

RESPONSES TO HEAT STRESS IN SLICK VS. NORMAL-HAIRED HOLSTEIN  
COWS

By

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To my mom and dad

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Abstract of Thesis Presented to the Graduate School  
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RESPONSES TO HEAT STRESS SLICK vs. NORMAL-HAIRED HOLSTEIN COWS

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Heat stress plays a significant role in cattle performance and likely will be of even greater importance in the future. Selection of cattle adapted to warm environments represents one strategy to mitigate the effects of heat stress. The objectives of this study were to determine the physical, metabolic and production responses of slick (n=11) and normal-haired (n=10), lactating Holstein cows under heat stress conditions and was conducted at the Dairy Research Unit in Hague, FL. The slick-haired phenotype is produced in animals with the Slick hair gene which have very short, sleek, and, sometimes, glossy coats. Cows were maintained in a barn containing fans and sprinklers and equipped with Calan<sup>®</sup> gates. Animals were offered a total mixed ration (TMR) that contained corn silage, alfalfa hay and concentrate mixed at 35, 10 and 55% of the dietary DM respectively. Rectal temperature, respiration rate, and sweating rate were recorded weekly in the morning at 0800 h and in the afternoon at 1430 h each Saturday and Sunday for a four week period beginning in late August. Sweating rate was measured on a clipped and unclipped area of the right shoulder of each cow. Vaginal temperature was recorded continuously for a five day period during two different periods of the study. The first vaginal temperature measurements were taken from

September 5 - 10, 2007 at the beginning of the trial, and the second measurement period was from September 28 - October 3, 2007 at the end of the trial. An apparent digestibility trial was also conducted using chromic oxide ( $\text{Cr}_2\text{O}_3$ ) as an external marker. Intake and milk production were recorded daily for subsequent dry matter intake, feed efficiency and energy balance determinations.

Slick-haired Holstein cows had lower ( $P < 0.01$ ) rectal temperatures 38.6 vs. 39.0°C, and averaged 0.4°C less than those of the normal-haired ones throughout the study. In addition, slick-haired cows had a lower ( $P < 0.01$ ) respiration rate than the normal-haired cows while under heat stress, 61 vs. 72 breath per minute, respectively. There were no differences in sweating rates between slick and normal-haired Holstein for both clipped ( $P = 0.16$ ) and unclipped ( $P = 0.43$ ) areas (28.08 vs. 25.3 g/m<sup>2</sup>h; and 27.9 vs. 26.2 g/m<sup>2</sup>h, respectively). Slick-haired Holsteins had lower vaginal temperatures ( $P = 0.04$ ) than the normal-haired ones during week one averaging 39.3 vs. 39.6°C, respectively. In contrast, vaginal temperature did not differ ( $P = 0.56$ ) between slick and normal-haired Holsteins, averaging 39.1 vs. 39.2°C, respectively as the weather became cooler at the end of the trial. During both weeks, vaginal temperature showed a hair type by day by time of day ( $P < 0.01$ ) interaction. In week one, slick-haired Holsteins had lower vaginal temperatures compared to those of normal-haired ones during the day as the ambient temperature rose throughout the day and similarly, during the night time, as body temperature increased as part of the normal circadian rhythm presented during the trial. Slick-haired Holsteins were able to resist increases in vaginal temperatures throughout week one as ambient temperature varied among days. A similar phenomena occurred during week five to a lesser extent as the

weather conditions were cooler than in week one. Slick-haired Holsteins were able to control their vaginal temperature similarly to that of week one, but the differences were smaller in magnitude due to the improving weather conditions. Dry matter intake between the slick-haired lactating cows and the normal-haired ones did not differ ( $P = 0.41$ ) slick = 21.5, normal = 22.3 kg DM/day. However, during the experiment normal-haired Holstein cows had a tendency ( $P = 0.07$ ) to produce more milk than the slick-haired ones, 28.8 vs. 24.7 kg/day, respectively. Measurements of vaginal temperatures in this study also indicated that slick-haired cows maintained lower core body temperatures than normal-haired cows exposed to elevated ambient temperatures for a four day period of time. The improved thermotolerance of the slick-haired cows indicates that slick-haired Holstein cows are able to regulate body temperature more effectively than normal-haired ones under heat stress, but results in this study show that slick-haired cows did not achieve higher dry matter intake under heat stress. It seems that slick-haired cows have more efficient heat dissipation mechanisms than normal-haired cows.

## CHAPTER 1 INTRODUCTION

Heat stress plays a significant role in cattle performance and likely will be of even greater importance in the future as climate change continues. Cattle are produced in a wide range of environments, some of which present thermal challenges to productive performance, even survival in extreme cases. Selection of cattle adapted to warm environments represents one strategy to mitigate the effects of heat stress. There are a number of environmental factors that contribute to heat stress; these include high temperature, high humidity and solar radiation. In tropical regions animals must be able to balance heat production and heat gain from their environments with dissipation of heat through the skin and respiratory surfaces; simultaneously, they must avoid excessive thermal energy incoming from the environment (DaSilva, 1999). All animals have a range of ambient environmental temperatures termed the thermo-neutral zone. This is the range of temperatures where the animals are able to regulate their body temperature without heat production as temperature changes.

One of the greatest challenges that dairy farmers face in the Southeastern United States is heat stress and the strain that it causes the lactating dairy cow. Conditions in this region are characterized by a relatively long warm/hot season during which an extended period of solar radiation occurs daily and which is accompanied by the presence of high relative humidity. Lactating dairy cows generally generate a large amount of metabolic heat and, in addition, accumulate additional heat from solar radiation. It is assumed that dairy cows in temperate regions that are exposed to episodic thermal stress may experience serious problems due to lack of a physiological adaptation to heat stress conditions (Beede and Collier, 1986; Beede and Shearer,

1996). Heat stress markedly reduces dry matter intake (DMI) and milk yield and because the decreased DMI precedes the reduction in milk production, it is generally accepted that reduced nutrient intake is primarily responsible for the diminished milk synthesis (Fuquay, 1981; Beede and Collier, 1986; West, 2003). Pregnancy rates of dairy cows have been shown to decrease from 55 to 10% when the temperature-humidity index (THI) increased from 70 to 84 (Ingraham et al., 1974). Other reproductive problems in dairy cows during warm weather include a decrease in intensity of estrus, failure to ovulate, lack of implantation, embryo disintegration, and fetal abortion (West, 2002). It has been shown that adequate regulation of body temperature is essential for surviving excessive heat load and involves both physiological and behavioral responses. Studies conducted in Florida (Hammond and Olson, 1994; Hammond et al., 1996, 1998), Puerto Rico and Venezuela (Lucena and Olson, 2000) have shown that breeds of cattle with short, sleek hair coats possess good heat tolerance, maintaining body temperatures up to 0.50°C lower than those of unadapted breeds.

Many cooling systems and nutritional strategies have been developed as management tools to alleviate some of the negative impact of thermal stress on dairy cattle, but production continues to be reduced during the summer. Almost all such environmental modifications come at a relatively high cost, and in many cases those expenses cannot be economically justified (Collier, 2007). It is possible, however, to select for both production and heat tolerance as a substantial amount of genetic variability for heat tolerance exists in dairy cattle (Ravagnolo and Misztal, 2000). This could be an economic advantage to producers if improved thermotolerance could be

accomplished without adversely affecting production. Accurately identifying heat-stressed cows and understanding the biological mechanism(s) by which thermal stress reduces milk synthesis and reproductive indices is critical for developing novel approaches (i.e. genetic, managerial and nutritional) to maintain production or minimize the reduction in dairy cow productivity during stressful summer months (Baumgard et al., 2006).

Olson et al. (2003) identified a gene (the *Slick hair* gene, *Sk*) that controls hair length in Senepol and Carora cattle. The gene has been mapped to chromosome 20 (Mariasegaram et al., 2007) and cattle that carry this gene have slick hair coats and experience lower respiration rates and body temperatures under heat stress (Olson et al., 2003, Dikmen et al., 2008). The thermoregulatory characteristic of animals associated with this phenotype is probably due to a lower metabolic rate, increased sensible heat or evaporative heat loss, more efficient transfer of heat to the surface, or a combination of these adaptations (Dikmen et al., 2008). Milk yields of Carora x Holstein (75% Holstein) cows in Venezuela were higher for animals expressing this gene than for contemporary half-siblings with normal-hair type (Olson et al., 2003).

The objectives of this study are to evaluate certain physical, metabolic and production responses of slick and normal-haired lactating Holstein cows experiencing heat stress conditions. The hypotheses of this study are: 1) slick-haired lactating Holstein cows are better able to regulate body temperature in the presence of heat stress than normal-haired ones and 2) slick-haired lactating Holstein cows are able to achieve a higher dry matter intake than normal-haired ones under heat stress.

## CHAPTER 2 LITERATURE REVIEW

### **Heat Stress**

Heat stress is a major source of production losses in the both dairy and beef industries. It has become one of the greatest challenges, and a limiting factor to dairy production systems in hot climates. Yousef (1985) defined stress as the magnitude of forces external to the body which tend to displace its systems from their resting or ground state. Therefore, heat stress in the dairy cow can be understood to include all temperature related forces that induce adjustments occurring from the sub-cellular level to the whole animal (Kadzere et al., 2002). The effort made by homeotherms to stabilize body temperature within narrow limits is crucial to control biochemical reactions and the physiological processes associated with normal metabolism (Shearer and Beede, 1990).

In tropical regions, where animals are exposed to thermal stress for long periods of time, cattle must be able to dissipate heat excess through the skin and from the respiratory surfaces. According to DaSilva (2000), the morphological characteristics of the skin (sweat glands, color, thickness) and of the hair coat (thickness, number of hairs per unit area, diameter of the hairs, length of the hairs) influence the ability of cattle to exchange heat with the environment, with sweating being the most efficient mechanism under hot and dry conditions.

During hot weather, numerous physiological changes occur in the digestive system of the dairy cow including acid-base chemistry and blood hormones. Some of these changes occur in response to reduced nutrient intake. Neurons that are temperature sensitive are located throughout the animal's body and send information to

the hypothalamus, which invokes numerous physiological, anatomical or behavioral changes in the attempt to maintain heat balance (Curtis, 1983). West (2003) has discussed the many changes that occur as a result of the strain caused by heat stress in the cow. Strain refers to an internal displacement from the resting or basal state brought about by external stress (Finch, 1984; Silanikove, 2000). Environmental factors such as ambient temperature, solar radiation, and humidity have direct and indirect effects on animals (Collier et al., 1982) and cause a substantial impact on livestock production around the world (Neinabor et al., 1999). These environmental factors constitute the main strains imposed upon cattle and can cause adverse physiological effects that eventually result in a decline in production, such as weight loss or infertility (Finch, 1984).

The impact on production and reproduction due to heat stress effects on the biological mechanisms are partly explained by reduced feed intake, but also include altered endocrine status, reduction in rumination and nutrient absorption, and increased maintenance requirements (Collier and Beede, 1986; Collier et al., 2005) resulting in a net decrease in nutrient energy availability for production.

Indices of environmental measurements that have been developed to assess their effects on animal physiology range from simple measurements of air temperature and humidity (Finch, 1984) to weighted indices like temperature humidity index (THI) (Ravagnolo et al., 2000; Ravagnolo and Misztal, 2000). But, THI values arguably are not the most precise measurement for determining heat stress in livestock due to the limited number of indicators involved in their calculation. Dikmen and Hansen (2008)

reported that dry bulb temperature is nearly as good a predictor of rectal temperatures of lactating Holsteins in a subtropical environment as THI.

### **Thermoneutral Zone (TNZ)**

The severity of heat stress is correlated to both ambient temperature and humidity level. As with other homeotherms, cattle have optimal temperatures within which they perform best and lactating dairy cows prefer ambient temperatures of between -20°C and 25°C, known as the thermoneutral zone (Berman et al., 1985). This is the range of temperatures where cattle regulate their body temperature without altering heat production (HP). Generally, the TNZ range (from lower critical temperature (LCT) to upper critical temperature (UCT) depends on age, species, breed, feed intake, diet composition, previous state of temperature acclimation or acclimatization, production, specific housing and pen conditions, tissue insulation (fat, skin), external insulation (coat), and behavior of an animal (Yousef, 1985).

### **Lower Critical Temperature (LCT)**

The ambient temperature below which the rate of HP of a resting homeotherm is not sufficient to maintain thermal balance is the LCT. The lower critical temperatures in the dairy cow were estimated from calorimetric studies as 2°C for non lactating cows, -4°C when producing 10 kg FCM (Fat Corrected Milk), and -10°C when producing 20 kg FCM (Hamada, 1971). Similar results were found from calorimetric studies in steers (Blaxter and Wainmann, 1961).

