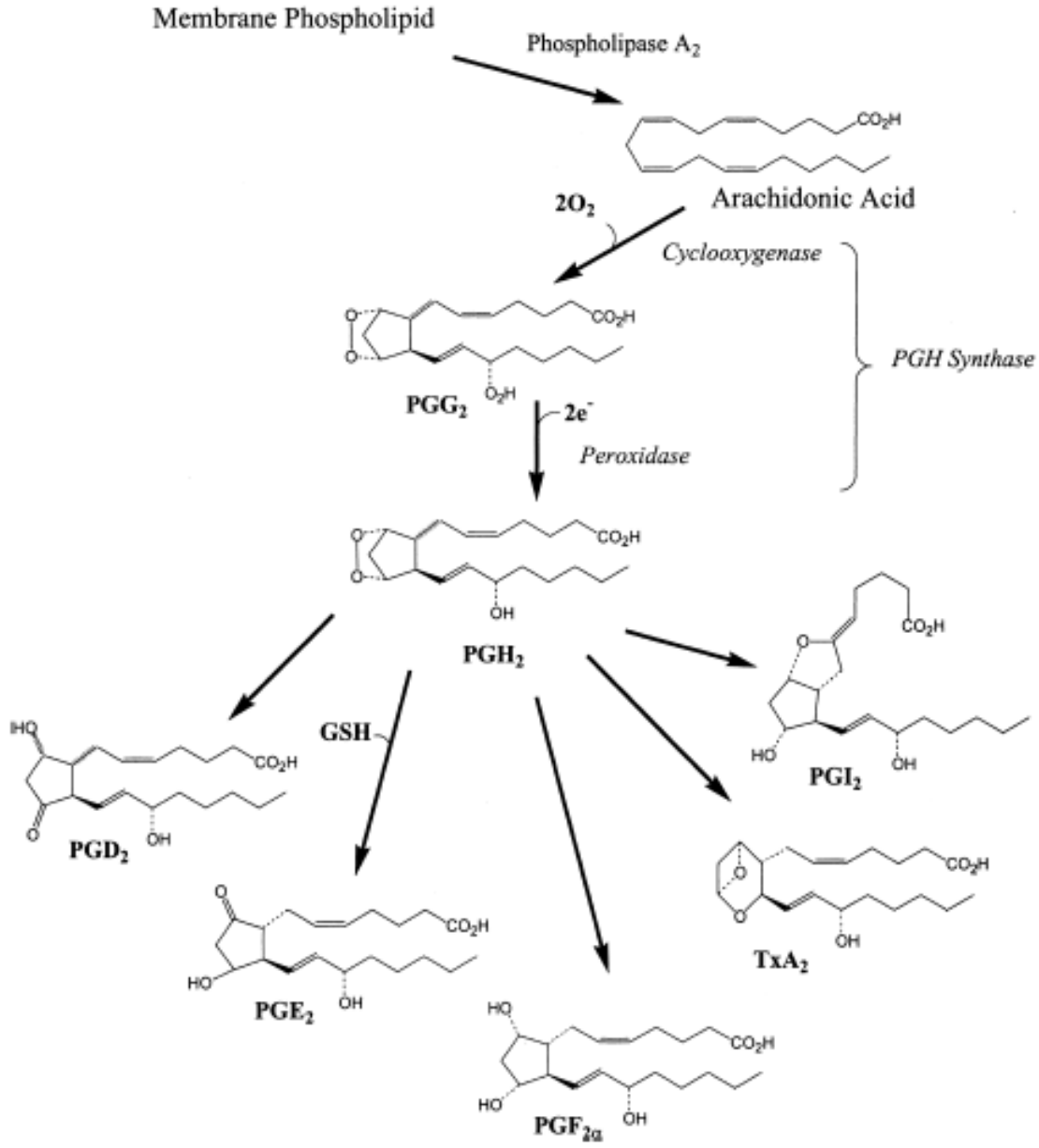


**Figure 4.1.** General pathway for synthesis of prostaglandins. Phospholipase $A_2$ catalyzes the release of arachidonic acid from membrane phospholipids. Arachidonic acid is then converted to the central prostanoid precursor $PGH_2$ via the action of the bifunctional enzyme PGH synthase. $PGH_2$ is then converted to protaglandins, thromboxane, or prostacyclin by specific synthase enzymes $PGE_2$ synthase, $PGD_2$ synthase etc. (**From Strauss and Glass, 2001**).

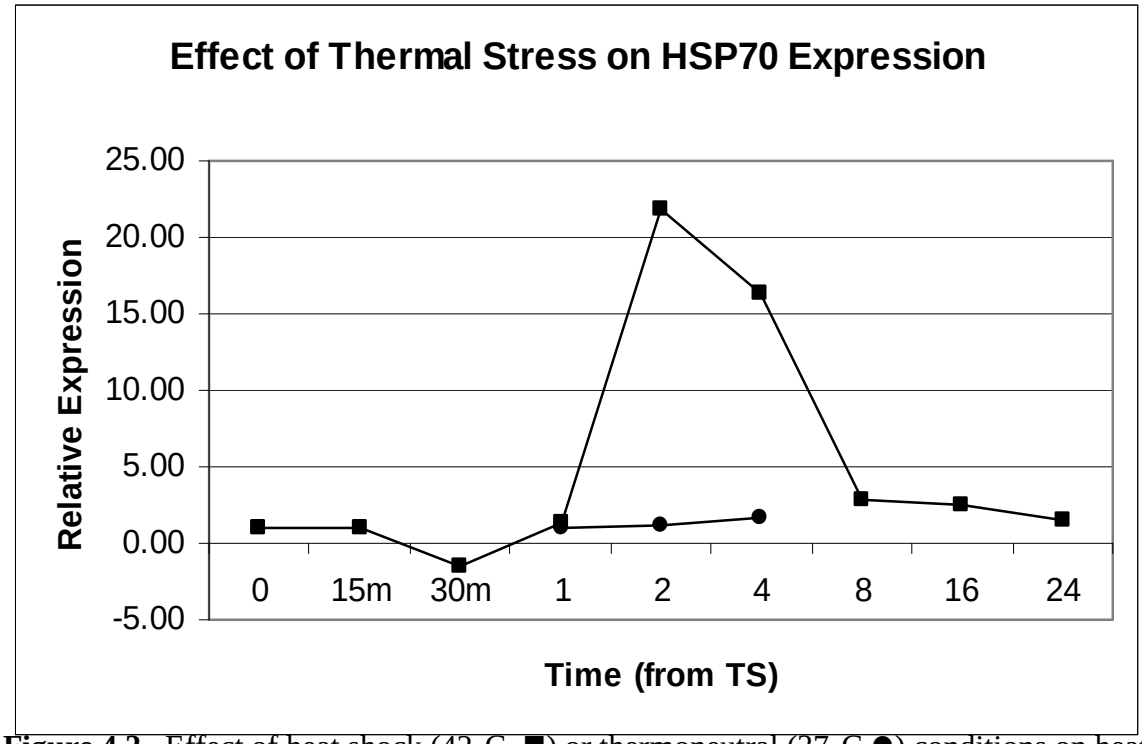


**Figure 4.2.** Effect of heat shock (42 C, ■) or thermoneutral (37 C,●) conditions on heat shock protein 70 gene expression in bovine mammary epithelial cells. From: Stiening, C.M, 2005.

