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The Evaluation of Holstein Heifer Calves Carrying the SLICK1 Allele of the Prolactin Receptor Gene for Growth, Physiological Responses to Heat Stress, and Reproduction

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The Evaluation of Holstein Heifer Calves Carrying the SLICK1 Allele of the Prolactin Receptor
Gene for Growth, Physiological Responses to Heat Stress, and Reproduction

By

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THESIS

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Abstract

Lactating Holstein cows carrying the SLICK1 allele of the prolactin receptor gene have demonstrated increased thermotolerance during periods of heat stress in tropical and subtropical climates. We aimed to evaluate Holstein heifers heterozygous for the SLICK1 allele for their (1) preweaning growth, (2) physiological responses to heat stress in low and high humidity climates at pre- and postweaned ages, and (3) reproductive performance as nulliparous heifers. Upon evaluation of 35 slick and 36 non-slick half-sister calves born in California during the cool season, birth weights of slick calves averaged 40.0 ± 0.9 kg while non-slick calves averaged 38.0 ± 1.0 kg ($P = 0.08$); however, weaning weights and average daily gain did not differ between slick and non-slick calves. In July 2020, 50 calves in Florida (21 slick and 29 non-slick half-sisters) and 222 calves in California (80 slick, 81 non-slick half-sisters, and 61 unrelated cohort control) were evaluated for physiological responses under environmental conditions where the average THI was 90 in Florida and 72 in California. Data were collected midday in Florida and at three times in California: morning and afternoon for all animals, and at an additional timepoint at midday for the pre-weaned calves. Two separate data analyses were performed. The first analysis combined the data from the measurements obtained in slick and non-slick heifer calves in Florida with the measurements obtained in the afternoon in the slick and non-slick heifer calves in California. Slick calves in Florida had rectal temperatures that were 0.4ºC lower than the non-slick calves in Florida while no difference between genotypes was detected in the calves in California. Regression analysis to test for a correlation between rectal temperature and THI revealed a stronger correlation between rectal temperature and THI of non-slick calves (Adj. $R^2 = 0.36$) compared to slick calves (Adj. $R^2 = 0.06$). Moreover, the calves in Florida had greater rectal temperature, respiration rate, and sweating rate than those in California regardless of genotypes. The calves in Florida experienced more severe heat stress conditions, under which the presence of the SLICK1 allele

was advantageous to improve thermotolerance in the calves. The second analysis was performed on the data obtained from slick, non-slick, and unrelated control cohort heifer calves in California in the morning, midday, and afternoon. Here again, regression analyses to test for a correlation between rectal temperature and THI during the afternoon when THI is highest, revealed a weaker correlation in slick calves (Group 1 Adj. R^2 = 0.01; Group 2 Adj. R^2 = -0.04) compared to non-slick (Group 1 Adj. R^2 = 0.12; Group 2 Adj. R^2 = 0.19) and control calves (Group 1 Adj. R^2 = 0.10; Group 2 Adj. R^2 = 0.07). This indicates that the slick heifers maintained a more consistent body temperature as THI rose compared to the non-slick and control animals. However, we did not detect differences between genotypes in the morning, midday, or afternoon. The preweaned calves began the day with lower rectal temperature, respiration rate, skin temperature, and sweating rate than the postweaned heifers, yet by the afternoon, preweaned calves had comparable or slightly higher measurements on all physiological parameters evaluated. We also evaluated the age at first breeding, pregnancy success, pregnancy success to first service, services per conception, age at calving, calving success, and pregnancy length for a subgroup of heifers in California. There were no differences in these reproductive parameters between heifers carrying the SLICK1 allele and their non-slick half-sisters. This project is the first to evaluate the preweaning growth of slick Holstein calves in a climate with low humidity. Additionally, this is the first evaluation of physiological responses to heat stress in relation to presence of the SLICK1 allele in young cattle (< 1 year of age) and cattle raised under heat with low humidity. Finally, this is the first report on the reproductive performance of nulliparous Holstein heifers carrying the SLICK1 allele.

Table of Contents

Figures

Tables

Chapter 1: Literature Review

1.   Introduction

Accounting for the inflation of the dollar from 2003 to 2022, heat stress results in the annual loss of over a billion dollars for the United States dairy industry (St-Pierre et al., 2003). When climate conditions exceed the threshold for an animal's ability to dissipate heat, energy must be diverted from other biological functions towards heat dissipation (West, 2003); in turn, dairy cows afflicted with heat stress experience lower milk production and reproductive efficiency, increased health disorders, and altered behavior that can further impair their welfare and productivity (Liu et al., 2019). To decrease these deleterious effects of heat stress, current mitigation methods include shelter modifications, utilization of cooling systems, altered nutrition, and incorporation of advantageous thermotolerant genetic traits into breeding programs. One promising genetic strategy for mitigation of heat stress in Holstein cattle is the SLICK1 allele of the prolactin receptor, known as the slick hair mutation. This naturally occurring, dominant mutation (Olsen et al., 2003) produces a short, sleek hair coat (Figure 1.1). Moreover, lactating Holstein cows carrying the SLICK1 allele in tropical and subtropical climates have been shown to maintain lower internal temperatures and higher milk yields during periods of heat stress when compared to wild-type, non-slick Holstein cows (Dikmen et al., 2008, 2014).

2.   Thermoregulation and heat exchange between cattle and their environment

Like other homeotherms, Holstein cattle (*Bos taurus*) need to maintain a relatively constant internal body temperature via a variety of adaptive mechanisms. In the thermal neutral zone, an animal uses a basal level of energy to maintain their body temperature. However, as temperature rises, the body of the animal partitions energy from other sources to dissipate heat and return to thermal neutrality. Generally, lactating cows with a high milk yield have a lower thermal neutral

zone due to their high metabolic heat load compared to young, non-lactating stock or lower producing cows; therefore, lactating cows are more prone to heat stress (West, 2003; Berman, 2005).

Heat is transferred between the body and the environment through sensible heat loss – conduction, convection, radiation – and latent heat loss – evaporation. The effectiveness of the method of heat loss depends on the magnitude of difference between the temperature of the animal and the environment (Gebremedhin, 1985). Solar radiation transfers heat from the environment to the animal, where limiting this exposure via a coat color that is lighter and more reflective helps the animal stay cool (Da Silva et al., 2003). Conduction occurs when two surfaces touch and heat transfers from one to the other. For example, a warm animal can lose heat by being in contact with a cooler surface such that heat flows from the animal. Convection works by air moving over the animal thus pulling heat from the surface of the animal if the air temperature is cooler. Both higher air velocity and shorter coats increase the effectiveness of convective heat loss (Berman, 2005). As the core temperature of the animal rises, vasodilation, which increases blood flow to the periphery, maximizes heat loss by both conduction and convection (Hansen, 2004). An increased surface area is advantageous for convective and conductive heat loss (Berman, 2005). However, if there is no protection from solar radiation, increased surface area increases the heat absorption (Mitchell et al., 2018).

Once ambient temperatures near or exceed the animal's body temperature, latent heat loss is the primary mode of heat loss while sensible heat loss can become a source of heat gain (Gebremedhin and Wu, 2001; Maia et al., 2005). Latent, also called evaporative, heat loss happens when animals pant or sweat. Panting removes heat through exhalation of warm water vapor, although this only accounts for approximately 20% of evaporative heat loss (Robertshaw and

Dmi'el, 1983; Maia et al., 2005). Sweating in cattle accounts for the remaining approximately 80% of latent heat loss. Environmental factors such as humidity and air velocity which have an inverse relationship can alter the efficiency of latent heat loss. At high humidity, the air in the microenvironment is saturated with water molecules making the evaporative heat loss – transfer of sweat from the skin to the air – more challenging; conversely, at high air velocities, latent heat loss becomes more effective because the moving air reduces the moisture gradient between skin and air (Berman, 2005).

In addition to environmental factors, sweating efficiency at a high environmental temperature relies on a multitude of animal characteristics. Longer hair coats decrease the movement of air across the skin, thereby making latent heat loss more difficult and decreasing the thermal threshold (Berman, 2005; Gebremedhin et al., 2008). A darker coat color enhances solar absorption which increases the heat load (Da Silva et al., 2003; Gebremedhin et al., 2008). Differences in sweat glands, like density and conformation, play a role in cutaneous evaporative heat loss, yet the exact contribution of each difference is still unclear. Some research shows a higher density of sweat glands in the heat tolerant Sahiwal, Red Sindhi (*Bos indicus*; Haymon and Nay, 1957; Jian et al., 2014), and Criollo Limonero (*B. taurus*; Landaeta-Hernandez et al., 2011) compared to Holstein and Jersey (*B. taurus*). Other research with Sahiwal and Jersey cattle, however, showed no difference in density of sweat glands (Pan et al., 1963). Additionally, the slick-haired Criollo Limonero and *B. indicus* appear to have a "baggier" shaped sweat gland with a thicker lumen and wider walls that contain an abundance of cells, increasing its volume (Pan et al., 1963; Landaeta-Hernandez et al., 2011; Jian et al., 2014). Meanwhile, *B. taurus* have smaller, more tubular shaped sweat glands that are thin with few cells (Pan et al., 1963; Jian et al., 2014). While some reports support the theory that thermotolerant *B. indicus* sweat more than *B. taurus*

3

cattle (Haymon and Nay, 1957; Jian et al., 2014), others have found the inverse with lower sweating rate in *B. indicus* and higher in *B. taurus* (Hansen, 2004; Thompson et al., 2011).