### **Upper Critical Temperature (UCT)**

The UCT is the air temperature at which the animal cannot control body temperature as a consequence of inadequate evaporative heat loss (Yousef, 1985). Berman et al. (1985) suggested that the upper limit of ambient temperatures at which

Holstein cows are able to maintain a stable body temperature is 25 to 26°C, and that above 25°C practices should be instituted to minimize the rise in body temperature.

The UCT can be inferred from thermoregulatory functions like increased sweating and respiratory water loss, and increased body temperature (Berman et al., 1985).

### **Effect of Heat Stress on Feed Intake**

Heat stress negatively impacts feed intake. A study conducted at the University of Arizona's ARC facility, however, showed that the reduction in DMI can only account for ~40-50% of the decrease in milk production when cows are heat-stressed and that ~50-60% can be explained by other heat stress-induced changes (Rhoads et al., 2007). The reduction in daily feed consumption caused by heat stress primarily occurs through a reduction in meal size, and sometimes even meal frequency (Nienaber et al., 1999).

In a early study conducted by Johnson and Ragsdale (1959), Holstein, Brown Swiss, and Jersey heifers were raised from one to thirteen months of age in environmental chambers with constant temperatures of 10 or 26.7°C. Holstein heifers raised in the 26.7°C environment were lighter than heifers in the cool environment by 8.2 kg at 3 months and 30.4 kg at 11 months of age. Also, it took the Holstein heifers in the warm environment 11.5 longer to reach 299 kg BW.

Collier et al. (1982) reported that the effects of heat stress in high producing lactating dairy cows result in dramatic reductions in roughage intake and rumination. Silanikove (1992) suggested that the reduction in appetite under heat stress is due to an increase in body temperature and may be related to gut fill

### **Effect of Heat Stress on Milk Production**

Heat stress reduces both DMI and milk yield; the decreased DMI precedes the reduction in milk production. It is generally accepted that reduced nutrient intake is

primarily responsible for the reduction in milk synthesis (Fuquay, 1981; Beede and Collier, 1986; West, 2003). Rhoads (2009), however, showed that the reduction in dry matter intake caused by heat stress accounted for only approximately 35% of the decrease in milk production. In addition to reduced nutrient intake, heat-stressed cows are thought to have increased  $\geq 30\%$  maintenance costs (Morrison, 1983; Huber, 1996; Fox and Tylutki, 1998) as maintaining homeothermia presumably has a large energetic cost.

Thermal load may directly affect milk yield by unknown mechanisms that are independent of reduced DMI, however, as heat-stressed cows do not display the typical metabolic profile of an animal on a lowered plane of nutrition (Shwartz et al., 2009). While it is known that heat stress affects cellular physiology and systemic metabolism, differentiation between the direct versus indirect (mediated by reduced feed intake) effects of thermal load is difficult.

Because of decreased energy availability and increased energy utilization, heat-stressed cows enter into a negative energy balance (EBAL; Moore et al., 2005). The heat stress-induced deficit in energy and nutrient availability is thought to limit milk synthesis during a thermal load. Milk synthesis and secretion are processes governed by several hormones and are sensitive to both physiological and environmental signals. Some of the most potent and well-characterized lactogenic hormones are members of the somatotrophic axis: somatotropin (ST) and IGF-I (Bauman and Vernon, 1993). During negative EBAL, the somatotrophic axis uncouples, meaning that hepatic IGF production decreases despite increased circulating ST concentrations (McGuire et al.,

1992). Thus, during times of somatotrophic axis uncoupling, ST remains galactopoietic via partitioning nutrients (dietary and tissue derived) toward milk synthesis.

### **Heat Dissipation Mechanisms**

Cattle regulate internal body temperature by matching the amount of heat produced by metabolism with the heat flow from the animal to the surrounding environment. Heat flow occurs through processes dependent on surrounding temperature (sensible heat loss, i.e. conduction, convection, radiation) and humidity (latent heat loss, i.e. cutaneous and respiratory evaporation). Under a low ambient temperature thermal energy is lost mainly as sensible heat due to the increasing temperature difference between the body and the environment (McLean 1963a; Gebremedhin and Wu 2001). On the other hand, if the environment is characterized by intense solar radiation, the body gains large amounts of heat by radiation (DaSilva 2000). In this situation the ability of the animal to withstand its environment is proportional to its ability to dissipate heat by evaporation from the skin surface as a result of sweating (Finch et al. 1982; McLean 1963b) or from the respiratory system by panting (Stevens, 1981; Maia et al., 2005).

### **Sensible Heat Loss**

Sensible heat losses from the body are governed by the temperature gradient and air velocity. The velocity of air movement affects the rate of convection and anything that resists air movement, such as excess hair in cattle, will decrease the rate of heat transfer by convection. The flow of heat by conduction depends on the temperature difference, the conductance (or inversely the resistivity) of the medium, and the area of contact (Schmidt-Nielsen, 1964). Heat exchange by radiation depends upon surface area as well as the reflective properties of the hair coat. Light-colored hair coats and

hair coats that are sleek and shiny reflect a greater proportion of incident solar radiation than hair coats that are dark in color or more dense and wooly (Hutchinson and Brown, 1969; Finch, 1986; Hansen, 1990).

### **Latent Heat Loss**

The proportion of metabolic heat that is dissipated from an animal's body by evaporation increases with rising environmental temperatures and a decreasing temperature gradient between the animal and air. Johnson (1976) showed that the differences in the ratio of evaporative cooling to total heat loss is species variable and that the evaporative ratio of cattle begins to increase markedly at 16.6 – 18.3°C. In hot climates the potential for non-evaporative heat loss is reduced and animals rely on the evaporation of water to dissipate any excess heat generated by metabolism (McArthur and Clark, 1988). However, the efficiency of evaporative cooling declines with an increase in relative humidity.

In cattle under heat load about 15% of the endogenous heat is lost directly from the body core via the respiratory tract (McDowell et al., 1976). In another study, Maia (2005) reported that heat loss by cutaneous evaporation accounted for 20–30% of the total heat loss when the air temperature remained between 10 - 20°C, the rest being lost as sensible heat. Under high environmental temperatures, close to or greater than body temperatures, as is often the case in tropical regions, however, sensible heat becomes a means of heat gain. Under this situation evaporation becomes the main avenue of heat loss, accounting for approximately 85% of the total heat loss, while the rest is lost by respiratory evaporation.

## **Thermoregulatory Reactions**

Dairy cows are adapted for optimum function within their particular thermal neutral environment. Any displacement from this ground state, as is the case during heat stress, threatens the normal metabolic balance of the animal. The further the animal moves away from its comfort zone, the greater its effect upon performance is likely to be. 'Metabolism and productivity run parallel' (Brody, 1945). Physiological measurements (heart rate, body temperature, respiration rate, etc.) can quantify the extent of the physiological displacement from the normal state; the greater the physiological response, the nearer the animal is to collapse (Yousef, 1985). Cows' physical responses to temperatures above the thermoneutral zone are varied. These include an increment in respiration rate and rectal temperature (Omar et al., 1996), panting, drooling, reduced heart rates, and profuse sweating (Blazquez et al., 1994), decreased feed intake (West, 2003), and reduced milk production (Abdel-Bary et al., 1992). The effects of hot, humid conditions are thought to be mediated through an effect on cow body temperature.

In addition to reducing feed intake during heat stress, cows also exhibit decreased activity, seek shade and wind, increase respiratory rate, and increase both peripheral blood flow and sweating. These responses have a deleterious effect on both the production and physiologic status of the cow (West, 2003).

### **Body Temperature**

Mammals have the homeothermic ability to control body temperature as a consequence of the thermoregulatory mechanisms of animals and the resistances to energy exchanges between them and the environment. Various parts of the body, however, have different temperatures. Rectal temperature (RT) and, to a lesser extent,

vaginal temperature (VT) are commonly used to assess bovine temperature. RT is an indicator of thermal balance and has been used to assess the adversity of the thermal environment such that growth, lactation, and reproduction of dairy cows can be affected (Johnson, 1980; Hahn, 1999; Hansen and Arechiga, 1999; West, 1999). A rise in body temperature of only 4.4°C above normal is often quickly fatal, and most mammals die at a core temperature of 42 - 45°C (Brody, 1948; Silanikove, 2000).

Core body temperature is tightly regulated such that even a small increase above 39°C is negatively correlated with milk production (Berman et al., 1985; Silanikove, 2000). Hahn (1995) showed that under hot environmental conditions, DMI is a function of core body temperature.

Finch (1986) determined differences between breeds in their ability to regulate RT, the mean RT is higher in *B. taurus* than in *B. indicus* cattle and *B. taurus* cattle are more sensitive to heat stress than their *B. indicus* counterparts. West et al. (2002) found that changes in cow body temperature (measured as milk temperature) were most sensitive to same day climatic factors.

The temperature of the central nervous system, in particular the brain, appears to be the most closely regulated deep body temperature as the function of the brain is very susceptible to temperature change. Tympanic temperature has been used as an indication of core temperature because the origin of the blood supply to the tympanic membrane and to the brain is the same (Robertshaw, 1985). Also, a high degree of association between tympanic temperatures and the feeding activities of cattle under varying ambient temperatures has been reported by Hahn (1999).

## Respiration Rate

Respiration rate (RR) is often used as a gross indicator of thermal load in cattle during hot weather and increases when animals need to maintain homeothermy by dissipating excess heat as other means became inadequate. Breaths per minute (BPM) from 20 to 60 are indicative of cattle under thermoneutral conditions, while 80 to 120 BPM is indicative of cattle under moderate thermal stress. Hahn et al. (1997) reported respiration rate responses from combined observations of *ad libitum*-fed crossbred *B. taurus* feeder cattle under ambient conditions. A threshold for markedly increasing RR was identified at an air temperature of 21.3°C, with RR increasing by 4.3 BPM per degree °C above a baseline of 60 BPM at the threshold temperature. Thatcher and Collier (1982) reported that the increase in RR from the thermoneutral phase to heat stress phase is a characteristic of heat-stressed animals that thermoregulate by increasing evaporative heat loss from the upper respiratory passages.

High ambient temperatures induce physiological adjustments, including increased respiration rate (Coppock et al., 1982). Johnston et al. (1959) reported increases from 20 BPM under cool conditions to 100 BPM or more at 32°C and above. During the summer, Holstein cows are less thermotolerant than either Jersey or Brown Swiss cows, having both higher body temperatures and respiration rates (Ruvuna et al. 1983; Correa-Calderón et al. 2004; Garcia-Peniche et al. 2005). Berman et al. (1985) found in high-producing dairy cows under a subtropical environment that the respiratory frequency started rising above 50–60 BPM at ambient temperatures higher than 25°C. Furthermore, increased respiration rate has been associated with increases in solar radiation (Spain and Spiers, 1996), with increased relative humidity (McLean, 1973), and with decreased wind speed (Mader and Davis, 2002). While individual variability

does exist among animals (Hahn et al., 1997), measurement of respiratory rates provides a noninvasive and practical assessment of stress in feedlot cattle due to hot environmental conditions.

Extreme respiratory ventilation, known as panting, is associated with very high levels of heat exposure (Robertshaw, 1985). Panting can be classified as rapid-shallow (first phase) or open-mouth (second phase) (NRC, 1981). A slight reduction in the respiration frequency during the days with highest environmental strains could be related to a shift in RR dynamics from rapid panting to a deep phase, open mouth panting which is slower (Gaughan et al., 2000).

### **Sweating Rate**

Sweating leads to evaporative heat loss from the skin surface, whereas in panting, heat is removed from the animal through the loss of vaporized moisture to the environment. Evaporation is an essential mechanism in thermoregulation in homeotherms. It could occur throughout the respiratory tract or at the surface of the skin, but there are large differences between species in the relative amounts of latent heat which can be lost via these two pathways. The proportion of latent heat which can be dissipated by sweating depends partly on the nature and activity of the sweat glands and partly on the extent to which the diffusion of water vapor into the air surrounding the animal is impeded by a hairy coat (Cena and Montieh, 1975).

At high temperatures, the most dominant mode of heat loss in dairy cows is through evaporative cooling (Gebremedhin and Wu, 2001). This mechanism is affected by wind velocity, ambient temperature, and relative humidity. There are other factors affecting the efficacy of evaporative cooling from the skin surface, such as fur and hair coat physical properties. Allen et al. (1970) recorded vapor pressure gradients in

sweating cattle coats and found that the hair close to the skin was seldom moist enough to contain free water. This implies that the evaporative cooling usually occurs at the skin surface. As air temperature approaches skin temperature, evaporation becomes the major route for heat exchange with the environment. Johnson (1976) showed differences between species in the ratio of evaporative cooling to total heat loss, and that the evaporative ratio of cattle begins to increase markedly at 16.6 – 18.3°C.