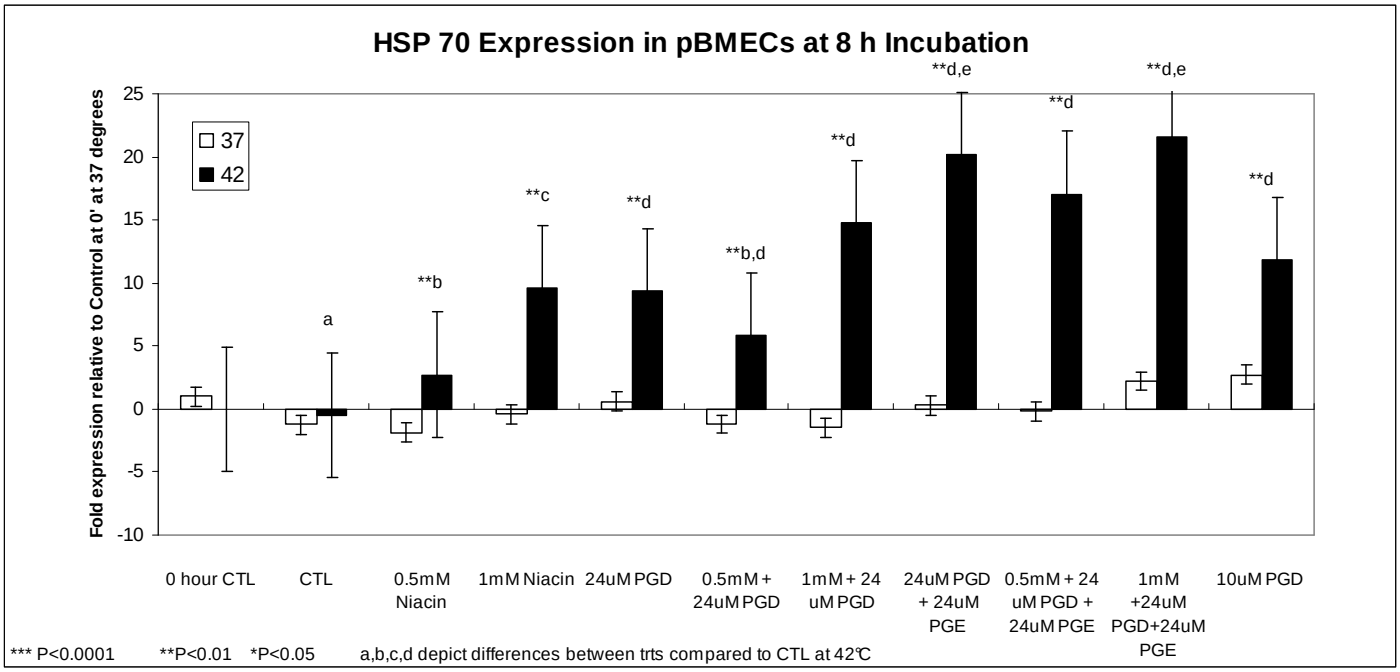


**Figure 4.3.** Heat shock protein 70 expression in primary Bovine Mammary Epithelial Cells incubated for 8 hours.

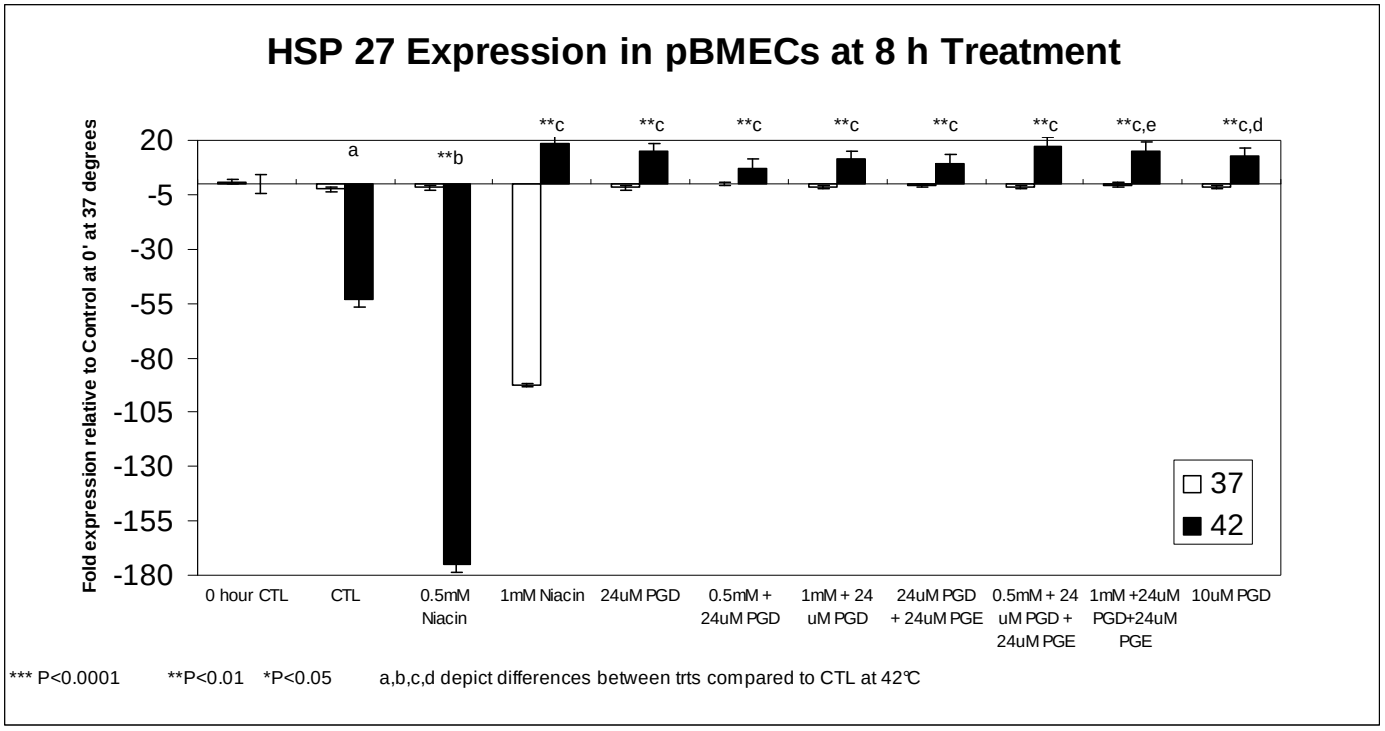


**Figure 4.4.** Heat shock protein 27 expression in primary Bovine Mammary Epithelial Cells incubated for 8 hours.

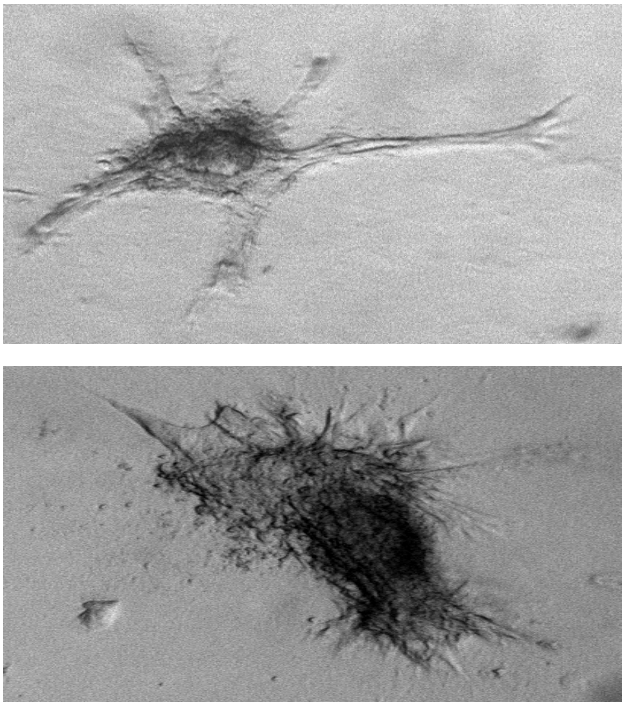

**Figure 4.5.** Primary bovine mammary epithelial cells cultured for 8 days. Treatment was with fresh growth media (control) on day 9. Incubation for 20 hours at either 37º C (upper photo) or 42º C (lower photo).