3.   Temperature Humidity Index (THI)

The temperature-humidity index (THI) combines heat and humidity information to describe the environmental conditions, which, compared to ambient temperature alone, provides a better evaluation of the environmental conditions that cattle are experiencing and can aid in identifying when to initiate cooling strategies to prevent drops in production and to maintain animal welfare. In 1958, Thoms designed the original THI for measuring human comfort levels. The index combined ambient temperature – measured via dry bulb temperature (T_{db}) – and relative humidity – measured via wet bulb temperature (T_{wb}). Bianca (1962) and Berry et al. (1964) wrote the first reports transitioning the equation for use in cattle. Since then, multiple THI equations have been derived and, with them, THI thresholds for dairy cattle that are continuously being refined based on findings from further research. However, selection of an appropriate index may impact calculation of THI thresholds based upon experimental climate conditions, which is particularly relevant when evaluating milk yield changes in response to THI. More specifically, subtropical and tropical climates characterized by high humidity should utilize a THI that places more weight on T_{wb} while more arid and temperate climates in which humidity most likely doesn't hinder evaporative cooling should choose a THI that places more weight on T_{db} (Bohmanova et al., 2007).

The THI threshold at which *B. taurus* may begin experiencing heat stress varies depending upon a multitude of variables. When evaluating milk yield, THI thresholds have been reported to range anywhere from 60 to 72 (Ravagnolo et al., 2000; Brugemann et al., 2012). When considering parity in subtropical Georgia, Ravanolo et al. (2000) found a THI threshold of 72 for first-parity lactating cows when using milk yield as an indicator of heat stress. Conversely, Zimbelman et al.

(2009) utilized environmental chambers mimicking the arid climate of the southwestern United States to measure physiological measurements such as rectal temperature, heart rate, and respiration rate of multiparous lactating Holstein cows. They concluded that the animals began experiencing heat stress beyond a THI threshold of 68. Additionally, THI thresholds for heat stress can range between 68 and 77 depending upon stage of lactation and the physiological measurement of heat stress. Pinto et al. (2020) evaluated multiparous early and mid-lactation Holstein-Friesian cows in Germany's temperate climate and found that standing cows had different THI heat stress thresholds depending upon the physiological parameter being measured: respiration rate and rectal temperature (THI = 70), and heart rate (THI = 72). Using conception rate (CR) as a measure of thermotolerance, Schüller et al. (2014) concluded that CR for early to mid-lactation Holstein cows in Germany had a THI threshold of 73. Similarly, in North Queensland, Australia, Morton et al. (2007) observed early to mid-lactation cows from predominantly Holstein-Friesian herds to have a THI threshold for CR of 72. These THI thresholds are lower than the 77 reported by Ouellet et al. (2021) for dry multiparous Holstein cows in a subtropical climate. Contrarily, Fabris et al. (2019) observed that dry Holstein cows in Florida's subtropical climate not cooled at THI > 68 had higher rectal temperatures and respiration rates, and had depressed milk, protein, and lactose yields in their subsequent lactation compared to dry cows cooled with sprinklers and fans. Furthermore, age may also contribute to the THI threshold for heat stress. In subtropical Florida, Dado-Senn et al. (2020) was the first to report THI thresholds for dairy calves under chronic heat stress conditions, which were 67 for rectal temperature and 65 for respiration rate when calves were subjected to chronic heat stress. Although lactating cows have received the most attention due to their higher sensitivity to heat stress, it is becoming clearer that more research is needed to evaluate THI thresholds and responses to heat stress in young animals due to the immediate and long-term

negative effects on health, welfare and production (Young, 2020; Laporta, 2021; Dado-Senn et al., 2022).

4.   Impacts of heat stress in dairy cows

Heat stress occurs when the combined external forces and basic metabolic functions exceed the cow's ability to dissipate heat. As she places more energy into cooling herself to return to thermoneutrality, other biological systems such as lactation performance, reproduction and immunological responses can be impaired; furthermore, the effects of heat stress go beyond the individual cow and affect the performance of subsequent generations (Laporta et al., 2017; Dado-Senn et al., 2020; Dado-Senn et al., 2021). Many strategies have been devised to mitigate these deleterious consequences like shelter alterations, implementation of cooling systems, nutritional modifications, and introgression of genetic traits that confer superior thermotolerance.

4.1   Reproductive performance

The detrimental effects of heat stress on reproductive function are multifactorial. A decrease in reproductive efficiency, oocyte quality, and survival of the early embryo can occur as a direct effect of heat, or more indirectly as a consequence of reduced feed intake, negative energy balance, and endocrine disruptions (Sammad et al., 2020). Expression of estrus can be reduced by as much as 20% in periods of heat stress (Kornmatitsuk et al., 2008). Mounting behavior decreases while licking and rubbing increases, which may be more difficult to detect than mounting and standing heat (Pennington et al., 1985). Along with decreased estrus detection, conception rates (CR) can be 50% lower during summer months versus cooler months (Peralta et al., 2005). When the THI exceeded 76, Japanese Black cows had a reduction of CR from 38.8% to 17.6% (Sakatani et al., 2016). Kornmatitsuk et al. (2008) found the mean artificial insemination (AI) per conception to be higher in the hot season at 2.6 inseminations per conception versus 2.0 inseminations per

conception in the cool season. Moreover, 9.8% of females conceived after the first AI in the hot season versus 23.1% in the cool season. Heat stress exposure 20-50 days prior to AI may cause a reduction in CR by 2.5-8.3% (Chebel et al., 2004). In that same study, multiparous cows were 13% less likely to conceive compared to primiparous cows during periods of heat stress.

Pregnancy rate and pregnancy loss are also affected by heat stress. Garcia-Ispierto and colleagues (2006) found that Holstein-Friesian cows in Spain had pregnancy rates of 55.5% and 44.5% during the cool and warm seasons, respectively. Similarly, Kornmatitsuk et al. (2008) reported pregnancy rates of 61.5% and 33.3% for Holstein-Friesian cows in Thailand during the cool and warm seasons, respectively. Peralta et al. (2005) reported that lactating Holstein cows exposed to THI above 76 had a pregnancy rate of 17.6% whereas cows exposed to THI less than or equal to 76 had a 38.8% pregnancy rate. At THI above 85 during days 11-20 of gestation, dairy cows had a 1.57-fold increase in pregnancy loss (Santolaria et al., 2010). Additionally, between seasons, pregnancy losses of mature cows rose from 2.1% during the cool season to 12.3% during the warm season (Garcia-Ispierto et al., 2006). Cows with a twin pregnancy are 3.4 to 3.98 times more likely to lose the pregnancy and, during the warm season, twin pregnancies are more common (Garcia-Ispierto et al., 2006; Santolaria et al., 2010). Pregnancy loss during months of heat stress is higher for lactating cows at 10.0% than for heifers at 4.7% (Al-Katanani et al., 2002; Jousan et al., 2005).

Long-term exposure to heat stress can reduce plasma progesterone (Howell et al., 1994) and estradiol concentrations (Badinga et al., 1993). Additionally, the concentration of estradiol in follicular fluid is reduced during heat stress (Badinga et al., 1993). Supplementation of progesterone to Holstein cows from d5-13 post-insemination during the summer months improves their fertility, thus supporting the proposal that low fertility during periods of heat stress can be

attributed in part to lower progesterone concentrations (Friedman et al., 2012). Conversely, cows with short term exposure to heat stress have been reported to either have an increase in plasma progesterone concentrations (Trout et al., 1998; Wilson et al., 1998) or no effect (Wise et al., 1988; Wolfenson et al., 1995; Roth et al., 2000). The variation in findings for plasma progesterone concentrations could be due to the severity of heat stress exposure as well as the multitude of additional physiological functions affected by heat stress in a way that may impact progesterone levels (Trout et al., 1998).

While the duration of the estrous cycle is not affected by heat stress (Trout et al., 1998; Roth et al., 2000), Wolfenson et al. (1995) reported an earlier emergence of the dominant follicle in the second wave by two days and of the preovulatory follicle as much as 4 days early for heat stressed cows. The early emergence – and therefore increased duration of follicular dominance – results in pregnancy rates decreasing to less than 20% in nulliparous heifers (Mihm et al., 1994; Austin et al., 1999). Several studies reported that heat stress impairs follicular dominance as shown by a reduction in size of the dominant follicle during both the first and second wave (Badinga et al., 1993; Wilson et al., 1998; Schuller et al., 2017) and in a lack of suppression of the subordinate follicles – represented by their longer duration and larger size (Badinga et al., 1993; Wolfenson et al., 1995; Roth et al., 2000). Besides the short-term effects on the follicular wave and dominant follicle, heat stress seems to have longer-term carry over effects on fertility. After summer, two to three estrus cycles are needed for the ovarian pool of oocytes to recover from heat-associated damage (Roth et al., 2001). Moreover, exposure of the ovarian pool of oocytes to environmental heat stress during the summer may impair the maternal mRNA storage and/or the mechanism of transcription thereby affecting gene expression in the developing embryo (Gendelman and Roth, 2012).

After fertilization of the oocyte, the newly formed embryo is still susceptible to heat stress during the first few cell divisions, becoming more resistant afterwards (Hansen, 2007). Heifers exposed to heat stress between the onset of estrus and insemination subsequently had decreased embryonic development and higher rates of abnormal and retarded embryos (Putney et al., 1989). For lactating cows, exposure to heat stress one day after estrus caused the embryos, at the one to two cell stage, to have reduced probability of developing into a blastocyst; however, heat stress exposure of embryos at the later stages of 8-16 cells, morula, or d7 blastocyst did not have an effect of the proportion of embryos that developed into a blastocyst on d8 (Ealy et al., 1993). As the embryo progresses through development, production of harmful reactive oxygen species decreases and acquisition of protective antioxidants increases, which can assist in reducing the damaging effects of heat shock (Sakatani et al, 2004; Boni, 2019).