The proportion of metabolic heat that is dissipated from an animal's body by evaporation increases with rising environmental temperatures and a decreasing temperature gradient between animal and air (Silanikove, 2000). The greater evaporation rates required at higher ambient temperatures are decreased when air velocity on the body surface is low. Berman (2005) reported that at air velocities of 0.6 and 0.2 m/s, skin water loss is decreased to 88 and 60% of the maximal value, respectively. Ittner et al. (1951) showed a reduction in the skin and body temperatures by increasing the air velocities over pigs and cattle subjected to high ambient temperatures.

Allen (1962) showed that *B. indicus* cattle had significantly higher sweating rates than breeds from temperate regions. Schmidt-Nielsen (1964) reported that as the environmental temperatures rose, *B. taurus* cattle showed a noticeable increase in evaporation between 15 and 20°C, with a maximum rate of evaporation before 30°C. On the other hand, Brahman cows (*B. indicus*) had initially lower evaporation rates, but rapid evaporation rates occurred when temperatures were between 25 and 30°C, and continued increasing up to 40°C. Finch et al. (1982) later showed that sweating rates

increased exponentially in response to increased in body temperature in *B. indicus* cattle, while *B. taurus* sweating rates tend to plateau after an initial increase.

Gabremedhin and Hilman (2007) showed that direct sunlight exposure to the skin in cattle caused by shaving off areas of the hair coat profoundly increased the sweating rate. The sweating rate of black-shaved skin was 1.84 times higher than that of an unshaved black hair coat. Similarly, sweating rate of the white shaved skin was 1.64 times that of unshaved the white hair coat. According to this study, hair coat acts as a barrier, becoming an obstruction to evaporation by holding a thin film of water close to the skin, thus decreasing the moisture gradient between the skin surface and ambient air. Dikmen et al. (2008) reported differences in sweating rate between hair types were a direct result of hair length. Sweating rate was greater for the clipped area than for the unclipped area, and greater in an outdoor, more stressful, environment.

## **Hormones**

Hormones known to be homeorhetic regulators are also implicated in acclimatory responses to thermal stress. These include thyroid hormones, prolactin, somatotropin, glucocorticoids and mineralcorticoids. Triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) are hormones associated with metabolic homeostasis and susceptible to climatic changes (Perera et al., 1985). Johnson et al. (1988) also showed declines in thyroid hormones  $T_3$  and  $T_4$  in response to heat stress which are an attempt to reduce metabolic heat production in the cow. Scott et al. (1983) reported a negative relationship between plasma  $T_4$  concentration and rectal temperature and also that initiation of night cooling at the time that rectal temperature was highest was the most beneficial mechanism to maintain thermoneutral plasma  $T_4$  concentration. McGuire et al. (1991) reported a tendency for plasma somatotropin to decline with heat stress but no difference due to

restricted DMI, while  $T_3$  concentration declined with both heat stress and with restricted intake. Other studies have shown declines in  $T_3$  and  $T_4$  when cows were exposed to high ambient temperatures (Magdub et al., 1982).

Stress response hormones (glucocorticoids) are elevated during initial heat stress exposure and then become depressed with prolonged periods of thermal stress. Recent studies (Itoh et al., 1998; Wheelock et al., 2006) have demonstrated that heat stress elevates plasma insulin concentrations in lactating dairy cows and this may be important for glucose disposal in peripheral tissues.

### **Blood Flow**

Cattle under heat stress lose part of the heat load through the respiratory tract. Another substantial portion is transferred to the skin where it is then dispelled by various mechanisms. A cow reaches thermal balance under hot conditions first by vasodilatation, primarily on the trunk (Ehrlemark and Sallvik, 1996), and then by increasing evaporative heat loss from the skin surface through sweating. Blazquez et al. (1994) reported that increased blood flow to the skin is positively correlated with the sweating rate. As the skin temperature approaches core temperature, the flow of heat to the environment decreases and heat amasses, elevating body temperature (Finch, 1986).

Cows that were fed *ad libitum* in a thermal comfort environment, fed *ad libitum* in a thermal stress environment, or fed a restricted intake in a thermal comfort environment had similar milk yields in both the restricted intake and thermal stress treatments, and mammary blood flow tended to be lower as compared with *ad libitum*-fed cows maintained in thermal comfort, suggesting blood flow was responsive to level of DMI (Lough et al., 1990). For cows exposed to similar treatments as those of Lough

et al. (1990), portal plasma flow was reduced about 14% for cows in thermal comfort with restricted intake and in thermal stress when compared with thermal comfort, *ad libitum* fed cows (McGuire et al., 1989).

### **Coat**

Hair coat characteristics affect the transfer of thermal energy from the skin to the environment and consequently, body temperature control. Dowling (1955) reported differences in temperature control between groups of cattle which were associated with differences in coat type. The “hairier” group showed a very low degree of hair medullation which was, in a subsequent study, highly correlated with rectal temperature. Dowling further suggested that shorter, thicker, medullated hairs, which also were stiffer, would improve air movement to the skin surface and, thus, allow greater opportunity for evaporative cooling. Turner and Schleger (1960) have indicated that visual hair scores (Table A-1) are highly heritable and associated with growth in warm climates. The heritability of hair score within cattle of British breeding, however, was found to be much higher (63%) than that found for zebu crossbred cattle (24%).

Cena and Monteith (1975) showed that in a less-dense coat, the transmission coefficient of solar radiation was greater than in denser ones. However, contrary results occurred with the absorption coefficient. Thus, these authors defined the effective reflectivity of the coat as the fraction of the incident energy that is reflected by coat and skin together; the effective absorptivity as the amount of incident energy that is absorbed by the coat; and the effective transmissivity as the amount of energy that is transmitted through the coat to the skin surface.

When an animal is exposed to the sun, an extremely steep temperature gradient is established between the hair coat surface and the skin. A short, sleek, thin coat

improves heat and water vapor conductance through the coat layer in stressful hot, humid environments (Gebremedhin et al., 1997; Turnpenny et al., 2000). The thickness of the coat layer is greater for animals bred in temperate regions, sometimes more than 15 mm. However, those of the same breed but acclimatized to tropical climates present very thin coats, less than 8 mm deep (Berry and Shanklin, 1962; Veiga et al., 1964; Pinheiro and DaSilva, 2000). Coat depth also seems to be important, Turnpenny et al. (2000) confirmed through sensitivity analysis that, in addition to air temperature, coat depth and wind speed are significant determinants of heat loss from both sheep and cattle. For both species, the effects of changing coat depth are most marked at low depths. An increase in coat depth from 3 to 10mm reduced the sensible heat loss from cattle by 17% at 20°C.

Hair density is another characteristic of the hair coat and is quantified as the number of hairs per unit of skin surface area. The density of the hair coat has been demonstrated to be an important factor in resistance to the attack of ectoparasites which is important in many tropical regions of the world (Peters et al., 1982). Observations made in the field show that Zebu and Criollo (shorter-haired) cattle, suffer less tick infestation than European *B. taurus* cattle (longer-haired) (Bonsma, 1981). Studies conducted in Arkansas found that an increase of hairs per cm<sup>2</sup> and the corresponding increase in the amount of sebum present on cattle skin and hair help to reduce horn fly infestation in steers from different breeds under feedlot conditions (Steelman et al., 1997).

There is evidence that hair color influences the susceptibility of the cow to heat stress because coat color is related to the amount of heat absorbed from solar radiation.

Borges and Silva (1986) observed that cows with predominantly white coats spent less time grazing under sunlight in tropical regions; this finding was explained by the association of white and coat of Holstein cows with absence of skin pigmentation. On the other hand, in Florida, Hansen (1990) conducted a study where he characterized Holsteins cows into two categories: greater than 70% white or greater than 70% black, and the white cows had slightly lower body temperatures and greater milk yield, regardless of whether they were under shade or no shade conditions (Hansen, 1990).

The consensus of several researchers is that a short, sleek or glossy hair coat over a pigmented skin that is kept soft and flexible is most appropriate for warm climates, and is a common characteristic of Criollo breeds of cattle from the tropical Americas. Some of such breeds are the Barroso from Guatemala (Melgar et al., 1984); the Romosinuano, the Blancorejinegro, and the Sanmartinero from Colombia (Martinez Correal, 1992, 1998; Martinez Correal and Gonzales Henao, 2000), the Yacumeño in Bolivia (Wilkins, 1985) and the Tropical Dairy Criollo from Costa Rica described by De Alba as cited by Wilkins (1985).

### **The Slick-Hair Gene**

A novel genetic approach to alleviate the impact of heat stress on cattle performance was suggested by Olson et al. (1997) who identified the existence of a major gene affecting hair length that appeared to be present in the Senepol breed and in some Criollo breeds. This gene seems to be responsible for the expression of an extremely short hair coat type that was called slick. Previous studies had shown that Senepol cattle are equal in heat tolerance to Brahman cattle and more heat tolerant than Angus and Hereford cattle (Hammond and Olson, 1994; Hammond et al., 1996). Rectal temperatures of Senepol cattle under heat stress were often 0.5° C lower than

Angus and Hereford cattle (Hammond et al., 1996). Senepol F1 crossbreds with temperate breeds showed heat tolerance comparable to those of Brahman and Brahman crossbreds (Hammond and Olson, 1994; Hammond et al., 1996; 1998). Crosses of Senepol with Angus and Hereford were subsequently found to be similar in heat tolerance to Senepol (Hammond et al., 1996; 1998). Observation of hair coat types in calves of Senepol x Angus crossbred cows indicated that they were segregating either for a short, sleek hair like that of purebred Senepol or for normal longer hair, typical of *B. taurus* cattle. This evidence led to the conclusion that a major gene for hair type that was dominant in mode of inheritance was responsible for the slick hair coats found in Senepol and likely, other cattle breeds (Olson et al., 2003).

The *Slick hair* gene also seems to be responsible for the greater heat tolerance of animals with this trait. Studies conducted in Florida (Hammond and Olson, 1994; Hammond et al., 1996, 1998), Puerto Rico and Venezuela (Lucena and Olson, 2000) have shown that breeds of cattle with short, sleek hair coats possess good heat tolerance, maintaining rectal temperatures up to 0.5°C lower than those of unadapted, temperate breeds. The increased tolerance to higher heat loads of cattle with this hair type was evident in cattle with better growth rates (Olson et al., 1997) and higher milk production compared to cattle of the same breed composition but with normal hair (Lucena and Olson, 2000). Weight gain from July to December of twenty-eight ¼ Senepol: ¼ Hereford: ½ Angus calves, normal-haired and slick-haired, was evaluated by Olson et al. (1997). They found the slick-haired animals heavier ( $P < 0.02$ ) by 13 kg than the contemporaneous normal-haired ones at the end of the trial.

Dikmen et al. (2008) reported the impact of the slick gene on sweating rate of ten slick-haired Holstein cows compared to ten normal-haired Holstein cows. Sweating rate was greater for slick-haired cows than for normal haired cows in unclipped areas, but lesser for slick-haired cows than for normal haired cows in clipped areas. Also, the difference between indoor and outdoor (greater heat stress) environments was greater in the clipped area than in the unclipped area.

### CHAPTER 3 MATERIALS AND METHODS

Data were collected at the Dairy Research Unit (DRU) of the University of Florida on slick (n=11) and normal-haired (n=10) lactating Holsteins cows from September to October 2007 to evaluate the effect of heat stress on their feed intake and other performance traits. Slick-haired cows averaged 164 days in milk (DIM), ranging from 45 to 253 DIM, while the normal-haired cows averaged 160 DIM, ranging from 56 to 226 DIM. Three of the slick-haired cows as well as one of the normal-haired cows were sired by the elite Holstein sire, Oman, and out of 7/8 Holstein slick-haired dams. An additional three of the slick-haired cows were sired by a 7/8 Holstein bull that was heterozygous for the *Slick hair* gene and out of 7/8 Holstein cows that were paternal half-siblings of some of the other cows in the study, both slick and normal-haired. There were no normal-haired cows in the study sired by this bull, however. The remainder of the slick-haired cows were from Holstein cows and were sired by three different 3/4 Holstein:1/4 Senepol bulls, each one heterozygous for *Slick hair* gene. Another three of the normal-haired cows used in the study were sired by two different unproven slick-haired Holstein bulls and thus siblings of the some of the slick-haired cows in the study while the remaining six normal-haired cows were unrelated cows from the DRU herd. The unrelated normal-haired cows were selected to be comparable in parity, stage of lactation, days in milk, and milk yield to the slick-haired cows that were available.