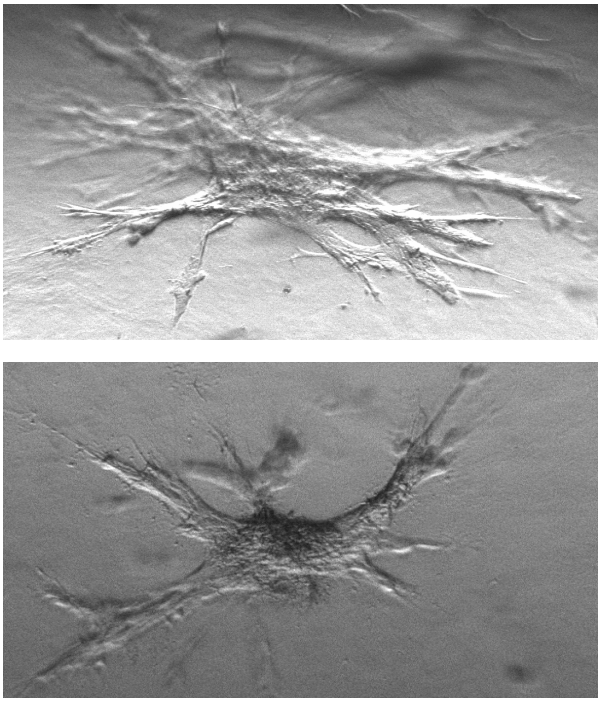

**Figure 4.6.** Primary bovine mammary epithelial cells cultured for 8 days. Treatment was with fresh media containing 24uM $PGA_1$ on day 9. Incubation at either 37º C (upper photo) or 42º C (lower photo). These cells are sister cultures to the control cultures shown above.

# CHAPTER FIVE: EFFECTS OF FEEDING ENCAPSULATED NIACIN ON CORE BODY TEMPERATURE, MILK PRODUCTION AND COMPOSITION IN LACTATING HOLSTEIN DAIRY COWS DURING HEAT STRESS

## ABSTRACT

Niacin has been shown to increase resistance to thermal stress in cattle by increasing whole body evaporative heat loss *in vivo* and cellular heat shock response by increasing the gene expression of heat shock proteins 27 and 70 during thermal stress *in vitro*. To determine effect of feeding encapsulated niacin on core body temperature, milk yield and composition, a total of 198 primiparous and 242 multiparous lactating Holstein cows were randomly assigned to a crossover design of either control (C; no feed additive n=213 or treatment (Trt; cows supplemented with 12g/d/cow of encapsulated niacin, n=214). Groups were balanced for DIM, milk yield, and parity prior to start of the study which was conducted between August 7 and October 7, 2007 on a commercial dairy in Arizona. Cows remained on their respective treatment for 30 d and then switched to the opposite treatment on d 31 and continued until d 60. Milk yield were recorded three times daily and a monthly milk sample was collected for milk component analysis. Feed samples were collected weekly from each group. Vaginal temperatures were collected in order to obtain core body temperatures, using temperature data loggers attached to a blank continuous intravaginal drug release device (CIDR) and inserted into a random sub-sample of cows (n=16) from each pen (n=2) with similar DIM, milk yields, and parity for 7 d. Core body temperatures were decreased for the Trt group during periods of peak thermal load from 1300 to 1600 h. Milk fat and protein percent was elevated in

the Trt versus C groups (3.74 vs. 2.99 % and 3.47 vs. 3.03 %, $P < 0.01$; respectively). Subsequently, both fat- and energy-corrected milk was greater for cows in the Trt group compared with cows in the C group (39.7 vs. 38.2 kg/d and 39.6 vs. 38.4 kg/d, respectively). Groups did not differ in milk yield for either test day (39.0 vs. 38.8 kg/d) or average daily milk samples (37.6 vs. 37.5 kg/d), and group fed dry matter intake (26.9 vs. 27.0 kg/d). In conclusion, supplementation of lactating cows with encapsulated niacin during heat stress reduced core body temperature and increased both fat and protein percent, in turn, elevating fat- and energy-corrected milk yields.

## INTRODUCTION

Heat stress negatively impacts production of lactating dairy cows. The economic impact of heat stress on the U.S. dairy industry has averaged annual losses of over $800 million as a result of reduced performance and increased incidence of disease (St. Pierre et al.., 2003). Milk production can be reduced significantly (10-35%) when the THI exceeds 72 (Thatcher et al., 1974; Schneider et al., 1984). A reduction in milk production of 0.26 kg for each unit increase of THI over 72 was reported by Johnson et al. (1962). A portion of the reduction in milk yield is due to a decrease in DMI (Collier and Beede, 1985; Rhoads et al., 2007). This is a result of an elevated animal body temperature, thus the animal reduces its intake to reduce the amount of metabolic heat produced (West, 1994). Furthermore, the maintenance requirements of lactating dairy cows increases during thermal stress; however, dry matter intake decreases not allowing the cow to successfully reach energy demands (Collier, 1985). Nutritional management strategies to help prevent the decrease in DMI have been previously described (Beede and Collier,

1986; Armstrong, 1994). These strategies are primarily aimed at reducing heat production by increasing dietary energy concentration and reducing roughage intake. A strategy that would be compatible with these strategies would be to increase heat loss in addition to reducing heat production (Collier et al, 2008). The primary route of heat loss in thermally stressed cows is evaporative. Therefore, nutritional management strategies which improve the ability of cattle to lose heat by evaporation (sweating rate) would likely be beneficial during periods of thermal stress. The vitamin niacin ($B_3$) is known to increase peripheral vasodilation which could increase sweat gland activity in humans and dairy cattle (Gille et al., 2008; Di Costanzo et al., 1997). However, unprotected niacin is rapidly metabolized in the rumen resulting in poor delivery to the small intestine (Campbell et al., 2004). Encapsulated niacin has been shown to escape rumen degradation with a large percentage reaching the small intestine (Balchem Corp., New Hampton, NY).

A recent study in controlled environment rooms with low animal numbers (n=12) demonstrated that cows supplemented with encapsulated niacin at a dose of 12 g/cow/d during acute thermal stress, displayed a lowered core body temperature and increased sweating rates (Zimbelman et al., 2007). The decrease in body temperature and increase in sweating rate are adaptive mechanisms of the body allowing for dissipation of more body heat to the surface area through peripheral or vasomotor function and (or) improved sweating rate. This prevents some of the decrease in DMI thus improving milk production (Di Constanzo et al., 1997). Animals undergoing mild to severe heat stress, when fed raw niacin at a dose of 12 and 24 g/cow/d, demonstrated a decrease in skin

temperatures and others have shown increased milk production during summer months (Muller et al., 1986; Di Constanzo et al., 1997).

In the past raw niacin in the form of nicotinic acid or nicotinamide, has been supplemented in studies during heat stress however, the majority of raw niacin is metabolized in the rumen via microbes and research has reported little (3-10%) niacin or nicotinamide is able to escape degradation in the rumen (Santschi et al., 2005; Campbell et al., 1994; Zinn et al., 1987 and Miller et al., 1986). Naturally both forms of niacin are normally synthesized in the rumen and have been demonstrated in past research (Santschi et al., 2005; Miller et al., 1986). The theory of ruminal wall absorption has been shown to be possible through increasing levels of free niacin (Rérat et al., 1958b) however, since these researchers reported very little escape of niacin from the rumen the likely beneficial factors seen when feeding niacin could be do to the absorption across the ruminal wall before reaching the duodenal cannula (Santschi et al., 2005). In order to increase the bioavailability of niacin in the small intestine more recently, technology has been able to encapsulate niacin and protect it from rumen degradation (Dueschler et al., 1998). Feeding a product of encapsulated niacin although perhaps not 100% pure niacin would result in increased stability in the rumen of 80% and the increase in bioavailability of niacin being 35% compared to raw niacin (Santschi et al., 2005; Deuschler et al., 1998).

The objective of this study was to examine the effects of encapsulated niacin during heat stress on milk production, milk composition and core body temperatures under commercial conditions.