Many *in vitro* studies utilizing heat shock (short duration exposure to temperatures $\geq$ 40.5ºC) as a tool to study the impact of heat on cattle oocytes at the cellular level have demonstrated a detrimental effect on the quality and developmental competence of the oocyte. One study found cumulus oocyte complexes (COC) in the germinal vesicle stage to be susceptible to heat shock (41 °C for 6 or 12h) such that fewer oocytes progressed to metaphase II, while blastocyst development *in vitro* was impaired (Payton et al., 2004). Heat shock of similar magnitude and duration during oocyte maturation *in vitro* has been associated with a disruption in the resumption of meiosis, increased rate of apoptosis, cytoskeletal modifications, and increased abundance of reactive oxygen species (Roth and Hansen, 2005; Wolfenson and Roth, 2019). Moreover, oocytes exposed to heat shock during *in vitro* maturation have lower chance of reaching the metaphase II stage, as well as a lower chance of cleaving and reaching the blastocyst stage following in vitro fertilization (Wolfenson and Roth, 2019). Further supporting these findings,

expression of apoptotic genes in oocytes of repeat breeder cows was higher in summer than winter (Ferreira et al., 2016), which appears to correspond with the 15-30% of oocytes that undergo apoptosis when exposed to elevated temperatures in vitro (Roth and Hansen, 2005).

Finally, maternal heat stress impacts fetal development. One such way is through alterations in the placenta. Cows exposed to heat stress during late gestation have lower placental mass and reduced vascularization which is associated with impaired oxygen and nutrient transfer to the fetus and a multitude of detrimental effects including but not limited to impaired immunity, growth, and metabolism (Tao and Dahl, 2013). During the first 7 months of gestation, the fetus only acquires approximately 40% of its birth weight, leaving the remaining 60% to be gained during the last 2 months of gestation (Bauman and Currie, 1980). Congruently, the majority of literature reports that pregnant dry cows (i.e., in the final 60 days of gestation) exposed to heat stress have fetuses or neonates with lower birth weights. One factor that potentially contributes to the lower birth weights is a decrease in gestation length for heat stressed cows and nulliparous heifers during the last two months of gestation (Almoosavi et al., 2021; Davidson et al., 2021). A study conducted by Mellado et al. (2017) found that a gestation length of less than 278 days leads to a 30% probability of resulting in a stillbirth. The authors also found that 19.3% of calves with birth weights of less than 35 kg were stillborn compared to 2.3% of calves born at or heavier than 35 kg. Moreover, cows exposed to a THI > 83 between 7 months of pregnancy and parturition had a 1.3 times higher risk of having a stillbirth than cows exposed to THI ≤ 83.

4.2   Milk yield and composition

Similar to reproductive performance, lactation performance notoriously decreases with increasing temperatures with or without high humidity. There is a lag in the effect of heat stress on milk yield such that a decline in daily milk yield is associated with THI values 1-3 days prior

(West, 2003; Li et al., 2021). Above a THI of 72 in the southeast United States (a climate characterized by high humidity heat), primiparous cows produce 0.2 kg less milk daily per increasing THI unit (Ravagnolo et al., 2000) while multiparous cows will have a daily milk yield decrease of 0.69 kg per THI unit increase (West et al., 2003). When examining milk yield data in Florida and Georgia from 1976 to 2002, de Vries and Risco (2005) found that milk yield for Holstein cows dropped 15% in the summer compared to the winter. Meanwhile, in China, Gao et al. (2017) found a 17% drop in milk output by multiparous Holstein cows during the summer. In environmental climate chambers mimicking the southwestern United States climate (a climate characterized by low humidity heat), heat-stressed cows had an average milk yield decrease of 33% per day for daily THI ranging from 73-82 or 2.2 kg per day when THI ranged from 65-73 (Rhoads et al., 2009; Zimbelman et al., 2009). Heat stress also reduces milk yield regardless of whether exposure happens in early (15-45), mid (100-180) or late lactation (180-300 days in milk), and the ability of cows to recover some of the milk yield loss after the cessation of heat stress seems to depend on the severity and duration of heat stress, as well as the stage of lactation when the insult began (Tao et al., 2018).

The effect of heat stress on milk composition is not as clear as for milk yield. Some studies have found that milk fat yield and composition reduces with increasing THI for cows at various stages of lactation and parity (Ravagnolo et al., 2000; Bouraoui et al., 2002; Bernabucci et al., 2014). Other reports, however, found no difference for milk fat content under heat stress conditions (Rhoads et al., 2009; Cowley et al., 2015; Gao et al., 2017). Further still, research by Smith et al. (2013) found milk fat percentage rose 0.2% in Holstein cows once the THI surpassed 79. As for milk protein, most research has found a reduction in protein yield and percentage under higher THI, with the magnitude of decrease depending on parity and stage of lactation (Ravagnolo et al.,

11

2000; Bouraoui et al., 2002; Bernabucci et al., 2014; Cowley et al., 2015). However, Smith et al. (2013) reported Holstein cows exposed to a THI of 90 or more had an increase of 0.1% in protein percentage.

The exact mechanisms behind the heat stress effects on protein and fat concentrations in milk are unclear. While a reduction in dry matter intake does play a role given that nutritional changes alter milk composition, this reduction only accounts for a portion of changes in milk compositions during heat stress (Baumgard and Rhoads, 2013). During early lactation, cows increase their mobilization of nonesterified fatty acids (NEFA) from adipose tissue for utilization as an energy source, thereby allowing glucose to remain available for milk synthesis in the mammary glands. Baumgard and Rhoads (2013) reported that cows that are heat stressed during early lactation have an impaired mobilization of NEFA compared to pair-fed thermoneutral cows resulting in less glucose being available for milk synthesis.

4.3   Health and immunity

Heat stress has a detrimental effect on the health and immune system of dairy cattle via direct and indirect mechanisms.

Heat stress severely impacts mammary gland health. One useful indicator of mammary gland health is milk somatic cell count (SCC) or somatic cell score (SCS), which provide a measurement of the cellular content in milk, including immune cells. As SCS increases, there is a corresponding decrease in milk yield and components (Igono et al., 1988; Guinn et al., 2019). Elevated THI is associated with increased SCS in temperate (Riekerink et al., 2007; Lambertz et al., 2013; Zeinhom et al., 2016; Safa et al., 2018), subtropical (Igono et al., 1988; Nasr and El-Tarabany, 2017), and tropical (Ferreira and De Vries, 2015; Negri et al., 2021) environments. For example, Nasr and El-Tarabany (2017) reported a SCC increase of 14% between a low (<70) to

medium THI (70-80), and a SCC increase of 36% between low THI and high THI (>80) in a subtropical environment. Negri et al. (2021) reported that the effect of THI impacts SCS most between days 5 and 180 of lactation during the period when milk yield is highest. High SCC values are also indicative of a higher prevalence of mastitis (Zeinhom et al., 2016). Higher pathogen loads causing mastitis are expected with higher ambient temperatures and humidity during summer, and indeed these heat-induced pathogen load increases have been reported to increase the incidence of mastitis (Godden et al., 2003; Thompson and Dahl, 2012).

Heat stress has also been associated with increased incidence of retained placenta. Seyed Almoosavi et al. (2021) reported that placenta expulsion was approximately 2 h longer for heat stressed cows, which they attributed to the shorter gestation length. Their findings are consistent with earlier research that shows a direct connection between short gestations and retained placenta (Han and Kim, 2005; Gernand et al., 2019).

The increase in health disorders like the ones mentioned above during periods of heat stress can be attributed at least in part to impaired immune functions (Bagath et al., 2019; Dahl et al., 2020). Broadly speaking, immune response to stress involves the two branches of the immune system: innate and adaptive. The innate immune response is nonspecific and is the first line of defense to an immune challenge. In contrast, the adaptive response requires more time to become functional due to its complexity and specificity (Carroll and Forsberg, 2007). Adaptive immunity can be divided into two responses: cell mediated and humoral. An integral part of the specificity of the adaptive immune response are antibodies, also called immunoglobulins, which are created through activation of humoral immunity via B lymphocyte cells (B cells). The immune response can be modified by the endocrine system, such as the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis (Carroll and Forsberg, 2007). Further

regulation of the immune response occurs by cytokines, which are small proteins released by the various immune cells that can have either proinflammatory or anti-inflammatory effects (Bagath et al., 2019).

Heat stress stimulates the HPA axis resulting in the production of glucocorticoids, primarily cortisol which is a common marker of stress in cattle. Higher THIs are positively correlated to higher plasma cortisol levels (Elvinger et al. 1991; Elvinger et al., 1992; Lacerta et al., 2005). Under acute heat stress, glucocorticoids are immunoenhancing in order to prime and stimulate the immune system; however, with chronic exposure, glucocorticoids alter cellular functions that result in a shift to immunosuppression, in part to help prevent tissue damage (Elenkov, 2004). During stress responses, the SAM axis invokes the production of the catecholamines epinephrine and norepinephrine, which act to suppress production of proinflammatory cytokines and enhance production of anti-inflammatory cytokines (Elenkov et al., 1996). A study by Alvarez and Johnson (1973) reported increased levels of epinephrine and norepinephrine by cattle under both acute and chronic heat exposure, such that an average increase of epinephrine by 45% and 91% and norepinephrine by 42% and 70% were found after short- and long-term exposure to heat, respectively.