Cows were maintained in a free-stall barn containing fans and sprinklers. The barn was equipped with Calan<sup>®</sup> gates (American Calan, Inc., Northwood, NH). Each Calan<sup>®</sup> gate was opened only by a single cow as determined by a transmitter suspended from its neck. Cows were trained to use Calan gates for 10 d prior to the

beginning of the trial. The diet was formulated to meet NRC requirements (NRC, 1989) for lactating dairy cows using a total mixed ration (TMR) composed of corn silage, legume hay and concentrate mixed at 38, 8 and 54% of the dietary DM, respectively (Table B-1). The TMR was fed individually to cows twice daily at about 0900 and 1400 h on an *ad libitum* basis; feed offered was adjusted daily to ensure approximately 10%orts. Two representative samples of the TMR were collected per week and dried using a forced air oven (60°C) for further DM determination. Feed refusals were removed daily at 0600 h, subsampled, and dried by forced air oven (60°C) for further DM determination. Diets were fed to the cows using 250-kg Calan Data Rangers (American Calan Inc., Northwood, NH). Intakes of individual cows were recorded daily for a 33 d period. Individual feed consumption was determined on a DM basis. Cows were milked at approximately 0930 and 2000 h daily

### **Environmental Measurements**

Daily environmental measurements were obtained from the FAWN station, located in Alachua, FL. On days when rectal temperature, respiration rate, and sweating rate were measured, air temperature, relative humidity, and wind speed were recorded with a Kestrel® 3000 Pocket Weather Meter (Nielsen-Kellerman. Boothwyn, PA). The temperature-humidity index (THI) was calculated according to the equation reported by Ravagnolo et al. (2000):

$$\text{THI} = (1.8 \times T + 32) - [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T - 26)]$$

where T = dry bulb temperature (°C) and RH = relative humidity (%).

### **Physical Measurements**

Rectal temperature (RT), respiration rate (RR), and sweating rate (SR) were recorded weekly in the morning at 0800 h and in the afternoon at 1430 h each Saturday

and Sunday for a four-week period. RT measurements were made with Hi-Performance Digital Thermometers (GLA M525/550, GLA Agricultural Electronics, San Luis Obispo, CA) using a 21 mm probe. RR was determined as breaths per minute based on observations of the number of flank movements in a 15 second period as recorded with a chronometer which were standardized to a minute. SR was measured on two areas of the right shoulder: an unclipped area and a closely clipped and brushed area on each of cows while under shade. Hair was clipped using an Oster Power Pro hair clipper with an Opti-Block blade kit (Oster Professional Products, McMinnville, TN). While these measurements were being taken, the cows were held in a shaded holding area near the Calan gate facility at the Dairy Research Unit in Hague, FL. Sweating rates were measured directly using a hand-held closed-chamber VapoMeter (Delfin Technologies Ltd., Kuopio, Finland). The VapoMeter was pressed directly on the animal where the evaporation measurement was desired. The instrument measured water evaporation in g/h-m<sup>2</sup>. Individual body weights (BW) were recorded once every week for a five-week period. Individual body condition score (BCS) of the cows was recorded twice, at the beginning of the trial and at the end of the trial; a scale from one to five was used to score the animals' degree of fatness.

Vaginal temperature was recorded using a iButton<sup>®</sup> (Data logger, Maxim Integrated Products, Inc., Sunnyvale, CA) attached to a blank (i.e., without progesterone) controlled, internal, drug-releasing device (CIDR<sup>®</sup>, Pfizer Animal Health, New York, NY) that was inserted into the vagina for a five-day period during two different periods of the study. The first vaginal temperature measurement period was from September 5 - 10, 2007 at the beginning of the trial and the second measurement

period was from September 28 - October 3, 2007 at the end of the trial. Vaginal temperatures were recorded continuously at approximately 15 minute intervals for each five day period.

### **Metabolic Measurements**

Chromic oxide ( $\text{Cr}_2\text{O}_3$ ) was used as an external marker for determination of apparent digestibility (AD). Chromic oxide powder (Fisher Scientific, Fairlawn, NJ) was weighed into gelatin capsules (Jorgensen Lab. Loveland, CO) and dosed twice daily with a balling gun (10 g/dose at 0700 and 1800 h) for 14 consecutive days. The assessment period was divided into seven days of adaptation followed by seven days of fecal collection. Fecal samples (approximately 150 g) were collected daily, AM and PM, during the last seven days of the dosing period. Feces were dried using a forced air oven at 60°C, ground to pass through a 1-mm screen in a Wiley mill, and a composite subsample was taken from all 14 fecal samples per cow. Chromium concentration in feces was determined using a Perkin Elmer 5000 (Wellesley, MA) atomic absorption spectrometer, according to the procedure described by Williams et al. (1962). Apparent digestibility of DM was calculated by the marker ratio technique (Schneider and Flatt, 1975). Individual cow's records for DMI, milk production, BW, lactation, days in milk were used to determine animals' energy balance (EB) status using the NRC Dairy Cattle Program Version 1.0 (NRC, 2000) software.

### **Production Measurements**

Individual milk production and DMI records from cows were grouped on a weekly basis to determine feed efficiency which was defined as kg of milk/kg of DMI. Voluntary feed intake was calculated from the difference between the weight of the feed offered and the refused portion on a dry matter basis. Individual milk production records

(kg/day) were obtained daily throughout the study using the PCDART Herd Manager (Dairy Records Management Systems, 2007) software.

### Statistical Analysis

Data were analyzed using the PROC MIXED and PROC GLM procedure of SAS (v.9.1.3.; SAS Institute Inc., Cary, NC). Rectal temperature, RR, SR were analyzed with SAS, using the MIXED procedure and the following mathematical model:

$Y_{ijkl} = \mu + T_i + D_j + M_k + TD_{ij} + TM_{ik} + TDM_{ijk} + C_l(T_i) + E_{ijkl}$  where:

$\mu$  = general mean,

$T_i$  = treatment effect (fixed effect),

$D_j$  = day effect (fixed),

$M_k$  = time effect (fixed),

$TD_{ij}$  = treatment by day interaction effect (fixed),

$TM_{ik}$  = treatment by time interaction effect (fixed),

$TDM_{ijk}$ : = treatment by day by time interaction effect (fixed),

$C_l$  = cow nested within T effect (random effect), and

$E_{ijkl}$  = experimental error.

Vaginal temperature was analyzed with SAS, using the MIXED procedure and the following mathematical model:

$Y_{ijkl} = \mu + T_i + D_j + TD_{ij} + C_k(T_i) + E_{ijk}$  where:

$\mu$  = general mean,

$T_i$  = treatment effect (fixed effect),

$D_j$  = day effect (fixed),

$TD_{ij}$  = treatment by day interaction effect (fixed),

$C_k$  = cow nested within T effect (random effect), and

$E_{ijk}$  = experimental error.

Feed efficiency and body weight were analyzed on a weekly basis with SAS, using the MIXED procedure and the following mathematical model:

$Y_{ijkl} = \mu + T_i + W_j + P_k + TW_{ij} + TP_{ik} + C_l(T_t) + E_{ijkl}$  where:

$\mu$  = general mean,

$T_i$  = treatment effect (fixed effect),

$W_j$  = week effect (fixed),

$P_k$  = parity effect (fixed),

$TW_{ij}$  = treatment by week interaction effect (fixed),

$TP_{ik}$  = treatment by parity interaction effect (fixed),

$C_l$  = cow nested within T effect (random effect), and

$E_{ijkl}$  = experimental error.

Dry matter intake and milk yield were analyzed on a daily basis with SAS, using the MIXED procedure and the following mathematical model:

$Y_{ijkl} = \mu + T_i + D_j + P_k + TD_{ij} + TP_{ik} + C_l(T_i) + E_{ijkl}$  where:

$\mu$  = general mean,

$T_i$  = treatment effect (fixed effect),

$D_j$  = day effect (fixed),

$P_k$  = parity effect (fixed),

$TD_{ij}$  = treatment by day interaction effect (fixed),

$TP_{ik}$  = treatment by parity interaction effect (fixed),

$C_l$  = cow nested within T effect (random effect), and

$E_{ijkl}$  = experimental error.

The covariance structures used in these analyses were auto regressive AR (1) and compound symmetry (CS).

Apparent DM digestibility was analyzed with SAS using the GLM procedure and the following mathematical model:

$Y_{ij} = \mu + T_i + P_j + TP_{ij} + E_{ij}$  where:

$\mu$  = general mean (fixed),

$T_i$  = treatment effect (fixed),

$P_j$  = parity effect (fixed),

$TP_{ij}$  = treatment by parity effect (fixed), and

$E_{ij}$  = experimental error (fixed)

A PDIFF statement was used to detect differences between means. Significance was declared at  $P < 0.05$  and tendencies at  $P < 0.10$ .

## CHAPTER 4 RESULTS

### **Environmental Conditions**

Data collected over a 33 day period were used in the analyses. The highest ambient temperature during the trial was 33.1°C on September 13. That same day THI averaged 75 and the average relative humidity was 77%. The lowest ambient temperature was 16.7°C on September 30 when the average THI was 68 and the average relative humidity was 62%. Table C-1 shows daily values for ambient temperature, relative humidity, precipitation, solar radiation, wind speed, and THI throughout the experiment. Ambient temperature, THI, and relative humidity averaged 24.3°C, 74, and 80%, respectively, throughout the experiment.

### **Physiological Responses**

RT was lower ( $P < 0.01$ ) in slick-haired than normal-haired cows. The mean RT for the slick-haired cows was 38.6 vs. 39.0°C for the normal-haired cows. There were hair type by day ( $P < 0.01$ ), hair type by time of day ( $P = 0.08$ ) and hair type by day by time of day ( $P < 0.01$ ) interactions. Slick-haired Holsteins had lower RT each of the days of data collection. Slick-haired Holsteins had lower RT than normal-haired ones during both the AM and PM measurement times. The highest RTs were recorded in the afternoon for both slick and normal-haired cows, 38.8 vs. 39.2°C, respectively. The magnitude of the difference in both AM and PM in RT between the slick and normal-haired Holsteins, however, varied based on the daily ambient temperatures (Figure 4-1).

RR was lower ( $P < 0.01$ ) for the slick-haired Holsteins than for the normal-haired cows. The mean RR for the slick-haired cows was 61 vs. 72 BPM for the normal-haired ones. There was a day ( $P < 0.01$ ) and time of day ( $P < 0.01$ ) effect. Slick-haired cows

maintained lower RR than normal-haired ones each sampling day. Also, RR were greater in the afternoon as the temperature rose (slick = 67, normal = 78) than in the morning (slick = 55, normal = 66); open-mouth panting was not observed at any time. There were no hair types by time of day interactions as the slick-haired cows averaged 11 BPM less than the normal-haired cows both during both the AM and PM measurements, but there was a hair type by day by time of day ( $P < 0.01$ ) interaction (Figure 4-2). Slick-haired Holstein cows had lower RR than normal-haired cows each day regardless of the time of day that the measurements were taken so the three-way interaction apparently was caused by variations in the magnitude of the differences in RR due to daily fluctuations in ambient temperatures

There was no difference ( $P = 0.89$ ) in sweating rate between clipped and unclipped areas of the cows in this study (Figure 4-3). Slick-haired cows tended to sweat slightly more in the clipped areas than did the normal-haired cows ( $P = 0.16$ ) while in the unclipped areas the difference between hair type was much less, 28.1 vs. 25.3 g/m<sup>2</sup>h and 27.92 vs. 26.2 g/m<sup>2</sup>h, respectively ( $P = 0.43$ ). SR measured in both clipped and unclipped areas was affected by day ( $P < 0.01$ ). Time of day had also an impact ( $P < 0.01$ ) on SR in both the clipped areas (AM = 18.2 and PM = 35.2 g/m<sup>2</sup>h) and unclipped areas (AM = 18.4 and PM = 35.8 g/m<sup>2</sup>h). On the other hand, hair type by day interaction did not have an effect on SR in the clipped areas ( $P = 0.18$ ), but it did have an effect on SR in the unclipped areas ( $P = 0.03$ ). SR in the unclipped areas was greater during the hotter days for slick-haired cows than for normal-haired in unclipped areas, except on two data collection days when the average temperatures were cooler relative to other sampling days. Furthermore, there was a hair type by day by time of

day interaction ( $P < 0.01$ ) in both the clipped and unclipped areas. Slick-haired cows increased their sweat production more than normal-haired cows from the AM to the PM measurements, except on two days where temperatures were cooler (Figure 4-4).