## MATERIALS AND METHODS

The study was conducted from August 7th to October 7th, 2007 on a 10,000 cow commercial dairy in Stanfield, Arizona. The University of Arizona's Institute of Animal Care and Use Committee approved all protocols and use of animals in the current study. Four hundred and twenty seven lactating primiparous and multiparous Holstein cows were balanced for DIM (166 ± 11), milk yield (95.90 ± 23.1 kg/d) and parity (1.73 ± 0.2) prior to start of the study and assigned to either a control (**C**; n=213) with 0 g encapsulated niacin/d or treatment (**Trt**; n=214) consisting of 12 g encapsulated niacin/d (NIASHURE™®; Balchem Corp.; New Hampton, NY) in a crossover design. The form in which niacin was supplemented was encapsulated and therefore only 68% pure niacin resulting in an actual dosage of 8.2 g of raw niacin per day (NIASHURE™®; Balchem Corp.; New Hampton, NY). All cows were fed a totally mixed ration three times daily. A separate premix of encapsulated niacin was made prior to feed mixing. The diet analysis was conducted by Chandler Analytical Laboratories (Chandler, AZ; Table 1). Cows were fed for ad libitum intake to allow 3-5 % feed refusal daily. Cows remained on C or Trt for 30 d, and then on d 31 were assigned to the opposite group for an additional 30 d. Milk yields were individually measured three times daily (~0200, 1000 and 1800 h) and sampled once during each 30 d period for composition analysis. Cows spent approximately 45-60 minutes in the milking parlor where cooling was provided via Korral Kool coolers in the holding pen. Individual milk samples were collected twice (once each 30 d period) and sent to the Dairy Herd Improvement Association of Arizona (DHIA; Tempe, AZ) for milk composition analysis. Milk samples were analyzed for

somatic cell count, fat, true protein, lactose and solids-non-fat using DHI standards and AOAC-approved (2000) mid infrared analysis equipment and procedures. Energy corrected milk yields (Orth, 1992) were calculated using (milk, kg x (0.327 + (7.2 *milk protein, %/100) + (12.95 x milk fat, %/100)) and the 4% fat-corrected milk yields were calculated using (milk, kg x (0.4255 + (16.425 x milk fat, %/100)); (NRC, 2001). Cows were fed three times daily and feed was pushed up every half hour. Feed was available at shaded bunks (~3.4 meters) from the shade and cooler structures; the entire pen was under shade structures except outside walking areas. There was no cooling over the feed bunks. All cows were housed in open dry-lot facilities with Saudi style shades, and pens were identical in size, location, and design. In addition, pens were located across from each other and housed with the same number of animals.

All cows were cooled using Korral Kool coolers (Korral Kool, Mesa, Arizona) which are reverse chimney evaporative cooling systems that were mounted into a conventional corral shade (Armstrong et al., 1993). These systems cool the environment surrounding the cow by injecting micron sized (30-65 microns @ 300 psi) water droplets into the air moving down the cooler (Ryan et al., 1992; Armstrong, 1994). Coolers are set to turn on and off depending upon ambient temperature. During the months of August and September coolers were turned on when temperature exceeded 25.6°C and off when temperature dropped below 23.9°C; however, during the month of October coolers turned on when temperature exceeded 26.7°C and off when temperature dropped below 25.6°C. In addition, curtains were suspended from the edge of the shades in order to prevent exposure to solar radiation during late afternoons. Curtains were scheduled to come down

at an ambient temperature exceeding 27.8°C and rolled up at a temperature below 25.5°C.

Temperature loggers (ibutton thermochrons, Maxim Dallas Semiconductor, TX) were used as a means to record and measure core body temperature. The manufacturer reports a measurable range of +15 to +46°C measuring 1/8°C increments with ±1°C accuracy. Although the manufacturer reports average accuracy for the data loggers each temperature logger was individually calibrated in our laboratories. Loggers were placed in one of two incubators set at 38.5 or 42°C for 24 hours after which they were kept at room temperature for 24 hours and then placed in the opposite incubator for 24 hours. During the period that temperature loggers were recording data, the digital temperatures for the incubator were recorded. Based on these calibrations we produced an offset for each logger which was then used as a co-variate in the statistical analysis of vaginal temperatures. Temperature loggers were then attached to blank cervical implant drug release (**CIDR;** Pfizer Animal Health, Kalamazoo, MI) devices and inserted into the vagina of a random sub-sample of animals (n=16; 8 primiparous and 8 multiparous cows) from each pen (n=2) with similar DIM, milk yield, and parity. The devices were inserted two weeks after being on either C or Trt during each 30 d environmental period. Data was collected from the temperature loggers for 7 d after insertion. Temperature loggers were set to record and store core body temperatures every five minutes throughout the 7 d insertion period; which was then downloaded onto a computer. Three temperature loggers (**HOBO**: H08-032-08; Onset Computer Corporation, Pocasset, MA) were placed in the north, center, and south side of both pens. Three were placed along the feed bunk

and three between coolers directly behind the feed bunks, approximately 8 feet from the ground. Loggers recorded the ambient temperature and relative humidity every 15 minutes beginning one week after study began until the end of the study.

Environmental conditions outside of the barn were recorded from Arizona Meteorological Network (**AZMET;** http://ag.arizona.edu/AZMET) site which was located near (5 km) the dairy. The AZMET uses several devices to record minimums, averages, and maximums of ambient temperatures, relative humidity's, dew and dew points daily.

***Statistical Analyses***

All data was analyzed using PROC MIXED for analysis of repeated measures and PROC GLM procedures of SAS for non continuous variables (SAS, 1999). Dependent variables tested were milk yield, DMI, core body temperatures, fat-corrected milk, energy-corrected milk, solids-not-fat (SNF), lactose, fat, somatic cell count and protein. The independent variables included trt, parity, period, sequence, and the respective interactions. Vaginal temperatures were analyzed as a dependent variable also using the Proc Mixed procedure of SAS (SAS, 1999). Covariates were used based on calibration analysis preformed in our laboratories and trt, period, and respective interactions were utilized as independent variables. The level of significance was set at $P < 0.05$ for all main effects and interactions and the LSMEANS test was conducted when significance was detected. If the higher order interactions were not significant then they were removed from the model.

## RESULTS

Dry matter intakes were 26.9 kg/d for the C group and 27.0 kg/d for the Trt group and were not different. Milk yield also did not differ for animals supplemented with encapsulated niacin or cows in the C group (37.6 vs. 37.5 kg/d ± 0.30; $P > 0.10$; Figure 1, Table 2). There was a parity affect ($P < 0.01$), multiparous cows produced 4.76 kg more compared to primiparous cows. Although treatment alone was not significant, treatment by time interactions were significantly different ($P < 0.01$) in that some days Trt milk yields were significantly increased or lowered however overall there was not effect on Trt alone. Milk protein percentage was increased for cows supplemented with encapsulated niacin compared to the C group (3.09 vs. 3.05% ± 0.01; $P < 0.01$; Table 2). In addition, average milk fat percentage was elevated for cows supplemented with encapsulated niacin compared to the C group (3.65 vs. 3.38% ± 0.04; $P < 0.01$; Table 2). Milk yields on the day of testing for milk components were not different between the Trt and C group (38.65 vs. 38.36 kg/d ± 0.72; $P > 0.10$). Somatic cell counts were also not affected by treatment or parity (115 vs. 116 cells/mL x 1000 ± 11.76; $P > 0.10$). Solids-not-fat were not different between treatments but were different between parity (Primiparous 8.82 vs. Multiparous 8.71% ± 0.02; $P < 0.01$). Lactose also did not differ between treatments; but, there was an effect of parity (Primiparous 4.87 vs. Multiparous 4.75% ± 0.01; $P < 0.01$). The 4% fat-corrected milk yield was elevated for cows in the Trt group compared to cows in the C group (39.62 vs. 38.14 ± 0.38 kg/d; $P < 0.01$; Table 2). There was also a difference in 4% fat-corrected milk yields due to parity (primiparous: 37.15 vs. multiparous: 40.6 kg/d ± 0.4; $P < 0.01$). In addition, cows in the Trt group had increased

energy-corrected milk (ECM) yields compared to cows in the C group (39.51 vs. 38.26 ± 0.34 kg/d; $P < 0.01$; Table 2); ECM was also affected by parity (primiparous: 37.2 vs. multiparous: 40.6 ± 0.4 kg/d; $P < 0.001$). There was no parity effects on intravaginal temperatures, therefore parity groups were combined within treatment. Core body temperatures during the hottest part of the day (1300 to 1600 h) were lower for cows in the Trt group compared to the cows in the C group (38.52 vs. 38.65°C ± 0.04; $P < 0.01$; Figure 2, Table 2).