Another reported effect of heat stress on the immune system is altered population counts, proliferation, and function of leukocytes. Most studies have found overall increases in leukocyte counts in response to seasonal heat stress (Bagath et al., 2019). More specifically, leukocytes arrange into two categories: polymorphonuclear (PMN) cells and peripheral blood mononuclear cells (PBMC). In response to physiologically relevant heat stress temperatures *in vitro*, bovine PMN cells – such as neutrophils, eosinophils, basophils, and mast cells – from cows at varying stages of lactation have been reported to have reduced phagocytosis and oxidative burst capacity

(Lacetera et al., 2006; do Amaral et al., 2011; Thompson et al., 2014; Lecchi et al., 2016). As for population counts of the varying cell types in response to heat stress, basophils are increased in livestock (Maxwell et al., 1992; Park et al., 2021). Conversely, eosinophil counts in livestock mostly decrease in response to heat stress (da Silva et al., 1992). Neutrophil population counts in response to heat stress are not as consistent in literature with both *in vitro* and *in vivo* studies reporting increases, decreases, or no change in the neutrophil counts (Bagath et al., 2019; Parker et al., 2021).

Generally, under heat stress conditions, PBMC – such as dendritic cells, monocytes, and lymphocytes – have decreased proliferation (Elvinger et al., 1991; Lacetera et al., 2005; do Amaral et al., 2010; do Amaral et al., 2011). Conversely, proliferation of lymphocytes in heat stressed animals is more nuanced. The two subclasses of T helper lymphocyte cells 1 and 2 (Th1 and Th2), for example, respond to the heat stress-induced increase of glucocorticoid secretions with a shift towards the production of more Th2 and fewer Th1 cells, which shifts the immune response towards increased humoral immunity (Nagai and Iriki, 2001; Elenkov, 2004). Since Th1 primarily secrete proinflammatory cytokines and Th2 primarily produce anti-inflammatory cytokines, shifting towards Th2 proliferation may be a protective shift to prevent potential damage to tissues from inflammation-causing leukocytes (Elenkov, 2004). Concordantly, proinflammatory cytokine production (e.g., TNFα) is reduced while anti-inflammatory cytokine production is increased under chronic heat stress conditions (do Amaral et al., 2010; Thompson et al., 2014). Within the udder, the suppression of cellular mediated immunity increases the risk of infections, apoptosis of mammary cells, and a reduction in milk production (Thompson-Crispi et al., 2014).

4.4   Behavior

Cattle often alter their behavior in an attempt to decrease the heat load. When temperatures are in the thermal neutral zone, dairy cows typically spend 10 to 14 h per day lying down (Wechsler et al., 2000; Cook et al., 2005). While above the thermal neutral zone, dairy cattle will increase standing time by 30-35% to increase surface area for maximal heat loss (Schutz et al., 2011; Nordlund et al., 2019).

A notable alteration in behavior is a reduced feed intake in an attempt to lower core body temperature by decreasing the heat produced from digestion (Chang-Fung-Martel et al., 2021). For every unit increase of THI above 72, dry matter intake by Holstein cows drops 0.45 to 0.51 kg per day (West et al., 2003; Chang-Fung-Martel et al., 2021). A major consequence of the decrease in feed intake is a drop in milk yield; however, under chronic heat stress conditions, only 35% of the milk yield reduction is accounted for by the drop in feed intake (Rhodes et al., 2009). Inversely, cattle will increase their water intake during heat stress due to increased water loss via respiratory and cutaneous evaporative heat loss (Lamp et al., 2015), and in an attempt to decrease core temperature.

As THI increases, cows with access to a voluntary overhead water source (i.e. "cow shower") would use the shower an additional 0.3 h per day for every 1 ºC increase in ambient temperature, which translated to evening body temperatures that were 0.2 ºC lower than for cows without access to the shower (Legrend et al., 2011). Interestingly, Schutz and colleagues (2011) reported that dairy cattle preferred to use shade during summer even though heat load and insect avoidance behavior decreased more efficiently with sprinklers. When choosing shade, dairy cattle are more likely to utilize shade structures with strong blockage of radiation (Gaughan et al., 1998; Schutz et al., 2009).

Cows suffering from heat stress will spend more time standing, which limits blood flow to the udder compared to when they are lying, therefore reducing the amount of nutrient uptake by the mammary gland and further partially contributing to the reduction in milk yield and altered milk composition observed during summer (Rulquin and Caudal, 1992; Prosser et al., 1996; Delamaire and Guinard-Flament, 2006). Additionally, increased time standing has been reported to be positively correlated with an increase in the risk of lameness and hoof diseases (Cook, 2004; Ito et al., 2010; Grandin, 2016).

4.5   *Transgenerational impacts*

Maternal heat stress during late-gestation negatively impacts the birth and weaning weight of the resulting calf (Tao et al., 2012; Monteiro et al., 2016; Laporta et al., 2017; Dado-Senn et al., 2020). When cooled dry cows were pair-fed with heat stressed dry cows, calves of the heat stressed cows still had lower birth weights compared to the cooled cows (Almoosavi et al., 2020). Their finding supports the hypothesis that heat stress itself, and not just the reduced feed intake in response to heat stress, somehow causes a reduction in birth weight. Some potential explanations for the decreased birth weight of calves that were heat stressed *in utero* are decreased gestation length (Tao et al., 2012; Fabris et al., 2019; Almoosavi et al., 2021; Davidson et al., 2021), altered placental development (Tao and Dahl, 2013; Van Eetvelde et al., 2016), and increased fetal temperature (Ouellet et al., 2021). Notably, most papers evaluating late-gestation heat stress focus on multiparous cows. Although, in a late-gestation heat stress study in nulliparous heifers, birth weights, weaning weights, and ADG were not different between calves that were heat stressed (IUHT) or cooled (IUCL) while *in utero*; however, there was still a decrease in gestation length for the heat stressed heifers comparable to heat stressed multiparous cows (Davidson et al., 2021). Those authors suggested that nulliparous pregnant heifers and the developing fetus potentially

17

have increased thermotolerance and further suggested that the IUHT calves may have had a higher fetal growth rate.

Laporta et al. (2017) evaluated the rectal temperature of preweaned calves of multiparous cows exposed to late-gestation heat stress versus cooled and found the IUHT calves to have a 0.4 ºC higher temperature. A study of IUHT or IUCL calves heat stressed or cooled postnatally during the preweaning period showed that IUHT calves cooled postnatally had the lowest respiration rate in the morning and evening; furthermore, calves heat stressed both *in utero* and postnatally had the highest rectal temperature and respiratory rate during the afternoon measurements (Dado-Senn et al., 2020).

While these studies focused on preweaned calves, Ahmed et al. (2017) reported that lactating cows exposed to heat stress when in utero were more thermotolerant compared to control cows, as evidenced by lower sweating rates as well as tendencies for lower rectal temperatures and higher surface temperatures. They concluded that the IUHT cows may have increased skin blood flow and would thereby have increased conductive heat loss, which would decrease the necessity for activation of the sweating response.

Davidson et al. (2021) studied IUHT and IUCL offspring of nulliparous heifer dams. They found no difference for the sweating rate, rectal temperature, or respiratory rate between IUHT and IUCL offspring; however, there was a tendency for IUHT calves to have lower skin temperature at 24 h of age yet higher at day 56 compared to IUCL calves. While the cause of the shift in skin temperature is unknown, Davidson et al. (2021) proposed that the IUCL calves, while initially less able to thermoregulate after birth compared to IUHT calves, are actually the ones with the more advanced thermoregulatory ability by weaning age, potentially due to the IUCL

environment. The conflicting findings of the thermoregulatory ability of IUHT calves warrants further investigation.

Heat stress experienced *in utero* seems to impact future milk production, with most research being retrospective studies. Monteiro et al. (2016) reported IUHT cows produced 5.1 kg/d less milk than IUCL cows up to 35 wk of their first lactation. Looking further, Laporta et al. (2020) reported that milk production of IUCL cows was greater in the first (2.2 kg/d), second (2.3 kg/d), and third lactation (6.5 kg/d) compared to IUHT cows. Furthermore, the daughters of the IUHT cows– which derived from the germline of the IUHT fetus during their first-generation cow's exposure to heat stress – produced less milk than daughters of IUCL cows during first lactation (1.3 kg/day; Skibiel et al., 2018). Additionally, late-gestation cows exposed to THI ≥ 60 for the last 3 weeks before calving had offspring with reduced milk yield and, at THI ≥ 50 for the last 8 weeks before calving, reduced milk fat percentage (Kipp et al., 2021). This reduction in performance of calves born to IUHT cows may be partially due to altered mammary gland development. At birth and weaning, IUHT heifers have lighter mammary glands compared to IUCL heifers (Dado-Senn et al., 2021). Further, while IUHT heifers have the same number of alveoli as IUCL heifers at 21d and 42d of their first lactation, IUHT heifers have 46% smaller alveoli, fewer secretory cells, and 84% more connective tissue thereby decreasing capacity for milk storage and secretion (Skibiel et al., 2018a,b; Laporta et al., 2022).

Reproductive performance can also be reduced in IUHT calves compared to IUCL calves. In a recent study, IUHT heifer calves had smaller ovaries at weaning compared to IUCL heifer calves, which may impact their future reproductive ability (Dado-Senn et al., 2021). Consistently, IUHT calves have a smaller size of ovarian follicular reserve as indicated by lower concentrations of anti-Müllerian hormone (AMH) – which has been shown in multiple species to be highly

associated with size of the ovarian follicular reserve (Akbarinejad et al., 2017). In the study by Kipp et al. (2021), calves from dry cows exposed to THI ≥ 50 for 8 weeks pre-calving had an increased interval for calving to first breeding by approximately 2 days.