VT was measured continuously for five consecutive days at the beginning of the trial (week one) and at the end of the trial (week five). During week one average ambient temperature was 24.4°C, average relative humidity was 76%, and the average THI was 73. VT was lower ( $P = 0.04$ ) for slick-haired cows than the normal-haired ones, averaging 39.3 vs. 39.6°C, respectively. Day and time of day affected VT ( $P < 0.01$ ) throughout week one. During this period of time, VT showed a diphasic circadian pattern characterized by a higher body temperature from midnight to early morning, a decline around midmorning, a rise during afternoon until evening, followed by a second trough until it increased again about midnight. There was hair type by day by time of day interaction ( $P < 0.01$ ) as slick-haired cows had lower VT throughout the day during week one, averaging 0.3°C lower VT than the normal-haired cows, but the difference in magnitude of the difference in VT in slick- vs. normal-haired Holstein cows changed as the ambient temperature rose during the day and was not the same each day (Figure 4-5). In addition, a similar phenomenon occurred during the night, where the slick-haired Holstein cows maintained lower VT than the normal-haired cows as both types experienced an elevation in core body temperature due to circadian rhythms. In contrast, during week five, when average ambient temperature was 22.9°C, average relative humidity was 81%, and the average THI was 72, VT did not differ ( $P = 0.56$ ) between slick and normal-haired Holstein cows, averaging 39.1 vs. 39.2°C, respectively. But, there was a day and time of day effect ( $P < 0.01$ ). During this period

of time, VT also showed a diphasic circadian pattern similar to that found in week one. Again there was hair type by day by time of day interaction ( $P < 0.01$ ). Slick-haired Holsteins had lower VT compared to normal-haired ones as the ambient temperature rose during the day. Similarly, during the night time as the normal increase in body temperature took place due to circadian rhythm, the slick-haired Holstein were able to maintain a lower VT even as the average ambient temperatures were lower compared to week one. During the last week of the study daily ambient temperature fluctuated considerably. The difference in magnitude in VT between the slick and normal-haired Holstein did not vary as much as in week one as the ambient temperature rose during the day (Figure 4-6).

### **Metabolic Responses**

Dry matter digestibility did not differ ( $P = 0.91$ ) between slick-haired and normal-haired cows. In addition, parity did not affect ( $P = 0.49$ ) dry matter digestibility. Apparent DM digestibility for the slick and normal-haired Holstein cows were 72.5 vs. 72.4%, respectively.

An energy balance analysis was conducted to evaluate the energy status of the cows throughout the trial. Both treatments had a positive energy status. There were no statistical differences between hair types ( $P = 0.21$ ); slick = 9.1 and normal = 7.2 Mcal/day. Also, parity did not have an effect ( $P = 0.52$ ); primiparous = 7.7 and multiparous = 8.6 Mcal/day (Table 4-1).

### **Production Responses**

There were no differences ( $P = 0.89$ ) between hair types for digestible dry matter intake; slick = 16.3, normal = 16.2 kg DM/day. Similarly, voluntary DM intake did not differ ( $P = 0.40$ ) between the slick-haired and normal-haired cows, 21.5 vs. 22.3 kg

DM/day, respectively (Figure 4-7). As expected, parity had an effect ( $P = 0.01$ ) on DM intake; multiparous cows ate more than primiparous ones, 23.0 vs. 20.8 kg DM/day, respectively. Body weight change did not differ ( $P = 0.34$ ) between hair type treatments; slick = 0.10 vs normal = 0.45 kg/day. Daily milk production tended to be higher ( $P = 0.07$ ) for the normal-haired cows than the slick-haired cows, 28.8 vs. 24.7 kg/day, respectively, throughout the experiment (Figure 4-8), but there was no difference between the hair type groups among half-sibs ( $P = 0.31$ ). There was a day effect ( $P < 0.01$ ) on milk yield as the weather conditions were changing constantly throughout the trial. However, parity did not have an effect ( $P = 0.26$ ) on milk production, primiparous = 25.6 and multiparous = 27.9 kg/day. The hair type by parity interaction also failed to approach significance ( $P = 0.26$ ). Likewise, feed efficiency did not differ ( $P = 0.14$ ) between slick and normal-haired Holstein cows, 1.2 vs. 1.3 kg milk/kg DM intake, respectively. Week did have an effect ( $P < 0.01$ ) on feed efficiency throughout the study; both hair types were more efficient at the beginning of the trial, averaging 1.4 kg milk/kg DM intake, than at the end of the trial, 1.1 kg milk/kg DM intake as the cows continued into later stages of their lactations.

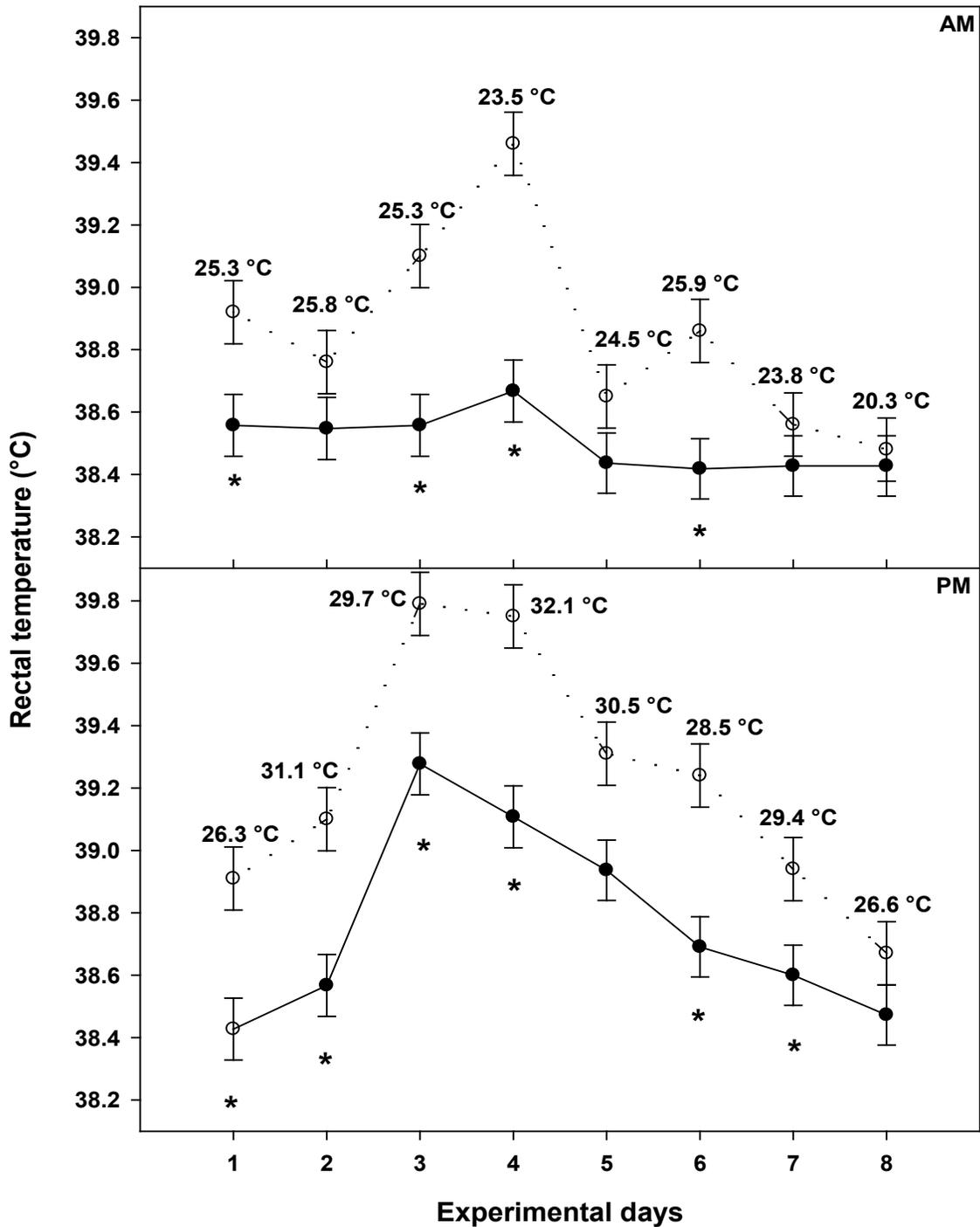


Figure 4-1. Rectal temperatures AM and PM of slick (closed circles, solid line) and normal-haired Holsteins (open circle, dashed line). Data are least square means  $\pm$  SEM. Rectal temperature was affected by hair type ( $P < 0.01$ ), day ( $P < 0.01$ ), time of day ( $P = 0.08$ ), and hair type by day by time of day ( $P < 0.01$ ).

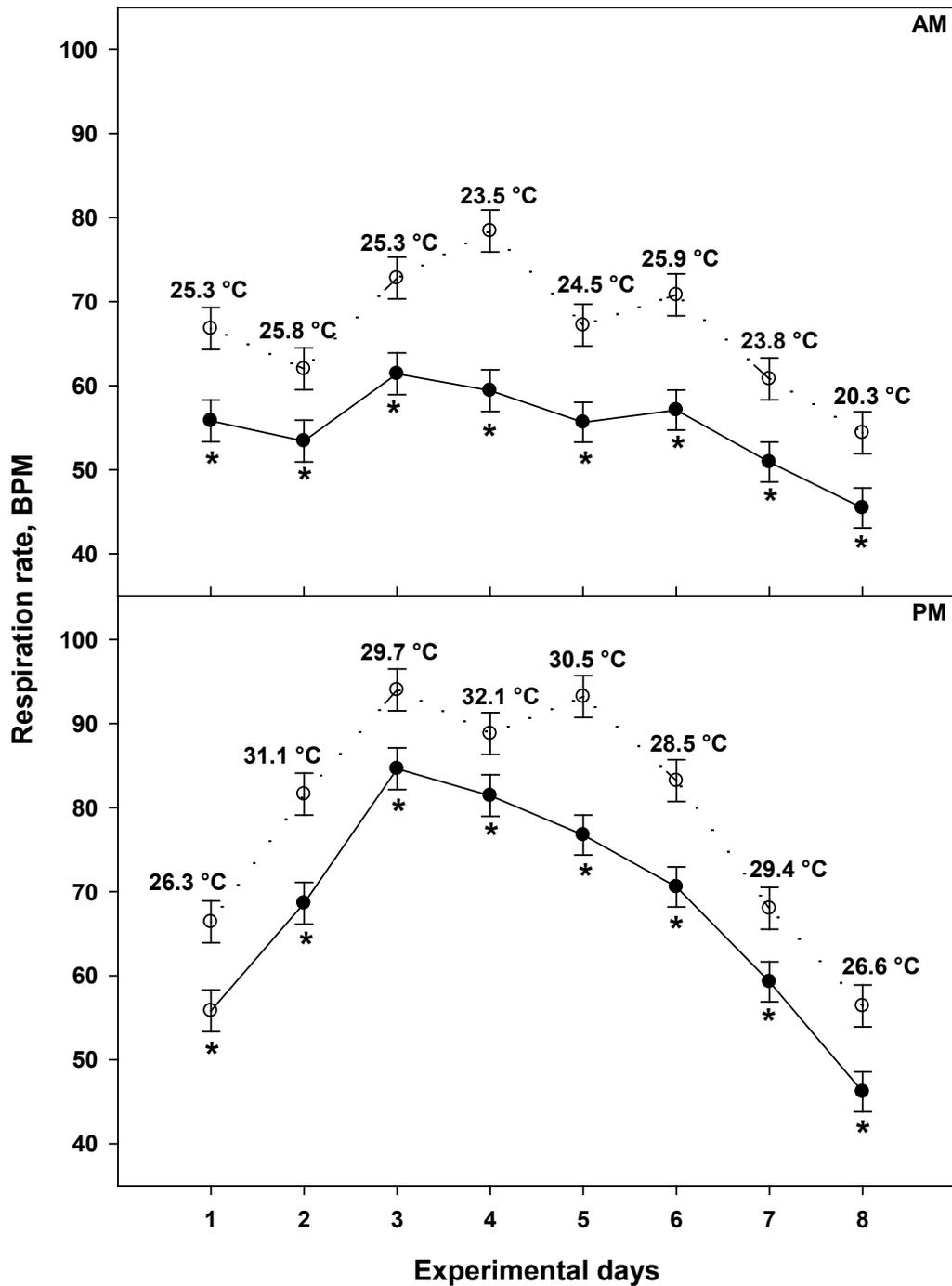


Figure 4-2. Respiration rates AM and PM of slick (closed circles, solid line) and normal-haired Holsteins (open circle, dashed line). Data are least square means  $\pm$  SEM. Respiration rate was affected by hair type ( $P < 0.01$ ), day ( $P < 0.01$ ), time of day ( $P < 0.01$ ), and hair type by day by time of day ( $P < 0.01$ ).