The THI is a well established measure for assessing cow comfort during thermal stress. The THI chart has been commonly utilized by the dairy industry as a means to predict the level of heat stress a cow is experiencing based upon a combination of ambient temperature and relative humidity (Whittier, 1993; Armstrong, 1994). Current standards show that when THI is greater than 72, dairy cows are in a heat stressed environment (Armstrong, 1994). The Livestock Conservation Institute, University of Missouri Extension, has currently categorized heat stress levels for cattle as mild, moderate, and severe (Whittier, 1993; Armstrong, 1994). Increasing milk yield elevates the sensitivity of animals to thermal stress and reduces the "threshold temperatures" at which milk losses can occur (Berman, 2005). It has also been reported that ambient temperature two days prior to recording milk yield had a strong correlation to decreases in production and dry matter intake (West et al., 2003) and the total number of hours when THI is greater than 72 or 80 over a four d period has the greatest correlation with milk yield (Linville and Pardue, 1992). In this study, THI was calculated for each day throughout the study, inside (Figure 3) the barns from the HOBO loggers or outside

(Figure 4) the barn from AZMET. Based on AZMET, THI was only below 72 (indicative of heat stress) four days of the 60 d study (Figure 4). For the months of August and September 2007, THI never decreased below 80 (Figure 4). Inside the barns, the environment for both pens throughout the study did not differ from each other (83 vs. 84 THI) therefore both C and Trt groups experienced similar environments. The walkway to the milking parlor was also with shade.

## DISCUSSION

Research studies evaluating milk yield responses to rations supplemented with raw niacin in the forms of nicotinamide and/or nicotinic acid have been inconclusive. Researchers have reported increases in milk yield when animals were supplemented with a form of raw niacin (Muller et al., 1986; Drackley et al., 1998) while others have reported no differences in milk yield (Di Constanzo et al., 1997; Madison-Anderson et al., 1997). Results published from a meta-analysis of 27 studies supplementing dietary nicotinic acid, summarized that approximately 12 g/d of nicotinic acid could perhaps improve lactation performance (Schwab et al., 2005). The differences between raw niacin and encapsulated niacin have been mostly attributed to the bioavailability of the product being fed to the animals. It is currently believed that encapsulated niacin has a greater bioavailability in the small intestine and therefore able to be used more efficiently (Balchem Corp., New Hampton, NY). In 2006, The University of Arizona conducted a study in environmental chambers and fed animals a control diet of no supplemental niacin per day or a treatment diet of 12 g of encapsulated niacin per day that was top-dressed on the ration during acute heat stress (Zimbelman et al., 2007). Although milk

yield was not increased, cows that had been supplemented with encapsulated niacin had increased average evaporative heat loss compared to animals in the control group, and the differences were more apparent during peak thermal stress. The difference was attributed to animals being able to dissipate more heat if supplemented with encapsulated niacin. Also in this study, cows in the treatment group had lowered rectal and core body temperatures compared to the control group when subjected to heat stress. When under heat stress, animals on treatment had reduced heat storage compared to cows in the control group which was calculated during the hottest part of the day (1400 to 1700 h). Based on these results, the current study was conducted to evaluate whether encapsulated niacin could reduce core body temperatures and improve production parameters during heat stress in a commercial field study with large numbers of animals.

Based on the environmental conditions from the nearby meteorological station, the combination of temperature and humidity were elevated inducing a THI above 72 (indicative of heat stress) in Arizona from August 7$^{th}$ to October 7$^{th}$ 2007 (Figure 4). Inside the barn, THI dropped below 72 for only four days throughout the study which could be due to a drop in ambient temperature or relative humidity during the end of September and beginning of October months. Despite those four days, THI values stayed similar throughout the study providing consistent heat stress for both C and Trt groups during the study.

In the current study milk yields were not increased when mid (beginning of study: 166 ± 11 DIM) to late (at the end of the study: 226 ± 11 DIM) lactation cows were supplemented with encapsulated niacin, however, treatment by time interactions were

significant (Fig. 1). When comparing days individually there was a statistical difference in increase or decrease in milk yields in nine of the 17 days analyzed. During d 17 through d 20 milk yields were increased for cows supplemented with encapsulated niacin compared to C group. From d 21 through d 25, milk yields were decreased for cows supplemented with encapsulated niacin. Reasons behind this decrease are not clear as it is not believed to be an adaptation response to the supplementation of encapsulated niacin, rather perhaps a management or environmental component that was not identified at the time of the study. An adaptation to encapsulated niacin is unlikely as cows had been supplemented with the product for two weeks prior to data being analyzed therefore an increase in milk yields for cows in the Trt group would not have been shown. From d 26 through d 30, there were no differences in milk yields between both groups however, on the last day (d 31) the Trt group had statistically increased milk yields compared to the control. This is consistent with previous research which found no effect on milk yield and attributed these findings to interactions with week or treatment by week, and (or) cows being well past peak lactation however, these studies supplemented with raw and not encapsulated niacin with only one being heat stressed and both with small numbers (Di Constanzo et al., 1997; Madison-Anderson et al., 1997). This warrants further investigation into stage of lactation effects on milk yield responses to oral niacin.

Energy- and 4% fat-corrected milk yield were increased due to an increase in milk protein and butter fat (Table 2). The reason behind the milk protein and butter fat increase cannot be explained by a dilution factor as milk yield difference on the day of testing for milk components was not different between Trt or C groups. Drackley et al., (1998)

reported a tendency for milk fat yields to be increased when fed nicotinic acid and supplemental fat from weeks 4 to 25 of their study along with a tendency for increased milk yields. In the current study, both rations contained supplemental by-pass fat. Furthermore, Harrison et al., (1995) showed an increase in milk fat when cows were fed whole cottonseed and calcium salts of long-chain fatty acids in combination of which both were included in the current studies rations, however, these were not controlled variables in our diets. Others have observed a tendency for milk fat and protein yields to be increased; however, the response was attributed to increased milk yields (Di Constanzo et al., 1997). Milk yields were not increased in this study, therefore the increase in milk protein and fat percentages maybe due to interactions with the supplemental fat in the diet. Other theories justifying the increase in production parameters with supplementation of nicotinic acid may be the effects of nicotinic acid on rumen fermentation and/or animal metabolism (Schwab et al., 2005). The mechanism behind an increase in milk fat and protein percentages when encapsulated niacin is fed and possible interactions with other feed additives such as supplemental fat is unknown.

Vaginal temperature probes were inserted for a 7 d during both 30 d periods measuring core body temperatures. During the hottest part of the day (1300 to 1600 hrs) cows supplemented with encapsulated niacin had reduced core body temperatures on average of 0.13°C (Table 2). Therefore, the vasodilation mechanism associated with niacin and the decrease in core body temperature is in agreement with recent studies observing the same effects with either feeding raw niacin at 12, 24, and 36 g/d for three consecutive periods or 12 g of encapsulated niacin during a mild to severe heat stress (Di

Constanzo et al., 1997; Zimbelman et al., 2007). Originally nicotinic acid was implemented in humans to treat dyslipidemia and functioned by reducing cholesterol levels of low density lipoproteins and increasing high density lipoproteins levels (Gille et al., 2008). Reports of human patients experiencing side effects of skin flushing resulting from a strong cutaneous reaction of vasodilation and characterized as a burning sensation have been most common (Gille et al., 2008). The mechanism behind this vasodilation is currently not known, however there is a tendency for patients to become adapted to the flushing effects weeks after nicotinic acid administration (Gille et al., 2008). Although the mechanism is not clear, studies have shown that raw niacin could be acting through the secretion of prostaglandin $D_2$ from the epidermal Langerhans cells and vascular endothelial prostaglandin D2 receptors in humans (Benyo et al., 2006; Cheng et al., 2006; Maciejewski et al., 2006; Meyers et al., 2006). Studies conducted using bovine mammary epithelial cells have reported an increase in heat shock proteins 27 and 70 when cells were administered niacin and prostaglandins alone or in combination (Zimbelman et al., 2008). This illustrates a potential thermo-protective mechanism for niacin at the cellular level by increasing cellular heat shock proteins, thus possibly elevating the thermotolerance level of the animal. We have demonstrated this protective effect on mammary epithelial cells in vitro (Zimbleman et al. 2008). Mammary epithelial cells in collagen gel cultures that also contained niacin and prostaglandin D-2 demonstrated increased viability when exposed to severe thermal shock (42 $^0$C) for 24 hours.