Immunity of calves exposed to heat stress *in utero* is sacrificed due to decreased efficacy of passive immunity as demonstrated by their decreased absorption IgG and lower IgG serum concentrations, especially within the first 24h after birth (Tao et al., 2012; Monteiro et al., 2014; Laporta et al., 2017; Dado-Senn et al., 2021; Davidson et al., 2021). The reason for decreased efficacy is still unclear, where current theories include but are not limited to: accelerated gut closure potentially due to increased rate of apoptosis in the jejunum, enterocytes not reaching full endocytotic capability due to decreased gestation length, and altered Fc receptor expression (Ouellet et al., 2020; Ahmed et al., 2021). *In utero* heat stressed calves also have altered cell mediated immunity such that they have lower peripheral blood mononuclear cell proliferation at up to 56d (Tao et al., 2012) and higher neutrophil and lymphocyte percentage at 42d (Monteiro et al., 2014). Further, compared to IUCL calves, IUHT calves have lower levels of TNFα and higher levels of IL-1 (Strong et al., 2015). Ahmed et al. (2021) reported lower spleen and thyroid weights proportional to body weight of IUHT calves compared to IUCL calves.

While the environment does not change the DNA sequence, it can influence the epigenome via histone modifications, non-coding RNA, or DNA methylation (Huber et al., 2020). During embryonic development, heat stress can influence epigenetic programming by altering the germline of the developing offspring which in turn alters the phenotype of the next generation (Skinner, 2011). Specifically within the mammary tissue, IUHT and IUCL calves have differentially methylated genes primarily involved in pathways for protein binding, enzyme and

cell activation, cell signaling, and inositol phosphate metabolism (Skibiel et al., 2018; Laporta et al., 2022).

Taking transgenerational effects one step further, Weller et al. (2021) examined four generations of dairy cows and the impacts of heat stress. The first generation (F_0) had been exposed to heat stress when they were pregnant with the second generation (F_1), meaning their F_1 may experience epigenetic reprogramming that would in turn affect the third generation (F_2) via the oocyte. Potentially, the epigenetic reprogramming from F_1 would pass on to the subsequent generations including the fourth generation (F_3), which was not exposed to heat stress. The authors concluded that F_2 and F_3 cows have reduced overall production and calving traits when the F_0 cows are in the second half of their pregnancy during the hot season; moreover, when both the first and second generation experience the hot season during the second half of gestation, the F_3 production and calving traits were the most detrimentally impacted. These findings support the theory that epigenetic modifications are maintained and that the different combinations of seasons of F_0 and F_1 yield intermediate values that could be due to the partial loss or restoration of the epigenetic modifications (Weller et al., 2021).

4.6   Calves and heifers

Extensive research has sought to define and quantify the effects of heat stress on mature cows to reduce production losses and improve welfare. However, less is known about young stock – especially growing heifers – due to their perceived increased tolerance to heat stress from lower metabolic heat load and higher surface area to mass ratio, and also due to their lower return on investment compared to lactating cows. Even though young stock may be more tolerant to heat stress than lactating cows, their welfare and future performance can still be affected by heat stress as explained in the above sections.

In previous research, the heat stress THI threshold for dairy heifers between 0 and 1 year of age was calculated at 77 by adapting measurements from finishing beef cattle (St. Pierre et al., 2003). In subtropical conditions, there is a strong positive correlation between THI and rectal temperature, respiration rate, and skin temperature in preweaned Holstein heifer and bull calves under chronic heat stress (Dado-Senn et al., 2020; Laporta et al., 2021). Thresholds for THI vary by the animal-based indicator: at or above a THI of 65, respiration rate increases 2.5 breaths per min per THI unit; at or above a THI of 67 rectal temperature increases 0.03ºC per THI unit; and at or above a THI of 82, milk intake decreases 0.29 L per day per THI unit (Dado-Senn et al., 2020). When short-term heat stressed in a more continental climate (characterized by less humidity), ambient temperature was better correlated with the animal-based indicators than THI for unshaded 7 week-old Holstein bull calves (Kovacs et al., 2018a). Lopez et al. (2018) reported a dramatic decrease in weaning weight of female Holstein calves when THI exceeded 75.

Similar to lactating cows, calves can also alter their behavior in an attempt to decrease heat stress. Cooled, group housed preweaned Holstein calves spent more time lying compared to heat stressed calves and, when provided cooling, calves spent more time lying under the location of maximum air flow (Kovacs et al., 2018; Dado-Senn et al., 2022). Heifers will similarly increase standing time and will actively try to access shade during increased thermal conditions (Pandey et al., 2017; Cardoso et al., 2021). Calves will also decrease their milk intake and dry matter intake to decrease their metabolic heat production (Lopez et al., 2018; Dado-Senn et al., 2022). This decreased intake is associated with lower ADG and weaning weight (Bateman et al., 2012; Lopez et al., 2018). Postweaned heifers will decrease their dry matter intake and increase water intake in response to heat stress (Kellawaya and Colditzab, 1975; Nonaka et al., 2007).

Few studies have examined the direct impact upon the immune system and future milk production of calves and growing heifers experiencing heat stress. Compared to thermoneutral calves, calves exposed to sustained high temperature had lower circulating IgG concentrations and peripheral blood lymphocyte count (Kelly et al., 1982). Calves exposed to heat stress were higher risk to contract bovine respiratory disease (Louie et al., 2018). In one study, heat stressed postweaned heifers had decreased oxidative burst capacity and lymphocyte proliferation response compared to thermoneutral Holstein heifers (Elvinger et al., 1991); conversely, another study reported comparable PBMC proliferation, neutrophil phagocytosis, and oxidative burst between heat stressed and thermoneutral Holstein heifers 98 ± 14 days old (Dahl et al., 2020). Srikanth et al. (2017) reported upregulation of immune-related signaling pathway genes in postweaned Holstein calves after acute heat stress. Similar to cows, pregnant nulliparous heifers exposed to heat stress in late gestation had reduced yields of milk, protein, and fat in their subsequent first lactation compared to cooled heifers (Davidson et al., 2021). Interestingly, a few studies have reported that calves born in summer ended up having higher first lactation performance than cows born in other seasons (Chester-Jones et al., 2017; Van Eetvelde et al., 2017; Chuck et al., 2018). More research is needed to decipher the direct effects of heat stress in the immune function of calves and growing heifers, future milk production, and mammary development.

5.   *Current methods of heat stress mitigation*

Heat stress mitigation takes many forms and can attempt to minimize heat gain from solar radiation, maximize heat loss via convection and evaporation, reduce metabolic heat load with nutrition, or utilize natural genetic advantages in breeding programs.

Properly designed shade can reduce the heat load from solar radiation 30-50% (Wang et al., 2020). As mentioned previously, dairy cattle will choose shade over sprinklers and will choose

shade structures that provide the greatest blockage of radiation (Gaughan et al., 1998; Shutz et al., 2009, 2011). Furthermore, less solar radiation experienced while in the shade translates to lower minimum body temperatures on days with high levels of ambient solar radiation (Kendall et al., 2007; Tucker et al., 2008). Lactating cows with access to shade have higher milk yield than cows without shade (Kendall et al., 2006).

Similarly, calves exposed to heat stress have lower rectal temperatures when utilizing shade (Kovacs et al., 2018a,b). Moreover, supplemental shade over individual plastic hutches resulted in calves having a less dramatic increase of rectal temperature than calves in hutches without supplemental shade (Lammers et al., 1996; Spain and Spears, 1996). Provision of shade for feedlot heifers resulted in higher dry matter intake, average daily gain, final body weight, and number of carcasses graded USDA Choice than unshaded heifers; furthermore, shaded heifers had lower rectal temperatures, respiration rates, circulating neutrophil concentrations, standing time, and antagonistic behavior (Mitlöhner et al., 2002; Gebremedhim et al., 2011).

As of 2014, 86.2% of dairy operations in the United States provided man-made shelter to supply shade for cows (USDA, 2014), so the vast majority of cooling systems are created in combination with shade. Unless otherwise specified, the following mitigation methods are used in some form of shaded environment.

To aid in convective cooling, fans and ventilation systems can be installed. One of the critical components of convective cooling is a fast enough airspeed to be effective at cooling the cows (Wang et al., 2018; Mondaca, 2019). Recommended airspeeds are between 2.5-3.0 m/s since high airflow speeds may raise dust and scatter feed, decreasing air quality (Wang et al., 2018). Spears et al. (2018) reported that cows continuously cooled via fans under environmental conditions consistent with heat stress had a less dramatic reduction in dry matter intake than cows

cooled for 8 hours in the morning or night. Lactating cows in an open-sided barn had lower milk yields and higher body temperatures than those in open-sided barns with fans (Folman et al., 1979; Takamitsu et al., 1987; Frazzi et al., 2000). Stowell et al. (2001) observed that tunnel-ventilated barns performed only slightly better than naturally ventilated barns with supplemental cooling fans in environments with THI peaks between 70 and 75; however, in hot and humid weather with little wind, tunnel ventilation was likely to be more beneficial. Lactating Holstein cows housed in tunnel-ventilated barns in north Florida had lower rectal temperature and less dramatic seasonal reduction in milk yield compared to cows in sprinkler-and-fan open-sided barns (3.5% vs. 5.8% milk yield decrease, respectively; Dikmen et al., 2020). Tunnel-ventilation is limited, however, as barns that are too long for the fan-exhaust system to be efficient may not provide proper air flow to all portions of the barn (Gooch and Stowell, 2003). Moreover, achieving sufficient air speeds in the stall microenvironments is challenging when only exhaust fans are utilized because of the structural components of the barn that redirect air flow away from stalls (Mondaca, 2019). Cross-ventilation yields the greatest convective heat loss (Wang et al., 2018), most often assisted by baffles which aid in increasing air speed and distribution of air of sufficient speed (Mondaca, 2019); further, cross-ventilated barns have a lower barn footprint per stall and overall are lower cost than tunnel-ventilated barns (Mondaca, 2019).