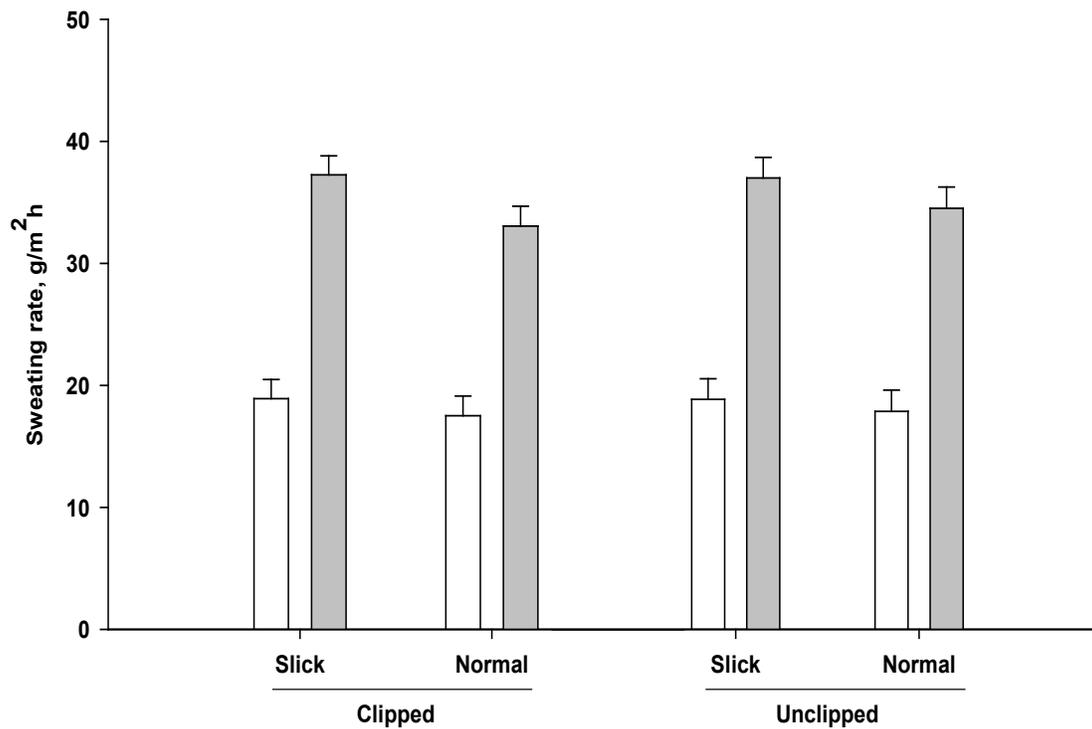


Figure 4-3. Sweating rates of clipped and unclipped area of slick and norma-haired Holstein cows. Data are least square means  $\pm$  SEM. Sweating rate was affected in both areas by day ( $P < 0.01$ ), and time of day ( $P < 0.01$ ).

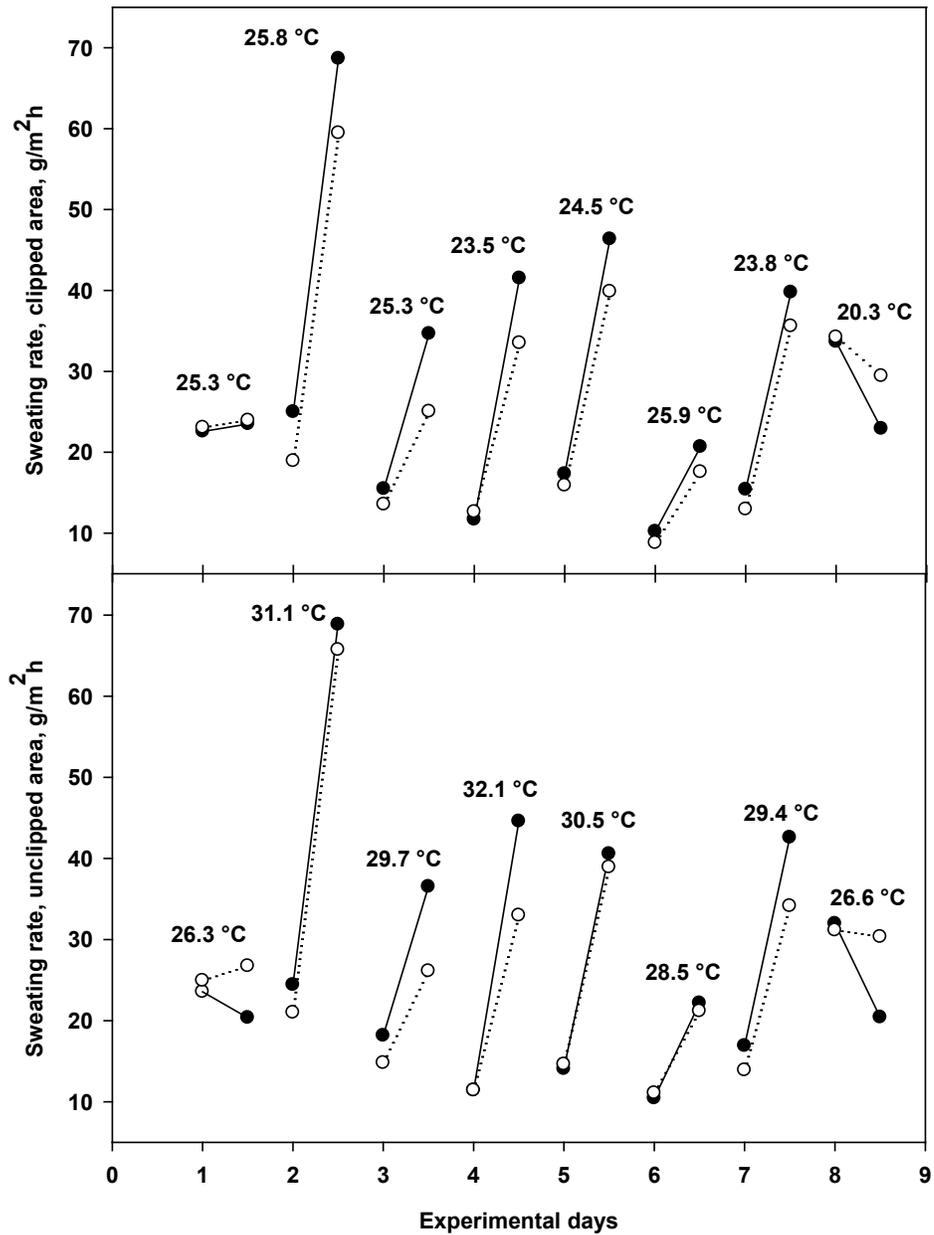


Figure 4-4. Sweating rates AM and PM of clipped and unclipped areas of slick (closed circles, solid line) and normal-haired (open circles, dashed line) Holstein cows. Data are least square means  $\pm$  SEM. Sweating rate was affected in both areas by hair type by day and by time of day ( $P < 0.01$ ).

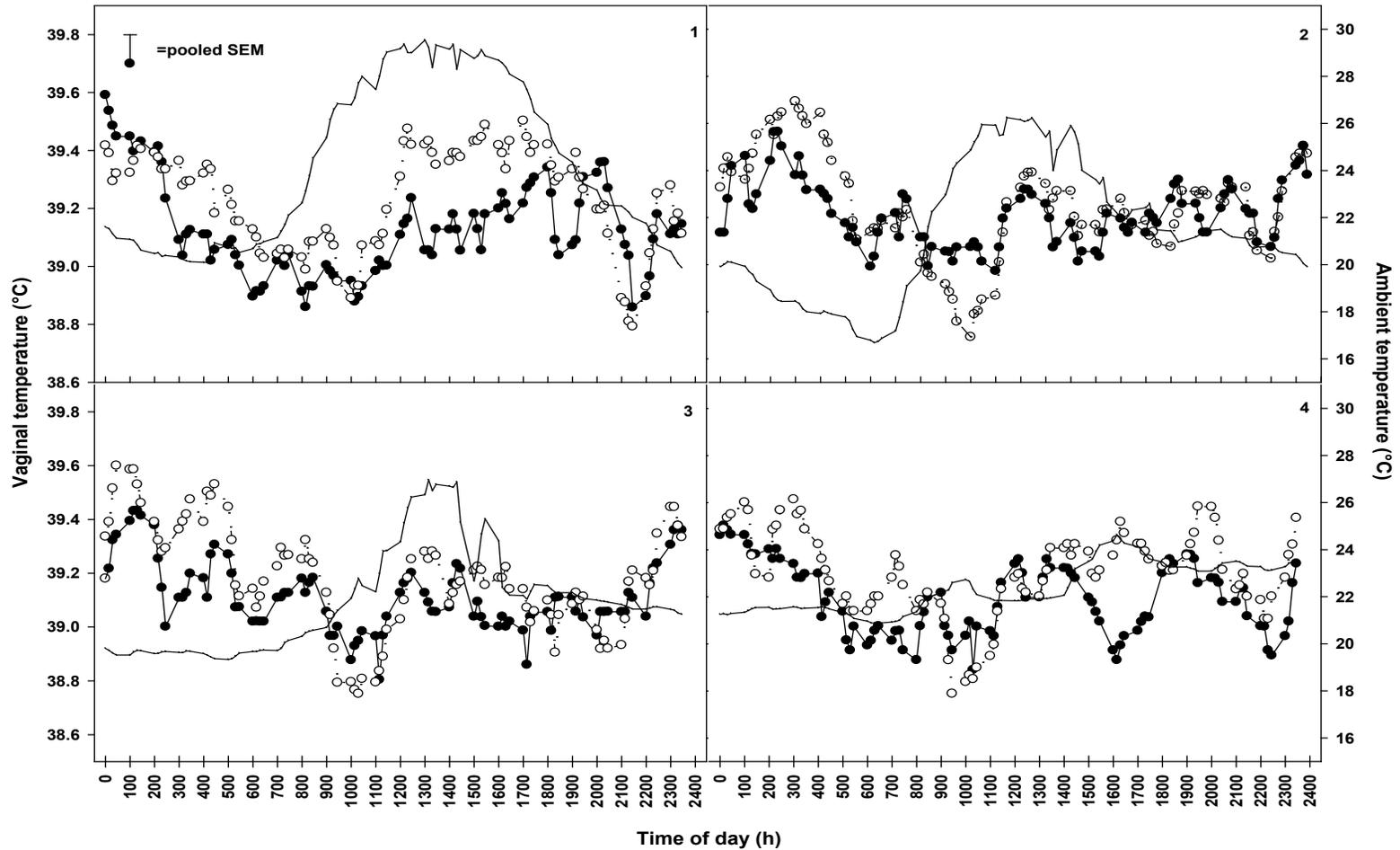


Figure 4-5. Vaginal temperatures during week one of slick (closed circle, solid line) and normal-haired (open circle, dashed line) Holstein cows. Ambient temperature dotted line. Data are the least square means  $\pm$  SEM. Vaginal temperature was affected by hair type ( $P < 0.01$ ), day ( $P < 0.01$ ), time of day ( $P < 0.01$ ), and hair type by day by time of day ( $P < 0.01$ ).