Research on effects of feeding encapsulated niacin during thermal stress is limited and studies utilizing raw niacin have been inconclusive. Due to differences in biological availability studies utilizing raw niacin cannot be compared to the effects of encapsulated niacin (Jaster and Ward, 1990; Kung et al., 1980; Madison-Anderson et al., 1997; Di Constanzo et al, 1997). Studies investigating encapsulated niacin at different dosage levels, physiological windows (transition and (or) breeding period), and effects on reproduction are warranted.

## CONCLUSION

Core body temperatures were moderately reduced when cows were supplemented with encapsulated niacin perhaps substantiating the increase in vasodilation and altering of blood flow to the periphery in turn reducing some of the negative effects of heat stress. Feeding encapsulated niacin did not increase milk yields; however, milk fat and protein percentages were increased thereby, increasing 4% fat- and energy-corrected milk yields significantly higher when fed encapsulated niacin.

## AKNOWLEDGMENTS

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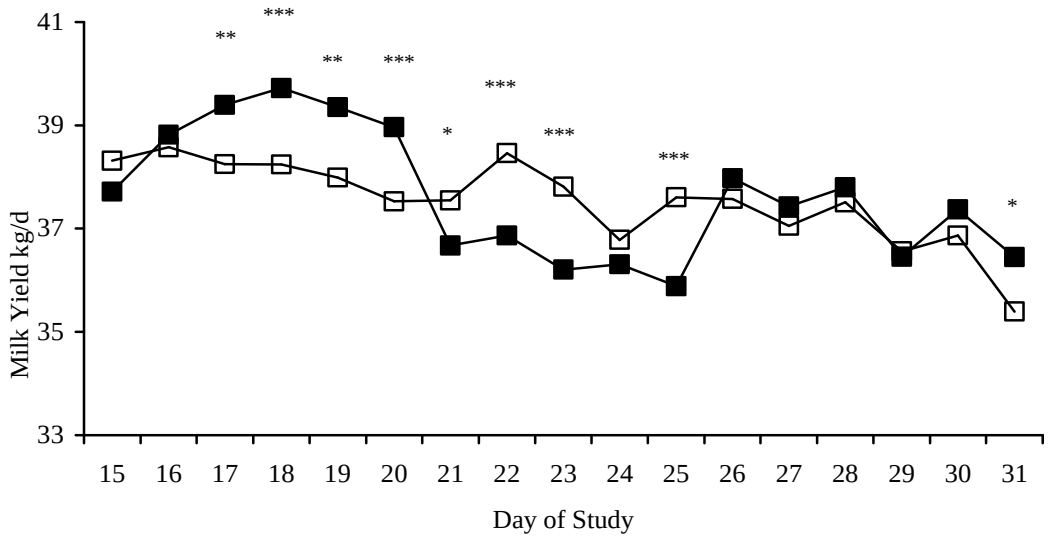


**Figure 5.1.** Temporal pattern of milk yields from d 15 to d 31 of the experiment period. Daily milk yields were not different ($P > 0.10$) for cows fed the control (□; 0 g/d/cow of encapsulated niacin) or treatment (■; 12 g/d/cow of encapsulated niacin) diets. Pooled standard errors for control = 0.31 and treated = 0.31 cows. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † $P < 0.10$.

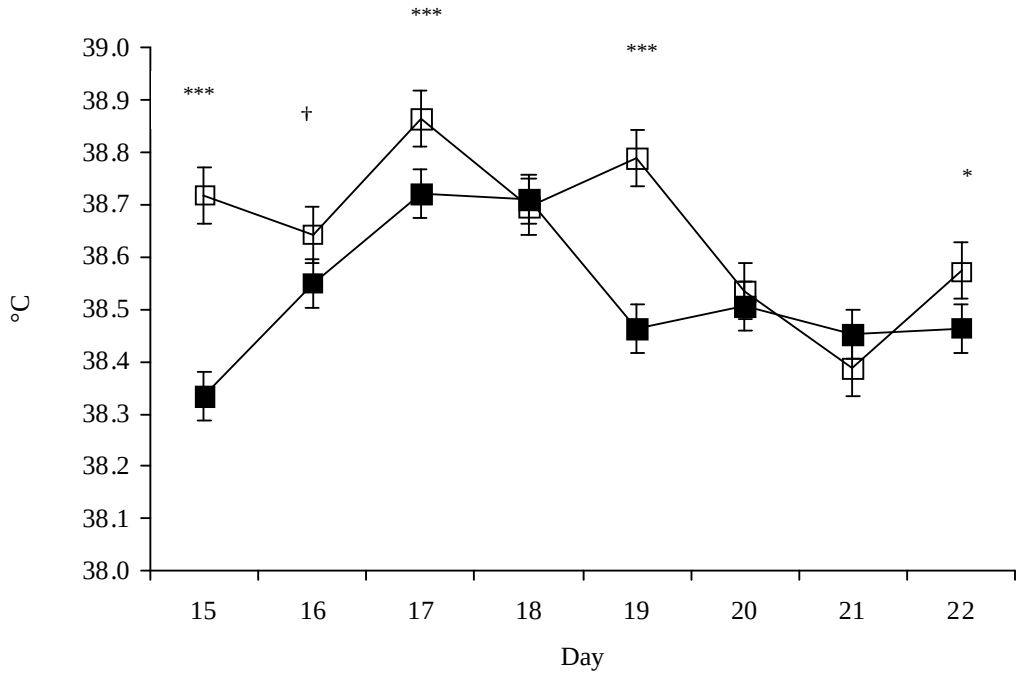


**Figure 5.2.** Average core body temperatures from 1300 to 1600 hours from d 15 to d 22 of the experiment period when analyzing daily differences between control (□; 0 g/d/cow of encapsulated niacin) and treatment (■; 12 g/d/cow of encapsulated niacin) groups. Vaginal probes were inserted into a subsample (n = 16) in each pen (n = 2). Core body temperatures were different during the entire course of the measurement period regardless of treatment ($P$ <0.01). * $P$ < 0.05; ** $P$ < 0.01; *** $P$ < 0.001; † $P$ < 0.10.

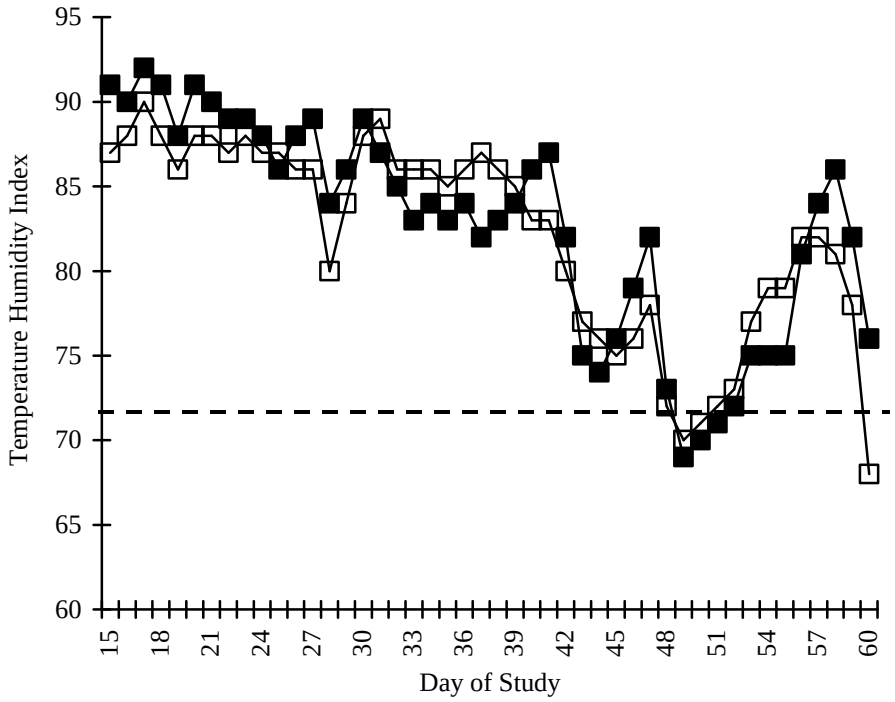


**Figure 5.3.** Temperature Humidity Index from d 15 to d 60 of the study inside the barns for cows in the control group (□; 0 g/d/cow of encapsulated niacin) or the treatment group (■; 12 g/d/cow of encapsulated niacin) with a line representing(----- ) 72 THI used for current initiation of heat stress.