Calves also can benefit from systems to increase convective cooling. Hill et al. (2011) reported that calves cooled with fans had lower respiration rates, 23% greater ADG, and 20% greater feed efficiency compared to calves without fans. To increase air movement in hutches, placement of blocks beneath the hutch can reduce interior temperature and carbon dioxide levels, which in turn helped decrease the respiratory rates of the calves (Moore et al., 2012).

Utilization of water to cool either the air or the cows, is another method to mitigate heat stress. One way to cool the air is to use a fogging system, which utilizes very fine water droplets that are immediately dispersed into a fan's air stream where they evaporate. The cooled air is blown over cows and does help cool cows during periods of heat stress (Worley, 2016). Two specific drawbacks to the fogging system are that the water filters need to be checked daily and cleaned, and the systems are more expensive than mister systems, which also work to cool the air. Mister systems work in the same manner, just with slightly larger water droplets. Dairy cows benefit from a mister system as seen by reduced rectal temperatures and increased daily milk yield (Lin et al., 1998; Calegari et al., 2011). While mister systems generally reduce ambient temperature 2-9ºC and THI anywhere from 1-5 units, relative humidity is increased 8-50 percentage points due to the added moisture to the air (Fournel et al., 2017; Almuhanna et al., 2021). This can be problematic in high humidity climates, making sprinkler systems a better choice (Collier et al., 2006). Sprinkler systems like soakers create large droplets of water that wet the cow's hair coat and skin. Usage of sprinklers on their own allows for similar or decreased body temperatures; however, data on the effect of only sprinklers on milk yield is variable and not consistent (Seath and Miller, 1948; Fuquay et al., 1979; Igono et al., 1985; Kendall et al., 2007; Chen et al., 2016).

To maximize heat loss, combinations of cooling systems generally work best (Grant, 2012). Mist-fan (MF) systems result in an average of 0.4 to 1.1 ºC decrease in body temperature, 12-48 fewer breaths per minute, and 0.4-4.2 kg per day increase in milk yield depending on breed and location (Fournel et al., 2017). Even with variations in duration and frequency of sprinklers, sprinkler-fan (SF) systems create lower THI (0.2-5.9 units) environments and result in reduced body temperatures (0.3-1.1 ºC), lower respiration rate (7-55 breaths per minute), higher dry matter intake (-.9-3.0 kg per day), and increased milk yield (1.0-4.1 kg per day per cow; Fournel et al.,

2017). Furthermore, implementation of a cooling system for dry cows can benefit their subsequent lactation. For example, do Amaral et al. (2011) utilized SF to cool cows from dry-off to calving and reported the cooled cows tended to have higher daily milk yield compared to the cows without the cooling system (34.5 vs 32.2 kg/day, respectively). Moreover, Thompson et al. (2014) reported a tendency for cows cooled via a SF system for the duration of their dry period to produce 3.8 kg more milk per day than cows without access to the cooling system.

Moghaddam et al. (2009) evaluated the effect of no cooling (control) with cooling heifers 2 hours before AI until 2 hours post-AI with sprinklers or SF on pregnancy rate 45 days after AI. Those authors reported that at the time of AI, rectal temperature was lower in heifers under sprinklers (39.5 ºC) and was even lower for heifers with SF (39.3ºC) than control heifers (39.9 ºC). However, at 1.5 and 3.5h post-AI, RT of control (39.3 and 39.3ºC) and sprinkler (39.3 and 39.2 ºC) heifers were similar, while heifers that also had access to SF were significantly lower (38.9 and 38.7 ºC). Heifers cooled with SF had a higher pregnancy rate than the control heifers (56.7% vs. 23.3%, respectively), with heifers cooled with sprinklers having an intermediate pregnancy rate of 40.0%.

While implementation of these cooling systems has positive effects on dairy cattle production and welfare, there are some drawbacks for installation of cooling systems. For one, adding water to an environment can potentially increase health problems like mastitis and respiratory disease, especially if the bedding is wet (Nienaber and Hahn, 2007). Another drawback is the increase in water usage for cooling systems on top of water required for drinking and, if applicable, cleaning systems. In Florida, Montoya et al. (1995) reported that each sprinkler nozzle would output 2.1 L of water for 1.5 min every 15 min once the cycle had been activated by temperatures exceeding 25.6 ºC. Bray et al. (1993) recommend a minimum practical application

rate of 25 gal (94.6 L) of water per cow per day for cooling systems alone. In locations where water is more challenging to acquire like California, these systems may not be feasible. Additionally, cooling systems require considerable amounts of electrical energy which increases the overall operational costs (Collier et al., 2006). From an environmental perspective, heifers cooled with sprinklers and shade in feedlots had 46% greater NH_3 emissions than heifers cooled with shade due to the hydrolysis of urea nitrogen from the manure interacting with water (Marcillac-Embertson et al., 2009).

In a cost analysis of different cooling systems, Perano et al. (2017) concluded that fans and SF systems are more economical to install and operate than MF systems. When combined with the profits from the varying efficacy of heat mitigation and its associated milk yield increase, SF produced the greatest positive return on investment. In climates with 2 mo/yr of heat stress, only fan ($10.37/cow/yr) and SF ($18.25/cow/yr) systems had a positive net return on investment while MF (-$29.78/cow/yr) had a negative return; however, in climates with 6 mo/yr of heat stress, fan, SF, and MF all had a positive return on investment ($45.41, $76.32, $7.34/cow/yr, respectively). These conclusions support the analysis of the economic feasibility of cooling dry cows performed by Ferreira et al. (2016) who found that implementation of a SF system for dry cows, while costly up front, prevented the loss of 290 kg of milk per cow in their subsequent lactation, which would amount to $87 per cow per year or $810 million in annual losses for the United States dairy industry.

Besides modifications to the environment, nutrition and genetic selection strategies can be utilized to alleviate some of the impacts of heat stress. Nutrition can be modified to account for the reduction in dry matter intake, the increased nutrient requirements, and the impact of dietary heat increment. The most common way to approach these changes is by feeding less forage, which

is bulky and may discourage intake, and more concentrate, which increases the energy density of the diet (West, 2003). Additionally, a plethora of supplementation methods exist to mitigate the detrimental effects of heat stress and in turn support milk production, immunity, and fertility (Toledo et al., 2020). Genetic selection for thermotolerance is highly desirable since alteration to genotype is permanent for the animal and influences their offspring. The traditional method has been crossbreeding high producing *B. taurus* cattle with more thermotolerant *B. indicus* cattle; however, doing so sacrifices production of the F1 offspring. Alternatively, transfer of known thermotolerant alleles may allow for high producing breeds to gain some thermotolerance without a drastic loss in milk production or potential drop in fertility (Hansen, 2020).

6.   *Slick hair mutation*

During the past 40 years, researchers have pursued the short sleek hair coat phenotype in cattle (Figure 1.1) caused by a mutation in the prolactin receptor (PRLR) gene as a potential heat stress mitigation method. Appropriately coined the "slick hair mutation" by Olson et al. in 2003, research into the phenotype soon associated it with an increased heat tolerance; slick Holstein cows have little seasonal variation in milk yield unlike wild-type Holsteins that typically have a drop in milk yield during the warmer months (Dikmen et al., 2014). The mutation is now known to be dominantly inherited with no apparent deleterious effects on embryonic or fetal survival since inheritance follows the Hardy-Weinberg equilibrium (Sosa et al., 2021).

6.1   History and physiology

There are several breeds of *B. taurus* cattle in central and south America that have been bred for adaptation to tropical conditions. These breeds include Senepol, Romosinuano, Criollo Limonero and Carora. Cattle from these breeds typically present one of two hair coats: one of normal length for a *B. taurus* breed, and the other with very short length, similar to *B. indicus*

cattle. One of the first studies examining the thermotolerance of *B. taurus* cattle from the tropics was performed by Hammond and Olson (1994) in subtropical Florida to compare the rectal temperature (RT) of Senepol, Angus, Hereford and Brahman cattle during summer. The authors found that Senepol and Brahman had similar temperature, which was lower than that of Hereford and Angus animals. Crossbreeding of Hereford and short hair Senepol revealed that the offspring inherited the short hair phenotype and lower RT typical of Senepol cattle. This research was expanded to include the Romosinuano breed, and again the results demonstrated that both Senepol and Romosinuano cattle were able to maintain lower RT and respiratory rates (RR) compared to Angus and Hereford cattle during summer (Hammond et al., 1996). When investigating this phenomenon further, Olson and colleagues (2003) performed backcross matings with Holsteins, Charolais, or Angus to Senepol or Carora crosses and found evidence of a major gene with dominant inheritance responsible for creating the short, sleek hair coat phenotype. They also reported lower RT in slick-haired, crossbred calves (0.18-0.4ºC) and lactating cows (0.61ºC) when compared to normal-haired contemporaries with RR being correlated to RT. In slick-haired 75% Holstein: 25% Carora lactating cows, they observed greater milk yields than Carora and Carora x Holstein F_1, and normal haired 75% Holstein: 25% Carora. Lastly, they did not detect a significant difference in weaning weights between slick- and normal-haired calves.