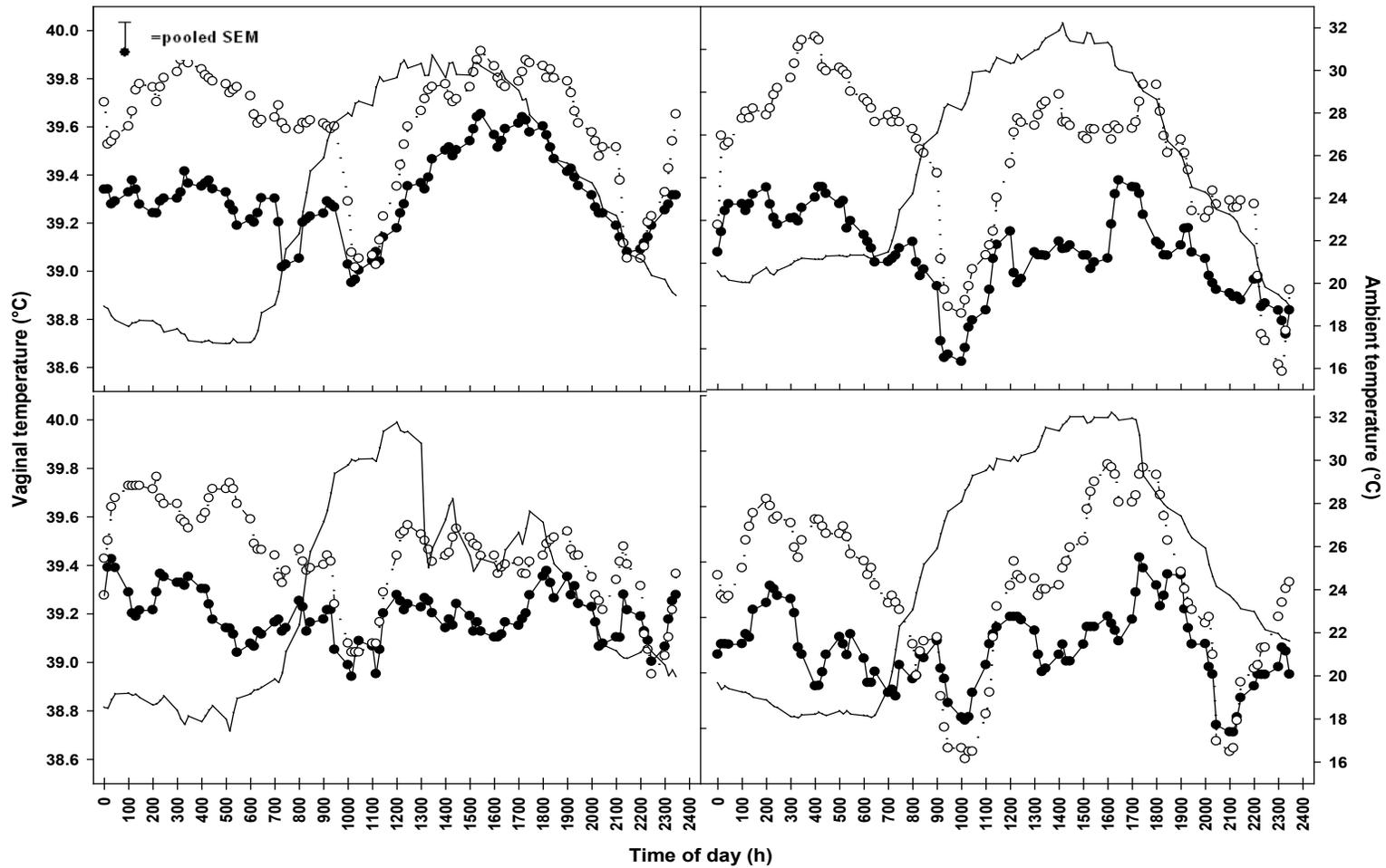


Figure 4-6. Vaginal temperatures during week five of slick (closed circle, solid line) and normal-haired (open circle, dashed line) Holstein cows. Ambient temperature dotted line. Data are the least square means  $\pm$  SEM. Vaginal temperature was affected by day ( $P < 0.01$ ), time of day ( $P < 0.01$ ), and hair type by day by time of day ( $P < 0.01$ ).

Table 4-1. Responses to heat stress for physiological traits by slick and normal-haired Holstein cows (LSM and  $\pm$  SEM)

Variable	N	Hair Type		SEM	P -value
		<i>Slick</i>	Normal		
Dry matter intake, Kg/day	21	21.54	22.28	0.070	0.41
Milk production, Kg/day	21	24.67	28.81	1.536	0.07
DM apparent digestibility, %	14	72.52	72.41	0.604	0.91
Energy balance, Mcal/day	21	9.09	7.19	1.021	0.21
Feed efficiency Kg Milk/Kg DM intake	21	1.15	1.30	0.066	0.14

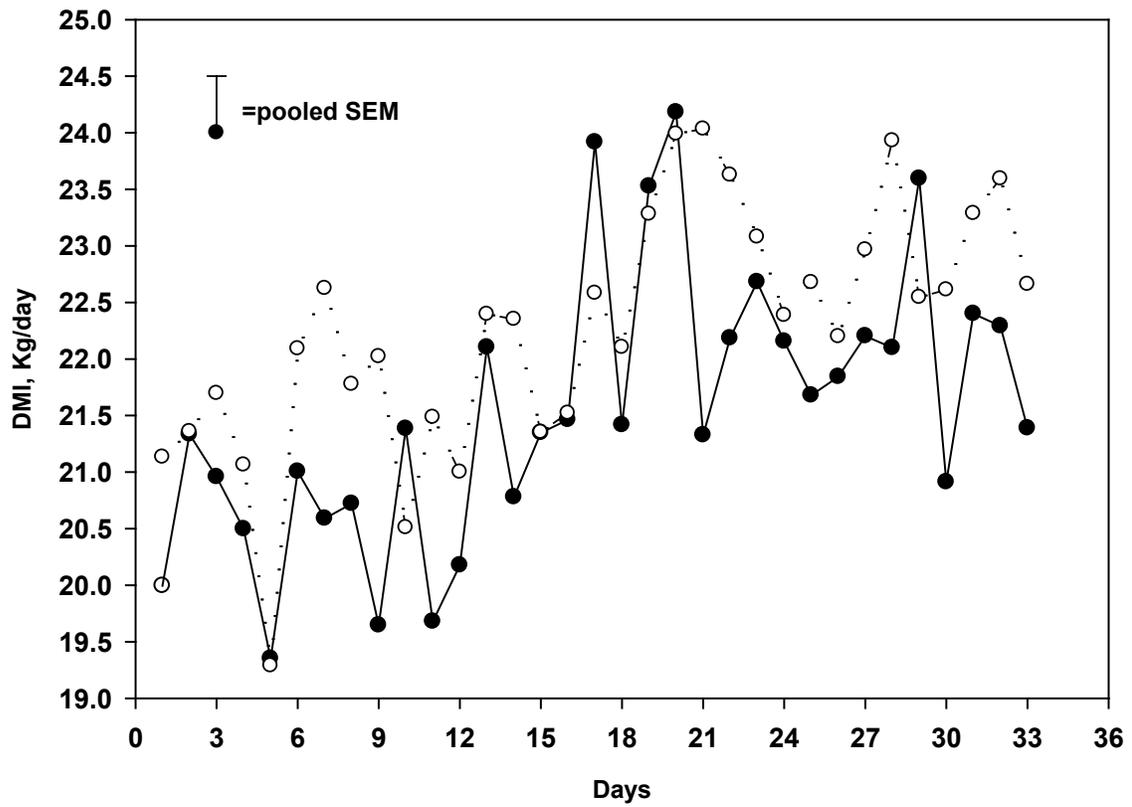


Figure 4-7. Dry matter intake of slick (closed circle, solid line) and normal-haired (open circle, dashed line) Holstein cows. Data are the least square means  $\pm$  SEM. Dry matter intake was affected by parity ( $P < 0.01$ ).

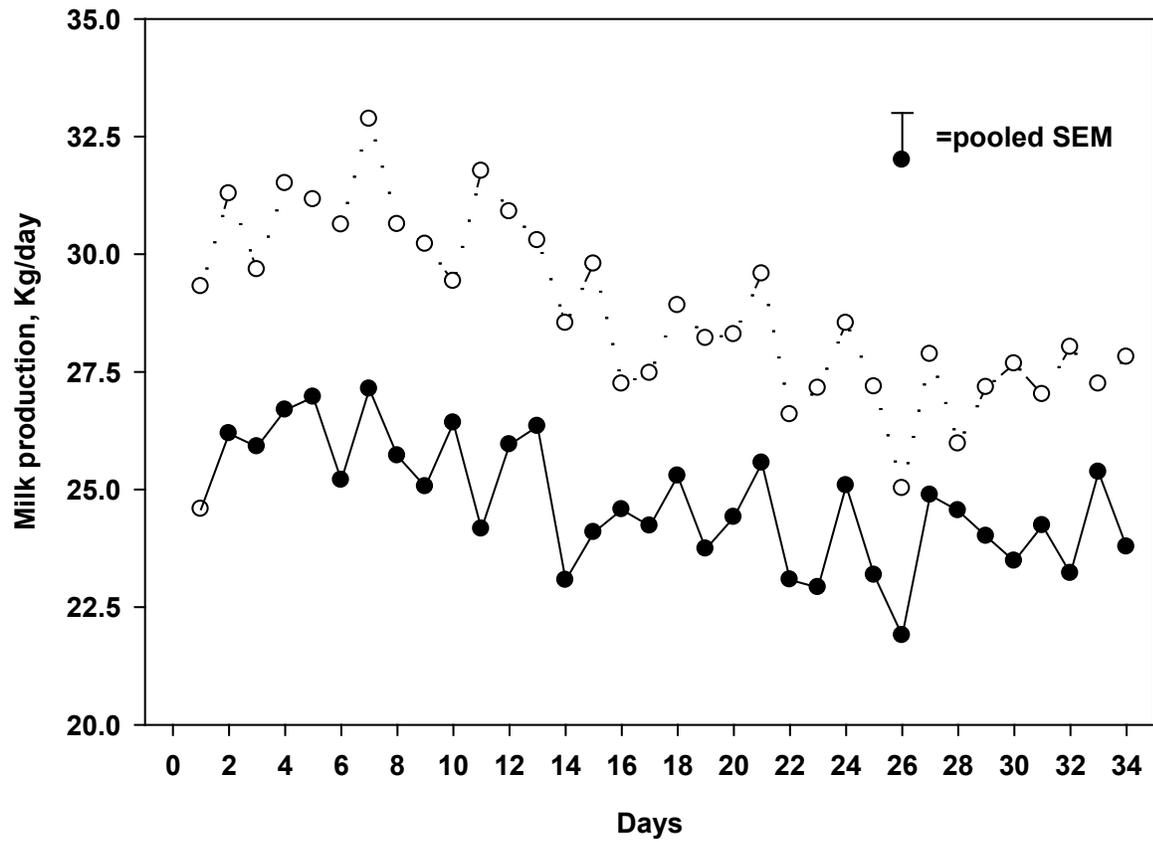


Figure 4-8. Milk production of slick (closed circle, solid line) and normal-haired (open circle, dashed line) Holstein cows. Data are the least square means  $\pm$  SEM. Milk production was affected by day ( $P < 0.01$ ).

## CHAPTER 5 DISCUSSION

In our study, exposure to a thermal stress environment confirmed previous studies (Olson et al., 2003; Dikmen et al., 2008) that lactating slick-haired Holstein cows were able to regulate body temperature better than their normal-haired contemporaries indicating the ability of the *Slick hair* gene to improve thermo-tolerance of lactating cows under hot conditions. These differences in thermal regulation between slick-haired and normal-haired Holstein cows were observed even though they were under a confinement system equipped with constant shade, fans and misters. During the trial cows were subject to modest thermal stress. Estimates of the degree of heat stress affecting these cows were estimated through mathematical formulae, combining different environmental variables. THI values ranged between 60 and 84 throughout the trial, values that considered above the threshold for heat stress (West, 2003). The ability to maintain lower rectal temperatures represents an advantage for slick-haired animals, given that even a small variation in rectal temperature can affect productive and reproductive processes (Brody, 1948). During this trial, however, cows were always exposed to some heat stress, therefore, it cannot be ruled out that the slick-haired cows will maintain lower body temperatures independent of ambient temperature. Olson et al. (2003) found lower body temperatures in slick-haired beef cattle even while grazing under cool weather in Florida.

Dikmen et al. (2008) stated that the physiological basis for improved thermoregulation of slick-haired animals must involve lower metabolic rate, increased sensible or evaporative heat loss, more efficient transfer of heat to the animal surface, or a combination of these adaptations. Differences in metabolic rate could have been a

factor in the temperature differences between the groups as even though dry matter intake was similar between groups, the normal-haired group had the tendency to produce more milk. The difference in milk yield probably occurred because most of the slick-haired Holstein cows were not sired by proven AI bulls, but instead by upgraded Holstein x Senepol sires (3/4 and 7/8). However, milk yield was similar between the slick-haired cows and their normal-haired paternal half-sibs. The slick-haired cows did have very short hair coats which Berman (2004) suggests would have allowed them to increase heat loss rate via convection and conduction, especially under confinement conditions with shade, fans and misters.