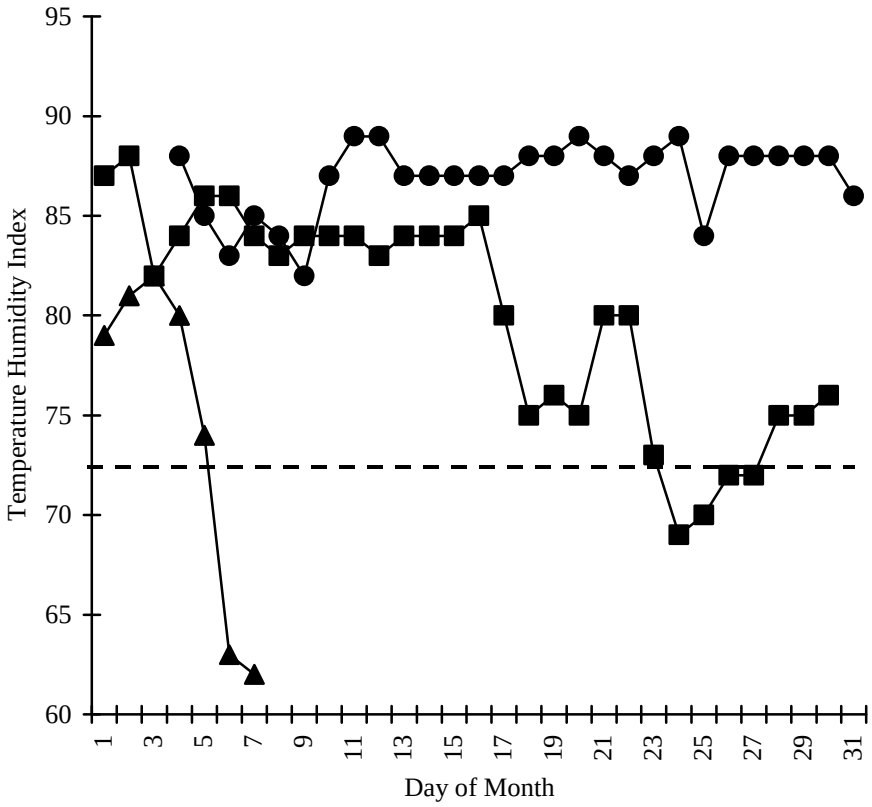


**Figure 5.4.** Temperature Humidity Index outside the barn, for each month of the study, taken from a meteorological weather station in Arizona (Arizona Meteorological Network; AZMET). Key: ● = August, ■ = September, ▲ = October, ----- Line represents 72 THI.

**Table 5.1.** Ingredient and chemical composition of diets containing 0 or 12 g of encapsulated niacin.

| Composition | C and Trt[4] |
|---|---|
| Ingredients, % of DM | |
| Alfalfa hay | 9.24 |
| Haylage | 3.85 |
| Corn silage | 11.08 |
| Alfalfa green chop | 10.15 |
| Millrun | 1.94 |
| Corn (steam flaked) | 16.65 |
| Barley | 24.01 |
| Amino plus | 2.53 |
| Corn distillers grain | 4.65 |
| Whole cottonseeds | 5.74 |
| Ammoniated cottonseed | 4.74 |
| Maxxer[2] | 2.79 |
| Molasses (cane) | 4.33 |
| Corn gluten feed | 2.60 |
| Mineral and vitamin mix[1] | 2.73 |
| Chemical composition | |
| $NE_L$ Mcal/lb of DM[3] | 0.84 |
| CP, % of DM | 16.78 |
| ADF, % of DM | 17.26 |
| NDF, % of DM | 26.69 |
| Fe, ppm of DM | 590 |
| Mn, ppm of DM | 153 |
| Cu, ppm of DM | 66 |
| Co, ppm of DM | 4 |
| Zn, ppm of DM | 134 |

[1] Mineral mix contained 17% CP, 5.26% fat, 1.12% Ca, 0.49% P, 0.37% Mg, 0.52% Na, 2.25% 0.33% S, 0.80 % Cl.
[2] **MAXXER™,** Tarome, Eloy, AZ
[3] Calculated using $NE_L$ values published by NRC (2001).
[4] Treatment group received added premix containing encapsulated niacin at 12 g/d/cow.

**Table 5.2.** Least square means and pooled SE for milk yield, energy-corrected milk, 4% fat-corrected milk, milk components and core body temperatures for cows either fed a control (0g/d/cow of encapsulated niacin) or treatment (12 g/d/cow of encapsulated niacin) diet. Milk tests were taken two weeks after the beginning of the study for each monthly experimental period (n=2).

| Item | C[1] | Trt[2] | S.E. | *P* value |
|---|---|---|---|---|
| Dry matter intake, kg/d | 26.9 | 27.0 | _____ | _____ |
| Milk yield, kg/d | 37.53 | 37.61 | 0.31 | NS |
| 4% Fat-corrected milk yield[3], kg/d | 37.02 | 38.33 | 1.08 | <0.01 |
| Energy-corrected milk yield[4], kg/d | 39.85 | 41.07 | 1.09 | <0.01 |
| Milk fat, % | 3.47 | 3.74 | 0.11 | <0.01 |
| Fat yields, kg | 1.39 | 1.49 | 0.05 | <0.01 |
| Milk protein, % | 2.99 | 3.03 | 0.03 | <0.05 |
| Protein yields, kg | 1.19 | 1.21 | 0.03 | NS |
| Somatic cell count, cells/mL x 1000 | 115.22 | 116.00 | 11.76 | NS |
| Solids-not-fat, % | 8.75 | 8.78 | 0.02 | NS |
| Core body temperature, °C | 38.65 | 38.52 | 0.04 | <0.01 |

[1]C= Control (0g/d/cow of encapsulated niacin)
[2]Trt = Treatment (12g/d/cow of encapsulated niacin)
[3] 4% Fat-corrected Milk = 0.4 x milk, kg + 15 x fat %/100 x milk, kg
[4] Energy-corrected Milk = 0.327 x milk, kg + 12.95 x fat, kg + 7.2 x protein, kg (Orth, 1992)

# CONCLUSIONS AND FUTURE RESEARCH

A re-evaluation of the THI confirmed that current THI values underestimate the severity of heat stress levels. Previously, a THI equal to or greater than 72 has been used to define onset of heat stress, this study demonstrated that a THI greater than or equal to 68 is low enough to cause adverse affects when cows suffer from heat stress. Furthermore we were able to demonstrate that when average THI is 68 or higher the milk yield loss becomes highly significant after 17 h of exposure to this THI, Fig 1 and Table 1. Results also confirmed that an animal heat and physiological parameters that are easily measurable correlate with the degree of heat stress that the animal is experiencing and is associated with given THI values.

Other reasons why new implementation and validity of a THI greater than or equal to 68 should be utilized is due to the experimental design. As discussed previously, past research used constant temperatures for two weeks prior to obtaining measurements. This study used circadian temperature fluctuations which are similar to those experienced by cattle under commercial conditions.

We evaluated 3 indices (THI, BGHI and STHI) to determine which of these indices provided the best estimates of heat stress threshold as evaluated by level of significance and correlation with physiological parameters and milk yield losses. We conclude that while some measures such as black globe humidity index are more tightly correlated with evaporative heat loss, the THI had the best relationship to milk yield loss.