First mapped to Chromosome 20 in Senepol and Senepol crosses (Flori et al., 2012; Mariasegaram et al., 2007), Littlejohn et al. (2014) later found a causative mutation in the *PRLR* responsible for the slick hair coat phenotype observed in Senepol cattle. A frameshift mutation from a single cystine deletion (chr20;39136558GC>G) causes a premature stop codon (p.Leu462*) in the resulting protein and the loss of 120 amino acids in the C-terminus, thereby truncating the cytoplasmic domain of the PRLR. This mutation became known as the SLICK1 allele and, since

then, additional research has found mutations in other regions of the PRLR resulting in truncation of the protein at different points and causing the similar slick phenotype observed in criollo-derived *B. taurus* breeds (Porto-Neto et al., 2018; Flórez Murillo et al., 2021). These alleles have been named SLICK2-SLICK6 (Flórez Murillo et al., 2021). The work presented in this thesis focuses on the SLICK1 allele.

The exact introduction of the SLICK1 allele into the Holstein population is unknown. However, matings between Senepol – who have so far only been found to carry the SLICK1 allele (Littlejohn et al., 2014; Porto-Neto et al., 2018; Flórez Murillo et al., 2021) – and Holsteins were performed in Florida and Puerto Rico since the 1980s, such that today there are registered Holstein animals that carry the SLICK1 allele. In Puerto Rico, crosses between Holsteins and other thermotolerant criollo breeds found in the Caribbean were practiced for many years before the introduction of the Senepol (Ortiz-Colon et al., 2018). As a result, Puerto Rican Holsteins are still genotyped as having the SLICK1 allele, but the mutation is most likely to have been introduced via a shared common ancestor between Senepol and the other criollo breeds in Puerto Rico (Hansen, 2020).

Initially demonstrated by Olson et al. (2003) and then further investigated by subsequent research, slick-haired cattle in subtropical and tropical environments are more tolerant to heat stress. In Florida, two publications from Dikmen et al. (2008, 2014) reported lower rectal and vaginal temperatures and RR of lactating Holstein cows with slick genotypes compared to non-slick contemporaries during summer. Similar findings were observed by Landaeta-Hernandez et al. (2021) when studying heat stress in Criollo Limonero non-pregnant heifers in Venezuela, such that the slick-haired heifers had lower RT and RR than normal-haired heifers. It is worth noting,

31

though, that Criollo Limonero have not been reported to carry the SLICK1 allele, but instead the SLICK2 and SLICK3 alleles (Porto-Neto et al., 2018).

Studies evaluating physiological responses to environmental conditions also measured sweating rate and surface temperature (Dikmen et al., 2008; Dikmen et al., 2014; Eisemann et al., 2020). Dikmen and colleagues (2008) reported that slick Holstein cows (n=10) had higher sweating rates only in areas of unshaved hair, but when the area was shaved, wild-type cows (n=10) had higher sweating rates. This led them to conclude that with shorter hair – whether by being clipped or naturally slick – the decrease in humid air trapped at the skin surface allowed for an increased sweating rate. The authors also reported no difference in skin temperature between genotypes. They postulated that heat delivery to the skin is enhanced in slick cows because, if slick and wild-type cows had comparable heat delivery, slick cows would have lower surface temperatures due to greater heat loss. A later study by Dikmen and colleagues (2014) also evaluated sweating rate and surface temperature. In that study, the authors found slick Holstein cows (n=13) to have higher sweating rates compared to wild-type Holstein cows (n=18), regardless of the area being shaved or unshaved; furthermore, slick cows had lower skin temperatures than wild-type cows. From these results, they first concluded that slick cows may have a greater density of sweat glands or an increased capacity of the individual glands for sweat production. They further posited that the slick cows have an increased rate of heat loss at the skin, which agrees with their finding of the increased sweating rate.

The inconsistent findings on sweating rates and skin temperature of slick cattle may have to do with project design. In their 2008 research, Dikmen et al. measured sweating rate only at the shoulder; however, in 2014, they took sweating measurements from the neck, loin, rump, and upper hind leg, which they suggested gave a more accurate representation compared to a singular

location. As for skin temperature, research by Eisemann et al. (2020) that evaluated surface temperature found that slick haired Angus-Senepol heifer crosses had higher surface temperature than the normal haired Angus-Senepol heifer crosses. Meanwhile, Dikmen et al. (2014) reported slick Holstein cows to have lower skin temperatures than non-slick cows and Dikmen et al. (2008) reported no difference between slick and non-slick Holstein cows.

While not a direct measurement of sweating rate, Landaeta-Hernandez et al. (2011) researched the skin morphology of slick- and normal-haired Criollo Limonero (most likely carrying an alternate slick mutation other than the SLICK1 allele), which provides critical phenotypic information that has the potential to be associated with sweat production. There were no differences between hair types for the number of hair follicles, sweat gland, or sebaceous glands per square centimeter, nor thickness of epidermis; moreover, there were no differences for the number of blood vessels per square centimeter between genotypes. The authors did find a difference in blood vessel number by age with the younger heifers having approximately 814 less vessels per square centimeter as compared to grown heifers. They also found that the slick-haired females had larger sweat glands with a different morphology compared to wild-type females. The slick animals had sweat glands more consistent with *B. indicus* while the normal haired females had the tubular shaped sweat glands primarily seen in *B. taurus*. Similarly, Puerto Rican slick Holstein cows had increased skin thickness and cross-sectional sweat gland area and perimeter compared to wild-type cows (Contreras-Correa et al., 2017). Also evaluating Puerto Rican Holstein cows, Muñiz-Cruz and colleagues (2018) reported that slick cows had a greater sweat gland cross-sectional area of average of approximately 2,000 μm^2 as compared to their wild-type counterparts; however, this difference is no longer significant when compared to Senepol cows as they had a 2,700 μm^2 greater sweat gland cross-sectional area as compared to slick Holstein cows.

To be noted, the exact slick allele responsible for the slick hair mutation in the Puerto Rican Holstein cows was not noted in these studies and therefore the results should be interpreted with some caution.

One of the major motivators for the continued investigation into the thermotolerance of slick cattle is the hope that it might result in a less dramatic drop in milk yield during summer months. Within the Carora-Holstein crossbred cows from an arid tropical area of Venezuela, slick haired 75% Holstein cows had greater 305-d milk yield than Carora, Carora x Holstein F_1, and normal haired 75% Holstein, but not purebred Holsteins (Olson et al., 2003). That study showed that within 75% Holstein cows, having slick hair was advantageous, to the point that these animals had equivalent production to purebred Holsteins despite likely having lower genetic merit for milk yield (although genetic merit was not reported in this study). Dikmen et al. (2014) found that slick Holstein cows from Senepol cross origin did not have as large of a depression in milk yield during the hot season, losing on average 1.3 kg/day in slick cows as compared to 3.7 kg/day in wildtype cows. In that same study, seasonal variation in milk composition was reported to be greater for slick cows, which could represent a drawback to utilizing the slick mutation. Those results have not been confirmed or refuted since. Research conducted in Puerto Rican Holstein cows by Sánchez-Rodríguez and colleagues (2021) reported that slick cows produced on average 4.27 kg/day more milk than non-slick cows; they also reported that the slick cows had an increased grazing time under sunlight. To be noted, we cannot rule out that some of the discrepancies between the findings of these studies could be caused by the different genetic makeup of the animals and study conditions, which includes the different slick mutations. Further research is needed to improve our understanding on how the slick mutation modifies milk yield and composition.

Slick-haired Puerto Rican Holstein heifer calves had comparable weekly body weight change to normal-haired Holstein calves during the first 8 weeks of life (Colón-Rodríguez et al., 2020). However, another study evaluating Puerto Rican Holstein heifers from 4 to 34 months of age reported a divergence in growth pattern such that slick heifers followed a quadratic curve and wild-type heifers followed a linear growth trend (Sánchez-Rodríguez et al., 2019). The authors hypothesized that at 34 months of age the wild-type heifers were still in the juvenile growth phase and had not yet neared maturation weight, and the slick heifers may have reached their mature body weight earlier, thereby reaching a smaller mature size earlier than the non-slick heifers. This finding supports the increased thermotolerance and the less dramatic decrease in milk production during periods of heat stress in the slick cows since a smaller mature weight will have increased surface area to mass ratio thereby having a lower metabolic heat load, an increased ability for heat dissipation, and lower energy maintenance requirements (Sánchez-Rodríguez et al., 2019). While these results are interesting, their interpretation should be taken with caution as variation in genetic background beyond inheritance of the slick allele(s) were not mentioned. Also, the exact allele responsible for the slick mutation in these Puerto Rican Holstein cows was not stated and may be due to an alternate *PRLR* mutation that results in an allele other than the SLICK1 allele.

In summary, studies have repeatedly demonstrated that animals carrying the slick mutation and presenting with a short hair phenotype maintain lower body temperature and other physiological variables that confer greater thermotolerance during periods of heat stress, although more research is needed to unravel the cellular and molecular mechanisms involved in these phenotypic changes.

6.2 Prolactin signaling and mutations

Prolactin (PRL) is a polypeptide hormone synthesized and secreted mainly by lactotroph cells in the anterior pituitary gland. Originally described as the hormone responsible for milk synthesis, lactation, and mammary gland development and function, PRL has since been associated with over 300 biological functions (Bole-Feysot et al., 1998). The focus of the PRL section of this review is mainly on thermoregulation; consequently, the effects of PRL on the mammary gland and reproductive performance, while important, are beyond the scope of this review. Besides the anterior pituitary, other tissues - such as the ovary and endothelial and immune cells - can produce extra pituitary PRL (ePRL). The PRLR is a single pass transmembrane receptor, part of the hematopoietic type 1 cytokine receptor superfamily; it has several isoforms that are either termed long, short, or intermediate (with varied lengths within the "intermediate" isoform) depending on the length of the receptor's intracellular region generally resulting from alternative splicing. Consistent with the diverse functions of PRL, PRLRs – both long and short – can be found throughout the body.