During this trial, differences in evaporative heat loss in both clipped and unclipped areas did not differ between genotypes in contrast to the results of Dikmen et al. (2008) where evaporative heat loss via sweating was greater in slick-haired cows than normal-haired cows. In our study both slick- and normal-haired Holstein cows exhibited lower sweating rates than those observed in other studies (Gebremedhin, 2007; Dikmen et al., 2008). This reduced evaporative heat loss may be explained by the season, late summer, and the fact that differences in hair length between the clipped and unclipped ones appeared to be very small. Moreover, the environmental conditions during the trial did not create the best environment to evaluate the sweating capacities of slick animals, as the average ambient temperature was 24.3°C with a relative humidity averaging 80% which likely affected the sweating responses. Gebremedhin et al. (2007) in Oahu, Hawaii reported a sweating rate at zero air velocity using the VaporMeter as 62 g/m<sup>2</sup>h when the ambient temperature was 33°C, relative humidity was 52% and solar load was 740 W/m<sup>2</sup>. This combination of environmental

factors of high ambient temperatures and low relative humidity resulted in a higher sweating rate response; allowing less humid air to be trapped at the skin surface. All these facts become relevant especially under hot conditions where 85% of heat loss from the skin to the environment is via evaporation when air temperature is greater than 30°C (Maia et al., 2005).

Our study showed that RT and RR responded similarly to heat stress. Slick-haired Holsteins had lower RT than normal-haired ones during both the AM and PM measurement times as well as lower RR. The increase in RR observed in the cows in this study may have allowed both the slick- and normal-haired cows to dissipate some of the rise in body temperature associated with the increase in THI. Respiration rate in slick-haired lactating cows was lower, averaging 11 breaths per minute less than the normal-haired ones when the THI was above 72. Even when the ambient temperature rose during the afternoons, and respiration rate increased for both slick and normal haired lactating cows, the slick-haired still had fewer BPM. The lower RR of slick-haired cows as compared to the normal-haired group can be explained by a likely increased rate of cutaneous heat loss for the slick-haired animals. Since slick-haired animals were able to maintain lower RT while at the same time breathing more slowly, a more efficient mechanism to maintain homeostasis is suggested.

The continuous measurements of vaginal VT allowed monitoring of thermoregulatory differences between the two genotypes. During the first week of the trial environmental conditions were characterized by high ambient temperatures and high relative humidity. Slick-haired cows were able to maintain lower VT compared to the normal-haired ones throughout the four-day period. This can also be explained by a

superior ability of the slick-haired cows to adapt their sensible and latent heat loss mechanisms, as mentioned earlier, as ambient temperature increased during the day and also as the normal increase in body temperature occurred during the night as a result of a circadian effect due to a second, subsequent milking (Kendall, 2008). On the other hand, during the last week of the trial there were no differences between VT of cows of the two genotypes even though the temperatures of both genotypes were slightly elevated, above 39°C. This may have occurred in response to a decline in ambient temperature to an average of 22.9°C during this later period of time. In general, these results tend to agree with currently accepted knowledge; i.e., that cows get hot when weather factors are severe enough to affect their ability to dissipate the heat which is generated or absorbed.

A digestibility component was included to determine if the slick- and normal-haired cows showed differences in their digestibility of the ration provided to them. The differences in body temperature regulation between slick and normal-haired lactating cows did not affect feed intake, even though slick-haired cows were often observed to be consuming the TMR during the hottest parts of the day. Variation in feed intake caused by heat stress normally occurs when homeostasis is disrupted (Hahn, 1999). Therefore, lack of difference probably happened because the ambient conditions were not particularly stressful. Possibly at higher levels of heat stress, the difference in feed intake between slick and normal-haired animals might have been more noticeable. Hammond and Olson (1994) had previously observed increased grazing time in Senepol during the daylight hours as compared with Hereford cows, however, the Hereford cows compensated by grazing more during the cooler evenings under tropical

summer conditions in Florida. On the other hand, feed efficiency analyses showed no differences between hair types, and showed that none of the cows were undergoing NEBAL during the trial. Normal-haired cows during the trial did not appear to be under particularly excessive heat stress and there was no particular reason to expect that the *Slick gene* would have a pleiotropic effect on the digestive tract, apart from what might have been caused by stress issues in the normal-haired cows. Production responses in this study also show no major differences between slick and normal-haired lactating cows.

Our results might have been different if the cows in this study had been under greater heat stress. This could have been achieved if more slick-haired cows in earlier stages of lactation had been available and the trial had been conducted earlier in the summer months. Greater heat stress could also have been caused by not allowing access of the cows to fans and misters. It would be of particular interest to be able to measure feed intake of slick- and normal-haired under grazing conditions with only limited access to shade, the conditions under which many dairy cows in the subtropical and tropical areas of the world must endure. In retrospect it might also have been useful to have observed the eating behavior of slick- vs normal-haired cows throughout the day and night. It may be possible to do so electronically through the use of electronic video recording devices or the the pedometer-plus equipment that may be available in the future.

## CHAPTER 6 CONCLUSIONS AND IMPLICATIONS

### Conclusions

The results of this study confirm earlier findings (Olson et al., 2003) that slick-haired cows are better able to regulate body temperature than normal-haired contemporaries. Rectal temperatures of slick-haired cows were significantly lower than those of normal-haired cows. Slick-haired cows showed an average rectal temperature of 0.4°C lower than of normal-haired cows. In addition, slick-haired cows had a lower respiration rate (11 BPM less) than the normal-haired cows while under heat stress. Similar results were found by Chytil et al. (2002) in slick vs. normal-haired Holstein heifers. Measurements of vaginal temperatures in this study also indicated that slick-haired cows were better able to regulate their core body temperatures compared to normal-haired cows exposed to elevated ambient temperatures; specifically they resisted the increase in body temperature as ambient temperature rose during the day and during the night due to the normal occurrence of circadian rhythm. Our studies also indicated that the data loggers used were an effective tool to monitor core body temperatures continuously over multiple days. Differences in coat type have important effects on the adaptation of dairy cattle in hot climates. The incorporation of the *Slick hair* gene into Holstein cattle did result in improved capacity for body temperature regulation under heat stress. However, our study did not show significant differences in sweating capacities of the slick-haired cows vs. normal-haired ones. Metabolic responses were not affected by hair type in this study; there were no effects of hair type on digestibility or energy balance.

While the advantage in heat tolerance of the slick-haired animals was expected, production responses to heat stress in this study were not as positive as we might have expected for the slick-haired cows. Weather conditions throughout the study apparently were not severe enough to determine whether slick-haired animals might have had higher productive parameters than normal-haired cows during summer months. There were no differences in dry matter intake and the milk production of normal-haired animals tended ( $P = 0.07$ ) to be higher under the experimental conditions in this trial.

### **Implications**

The *Slick hair* gene has the opportunity to increase the heat tolerance and, potentially, the productivity of *B. taurus* cattle raised in warm climates. This trait may become more important to dairy industries in countries located in tropical and subtropical regions under grazing production systems. Perhaps the addition of the slick-haired Holstein into the crossbreeding system would help to mitigate heat problems due to heat stress, by improving heat tolerance capacity through the use of the slick hair gene. In addition, these data suggest that the data loggers are an effective tool to monitor body core temperature continuously for several days. However, more research needs to be done to be able to understand the data and how this technology could be applied to particular dairy systems.

APPENDIX A  
SUPPLEMENTAL TABLE FOR CHAPTER 2

Table A-1. Hair scoring system (Turner and Schleger, 1960)

Score	Type	Description
1	Extremely short	Hairs extremely short and closely applied to the skin
2	Very short	Coat sleek, hairs short and coarse, lying flat
3	Fairly short	Generally smooth-coated, usually fairly coarse, easily lifted
4	Fairly long	Not completely smooth, coat easily ruffled
5	Long	Hairs distinctly long and lying loosely; predominantly coarse
6	Wooly	Hairs erect, giving a fur-like appearance.
7	Very wooly	More extreme expression of 6, with greater length and body over more of the body

APPENDIX B  
SUPPLEMENTAL TABLES AND FIGURES FOR CHAPTER 3

Table B-1. Diet composition

Feed Name	% of DM
Corn silage 2007	38.00
Legume hay, DRU	8.00
Citrus pulp dried	3.26
Brewers grains, wet	5.46
Corn gluten feed, dried	6.90
Soybean, meal, expellers	4.50
Corn grain, ground, dry	17.96
Soybean, meal, solvent extracted. 48% CP	5.20
Cottonseed, Whole with lint	3.26
Molasses, sugarcane	3.76
Vit/Min premix <sup>1</sup>	3.70

<sup>1</sup> Mineral Premix contained 0.75% Ca, 0.74% P, 0.23% Mg, 0.49% Cl, 1.32% K, 0.24% Na, and 0.36% S.

APPENDIX C  
SUPPLEMENTAL TABLES AND FIGURES FOR CHAPTER 4

Table C-1. Environmental conditions, Alachua, FI FAWN station, daily averages

Date	Avg. Temp	RH	Avg. THI	2m Rain Total (in)	SolRad avg 2m (w/m <sup>2</sup> )	10m Wind avg (mph)
01-Sep-07	24.7	89	76.84	0.36	115.60	43.21
02-Sep-07	25.1	86	76.39	0.03	113.00	33.76
03-Sep-07	25.4	79	76.38	0.00	196.78	14.38
04-Sep-07	24.7	71	73.89	0.00	261.05	36.8
05-Sep-07	24.4	76	73.33	0.00	197.71	45.82
06-Sep-07	24.8	74	73.34	0.00	225.09	25.79
07-Sep-07	25.2	71	74.24	0.00	242.95	30.54
08-Sep-07	22.5	83	71.29	0.01	156.80	44.21
09-Sep-07	24.8	74	73.52	0.00	236.08	15.95
10-Sep-07	25.5	74	74.97	0.00	226.75	28.18
11-Sep-07	24.6	80	75.00	0.00	157.00	0.77
12-Sep-07	25.3	77	75.22	0.00	217.51	0
13-Sep-07	25.1	77	74.87	0.29	233.00	0
14-Sep-07	25.1	82	75.53	0.69	201.13	23.34
15-Sep-07	26.0	81	76.94	0.31	193.43	5.05
16-Sep-07	25.9	77	76.86	0.01	230.19	9.31
17-Sep-07	23.4	82	71.55	0.02	112.71	7.47
18-Sep-07	22.8	77	71.16	0.01	191.30	8.07
19-Sep-07	21.3	81	68.88	0.53	50.18	6.84
20-Sep-07	24.5	82	74.44	0.73	161.76	8.36
21-Sep-07	25.6	75	75.74	0.85	213.76	7.62
22-Sep-07	24.8	82	75.09	0.01	166.24	4.53
23-Sep-07	24.1	87	74.53	0.13	95.78	4.41
24-Sep-07	25.5	76	75.53	0.00	198.24	7.11
25-Sep-07	23.1	85	73.35	0.08	139.91	4.75
26-Sep-07	23.7	85	74.22	0.46	183.86	4.25
27-Sep-07	24.1	80	73.93	0.00	209.87	2.62
28-Sep-07	24.9	78	74.32	0.00	207.65	3.07
29-Sep-07	24.1	72	73.15	0.00	189.88	7.03
30-Sep-07	21.3	78	68.11	0.06	136.75	6.34
01-Oct-07	21.8	86	71.20	0.07	137.35	7.2
02-Oct-07	22.4	91	71.08	1.10	38.50	7.55
03-Oct-07	25.9	83	77.77	0.03	159.84	5.32
04-Oct-07	24.9	89	76.49	0.23	126.32	5.33

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## BIOGRAPHICAL SKETCH

Eduardo Ignacio Alava Hidalgo was born in 1982, in Portoviejo, Ecuador, the only son of Eduardo Alava and Marja Hidalgo de Alava. He was raised with his two sisters in Guayaquil, Ecuador. During his youth, he was an avid and competitive athlete, competing in tennis, basketball, and swimming until the age of 18. He completed his secondary education at the Liceo Naval High School, a private school associated with the Ecuadorian Navy, graduating in 2000. Later that year, he was accepted at ESPOL University where he pursued his B.S. in “ingenieria agropecuaria” and graduated in 2005. During his last two years at the University, he started working with the family cattle ranch which continued until the end of 2006. He also participated actively in the Litoral y Galapagos Cattlemen’s Association, for the 2005–2006 periods, as a Brahman committee member and cattle show coordinator. In the spring of 2007, he began a graduate program under Dr. Timothy Olson at the University of Florida to study genetic improvement in cattle. Eduardo’s future plans are to continue his education and to pursue his PhD at the University of Florida, under the tutelage of Dr. Yoana Newman in the Department of Agronomy.