In the first niacin study, supplementation of lactating dairy cows with a Niashure dose of 12 g/d alleviated some affects of heat stress during acute thermal stress. This was observed through increased evaporative heat loss, increased water intake to support the increased sweating rate, decreased rectal and core temperatures. Although milk production was not altered in this study, the animal numbers utilized in the study (12) were not large enough to observe effects on milk yield. The increased evaporative heat loss, we hypothesize was induced through vasodilation effects documented in humans and lab animals (Di Constanzo et al.., 1997; Gille et al., 2008), in other words the "flushing effect" of increasing blood flow to the periphery enhanced sweating mechanisms leading to increased dissipation of heat through evaporation. Reductions in vaginal and rectal temperatures are also likely due to this alteration in insensible heat loss. Overall, physiological changes observed in this study warrant further research into mechanisms of niacin's effect on increasing evaporative heat loss and lowered rectal temperature. These studies should include a dose-range to determine the most efficacious commercial dose of Niashure that might be applicable on commercial dairies.

Past research demonstrated that the possible mechanism for vasodilation affects seen by niacin were most likely due to prostaglandin D secretions (Benyo et al., 2006; Cheng et al., 2006; Maciejewski et al., 2006; Meyers et al., 2006). Niacin acts through increased prostaglandin D and E production and secretion by these cells which then act upon vascular endothelial prostaglandin D receptors (Benyo et al., 2006; Cheng et al., 2006; Maciejewski et al., 2006; Meyers et al., 2006). Additionally, we and others have now shown that these prostaglandins induced elevated heat shock responses leading to,

increased heat shock protein gene expression in cells (Amici et al., 1992, Santoro, 2000, Kozawa et al., 2001, Ignoro et al., 2003; Collier et al., 2007; Zimbelman et al., 2008). Cellular viability is compromised under heat stress conditions and studies by Stiening, 2005 demonstrated that gene expression patterns leading to apoptosis were detected as early as 8 h after heat stress initiation. Additionally, after eight hours, heat shock protein gene expression is no longer elevated which is defined as a loss in thermotolerance, (Steining et al., 2005; Collier et al., 2006). Therefore, it was of interest to determine if niacin, alone or in combination of prostaglandins A and E could up-regulate Hsp gene expression and improve thermal tolerance of BMEC exposed to thermal shock. In fact, adding PGD to culture media increased Hsp-70 and 27 gene expression during heat stress. The addition of PGE increased Hsp-70 expression compared to the control and PGD alone. Niacin with PGD or PGD and PGE altered Hsp-70 and Hsp-27 expression in BMEC. Therefore, niacin had both systemic and cellular impacts on resistance to thermal stress by increasing heat dissipation through improved evaporative heat loss and increased protection at the cellular level by up-regulation of the heat shock family of proteins leading to improved cellular tolerance of heat stress. This suggests that niacin is playing a major role in improving resistance of the bovine to thermal stress. Further research is required to delineate the systemic and cellular mechanisms of this improved resistance and to identify nutritional management programs to utilize protected niacin to enhance performance and survival of cattle during periods of thermal stress.

Past studies from different researchers and our research discussed earlier did not focus on milk production responses or supplementing niacin on commercial dairies.

Therefore, the last study discussed was with the objective of examining the effects of encapsulated niacin during heat stress on milk production, milk composition and core body temperatures under commercial conditions. Past research had also only been conducted with raw niacin and this was the first commercial study to evaluate the effects on these parameters with encapsulated niacin. The differences between raw niacin and encapsulated niacin have been mostly attributed to the bioavailability of the product being fed to the animals. It is currently believed that encapsulated niacin has a greater bioavailability in the small intestine and therefore able to be used more efficiently (Balchem Corp., New Hampton, NY). Results from this study demonstrated chronic moderately reduced core body temperatures when cows were supplemented with encapsulated niacin perhaps substantiating the increase in vasodilation and altering of blood flow to the periphery in turn reducing some of the negative effects of heat stress, as discussed previously. We also concluded that feeding encapsulated niacin did not increase milk yields; however, milk fat and protein percentages were increased in this study thereby, increasing 4% fat- and energy-corrected milk yields significantly when animals were fed encapsulated niacin. Although the mechanisms behind these milk component increases and therefore, energy and fat corrected milk yields is not well understood it is clear that more research should be conducted to lead to the unraveling of niacin's mechanism not only physiologically however, metabolically as well.

Research conducted to evaluate the effects heat stress on lactating dairy cows either through the addition of niacin or the improvement of the accuracy of THI was demonstrated to be beneficial for milk producers. Re-evaluation of the temperature

humidity index was efficient in facilitating the evaluation of present high producing dairy cows affects from heat stress and to help producers manage effective cooling of the animal. Further research should be conducted to define the mechanism behind niacin's ability to alleviate heat stress and its interactions metabolically. Evaluating the black globe humidity index more closely would also be beneficial as accounting for the degree of solar radiation and convection affects during warm parts of the day. Correlations of black globe humidity index with physiological parameters would perhaps have greater potential for the management of animals during heat stress. Niacin's effects on the animal showed to induce possible increased evaporative heat loss and increase heat shock proteins to alleviate heat stress. Further research should be conducted to define the mechanism behind niacin's ability to alleviate heat stress and its interactions metabolically. Evaluating the black globe humidity index more closely would also be beneficial as accounting for the degree of solar radiation and convection affects during warm parts of the day. Correlations of black globe humidity index with physiological parameters would perhaps have greater potential for the management of animals during heat stress.

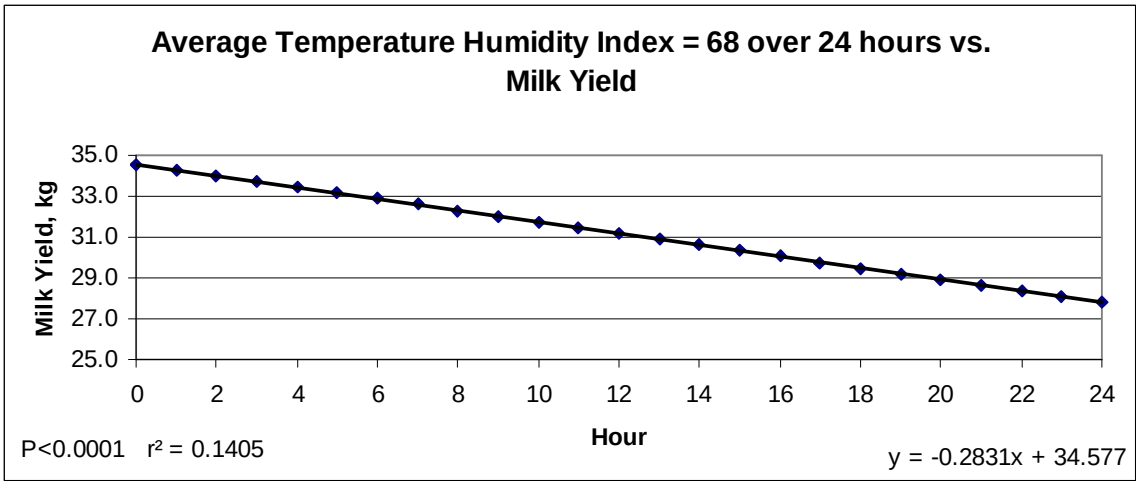


**Figure 6.1.** Average Temperature Humidity Index = 68 over 24 hours vs. Milk Yield.

Milk yield loss per 24 hours when THI is equal to 68.

**Table 6.1.** Amount of milk yield loss/gain when a given amount of hours are greater than THI = 68.

| **THI hours > 68** | **Slope** | **P-value** |
|---|---|---|
| 0 | -0.11 | 0.69 |
| 7 | -1.01 | 0.26 |
| 17 | -2.63 | 0.0007 |
| 19 | -0.09 | 0.86 |
| 21 | -2.04 | <0.0001 |
| 24 | -1.08 | 0.015 |

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