The primary pathway for signal transduction downstream of the PRLR is the JAK-STAT pathway (Freeman et al., 2000) which consists of the Janus kinase (JAKs) and signal transducers and activators of transcription proteins (STATs). Activation of the PRLR requires extracellular ligand-induced sequential receptor dimerization (Freeman et al., 2000; Radhakrishnan et al., 2012). When PRL binding dimerizes the receptor, the JAK-STAT pathway is initiated. First, JAK2 kinases that are constitutively associated with BOX1 of each PRLR monomer phosphorylate each other, and then aid in phosphorylating multiple tyrosine residues of the cytoplasmic region of the PRLR. These phosphotyrosines act as docking sites for STATs such as STAT5, which bind and activate via phosphorylation. The phosphorylated STATs dissociate from the PRLR and form a STAT dimer. This dimer translocates to the nucleus where it will bind specific response elements,

modulating transcription in target genes. Non-canonical signaling pathways activated by PRL include but are not limited to the mitogen activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K) pathways (Freeman et al., 2000; Radhakrishnan et al., 2012).

Much less information exists about the signaling mechanisms operating in the short and intermediate isoforms of the PRLR. As research has not yet shown how PRL signaling for the slick hair mutations (truncated forms of the receptor) is altered, we can look to what little is known about short and intermediate PRLR isoform signaling for potential indicators. There is some evidence that the intermediate PRLR in humans can activate the JAK-STAT pathway and can enhance cell survival, but it does not sufficiently promote cell proliferation (Kline et al., 1999). The short form was originally thought to prevent PRL signaling – mainly via JAK-STAT – through heterodimerization with the long isoform since the short PRLR lacks the region of the receptor that allows for interaction with STAT proteins. However, the short PRLR has also been shown in mice to form a homodimer that has its own signaling pathway, potentially activating MAPK (Devi et al., 2009, 2011). Further research to elucidate the signaling pathway for these PRLR isoforms, including those of the slick hair mutations, is warranted.

At the pituitary level, dopamine is a powerful suppressant of PRL secretion, and PRL will also suppress its own production via stimulation of dopamine synthesis and secretion (Freeman et al., 2000). Meanwhile, estrogen activates PRL expression and release while upregulating PRLR expression; further, thyrotropin-releasing hormone (TRH) stimulates PRL release, and oxytocin and vasoactive intestinal peptide both inhibit dopamine production thereby indirectly allowing for increased synthesis of PRL (Freeman et al., 2000). Additionally, the immune system is modulated via PRL signaling; while literature is not clear on the exact nature of their relation, under heat

stress conditions, PRL plasma concentrations are increased while mRNA expression of *PRLR* gene is decreased (do Amaral et al., 2010; do Amaral et al., 2011).

During seasonal or short-term heat stress, cattle have increased serum concentrations of PRL (Roman-Ponce et al., 1982; Ronchi et al., 2001; do Amaral et al., 2009; Fabris et al., 2020), but have decreased expression of the *PRLR* gene in the liver, mammary gland, and lymphocytes (do Amaral et al., 2010, 2011; Fabris et al., 2020). While the exact function of PRL during periods of heat stress is still unclear, there is potential for prolactin to play a role in thermoregulation due to evidence in livestock mammals of high PRL levels that may be associated with decreased RT, increased RR and expression of heat-shock proteins, down-regulated sweating, redistributed water towards the periphery, as well as increased water intake and absorption (Alamer, 2011).

Prolactin may aid in season thermoregulation by regulating hair follicle function in a photoperiod-dependent manner. During long photoperiods (i.e., spring/summer with long days, short nights), PRL levels are higher and PRLR expression is lower, and the inverse is observed for short photoperiods (i.e., fall/winter with short days, long nights; Collier et al., 2006). Increasaed levels of PRL are associated with hair follicles transitioning through catagen (involution) and entering the telogen (resting) phase of the hair cycle; appropriately, decreasing PRL levels are associated with hair follicles transitioning out of telogen and entering the anagen (growth) phase (Nixon et al., 2002; Geyfman et al., 2015; O'Brien et al., 2020; Zhang et al., 2021). As PRL increases with the elongating photoperiod (e.g. spring), an animal will molt its winter coat and be left with a shorter, less dense hair coat, and then, as PRL decreases with shortening photoperiod (e.g. autumn), an animal will grow a winter coat characteristically long and dense (O'Brien et al., 2020). In Angus x Senepol cattle fed forage with toxic endophyte (which normally causes growth of rough hair coat), slick haired offspring had more hair follicles in telogen and fewer in anagen

compared to normal haired offspring during the warm season (Eisemann et al., 2020). It is possible that the slick PRLR isoform inherited from the Senepol increases the proportion of hair follicles in telogen versus anagen resulting in the characteristic short, sleek hair coat.

In addition to systemic PRL affecting skin and hair, the skin produces ePRL and may have its own regulatory system (Foizik et al., 2003). Little is known about regulation of ePRL and PRLR at the skin level, and, while there are some studies of regulation in other mammals, caution should be taken in extrapolating the findings from these species to cattle. In humans, Langan et al. (2010) reported that estrogen acts to stimulate ePRL and PRLR similarly in skin as in pituitary, such that estrogen increases gene and protein expression of ePRL and PRLR. The authors also found that *in vitro* treatment of hair follicles with TRH increased PRL expression at the gene and protein level and *PRLR* gene expression; however, TRH decreased PRLR protein expression. In a later study evaluating serum-free organ culture of human scalp skin, dopamine – the classic hypothalamic PRL inhibitor – had no effect upon ePRL; however, in the hair follicles, interferon gamma (IFNγ) increased PRL immunoreactivity while tumor necrosis factor alpha (TNFα) decreased PRL and PRLR immunoreactivity thereby providing evidence for the existence of two potential regulators of ePRL within the skin (Langan et al., 2013).

As mentioned previously, multiple PRLR mutations have been found that result in the slick hair phenotype. While there are short and long isoforms of the PRLR that confer normal haired animals, slick isoforms are an intermediate length ranging from 427-479 amino acids long due to premature stop codons. All six known PRLR isoforms that result in slick phenotypes are dominant potentially due to the truncated receptor eliminating a secondary tyrosine domain needed for JAK2/STAT5 activation – one of the primary PRLR signaling pathways (Freeman et al., 2000). The SLICK1 allele arises from a frameshift mutation (chr20;39136558GC>G; Littlejohn et al.,

2014) resulting in a nonsense mutation, like the other five slick isoforms (SLICK2,3,4,5 and SLICK6) (Porto-Neto et al., 2018; Flórez Murillo et al., 2021). Except for SLICK4 – the rarest allele, which has only appeared in one slick animal of Criollo Lechero Tropical breed – the premature stop codons of the SLICK alleles occur after a highly conserved asparagine at N435 (Flórez Murillo et al., 2021).

Other PRL and PRLR mutations provide insight into potential mechanisms of the slick mutations. A mutation in the *PRL* gene in dairy cattle generated animals with an abnormally long and hairy coat that decreased thermotolerance and resulted in substantially less milk yield or lactation failure (Littlejohn et al., 2014). Besides the increased thermal insulation from increased coat length, the decreased thermotolerance most likely resulted from dysfunctional sweat glands, for hairy cattle produced half the weight of sweat when compared to normal haired cattle at 28ºC (Littlejohn et al., 2014). Additionally, in turkeys, a 5-bp frameshift deletion in the *PRLR* gene introduced a premature stop codon yielding a 98 C-terminal amino acid truncation and caused a reduction in feathering rate for turkeys (Derks et al., 2018). Besides expressing a wild-type *PRLR*, late feathering chickens co-express a functional intracellularly-truncated PRLR from a partial duplication of the *PRLR* (Bu et al., 2013). In the same study, both wild-type and truncated PRLR were found to be expressed in the skin.

7.   Conclusion

Heat stress is detrimental to the health, welfare, and performance of dairy cattle, and while mitigation methods exist, they can be costly and have some undesirable effects such as increased environmental impact and risk of health issues. One desirable mitigation method is utilization of genetic selection for thermotolerant alleles as this could provide a more permanent solution for the animal and can be passed on to future generations. The slick hair mutation caused by the SLICK1

and other alleles has shown promise as a tool to mitigate the detrimental effects of heat stress in tropical and subtropical climates with the majority of the research focusing mostly on lactating cows. The primary objective of our work was to provide the first evaluation of growing Holstein heifers carrying the SLICK1 allele in both high (Florida) and low (California) humidity heat. We hypothesized that Holstein heifers carrying the SLICK1 allele would have comparable growth and reproductive efficiency to non-slick half-sisters in low humidity, and increased thermotolerance during periods of heat stress in both low and high humidity climates.

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Figure 1.1. Holstein postweaned slick (background) and non-slick (foreground) heifers.

Chapter 2: Preweaning growth and reproductive performance of Holstein dairy heifers carrying the SLICK1 allele

ABSTRACT

Lactating Holstein cows carrying the SLICK1 allele of the prolactin receptor gene have improved thermotolerance under humid heat stress conditions. The objective of this study was to investigate the preweaning growth and reproductive performance of Holstein heifers heterozygous for the dominant SLICK1 allele in a low humidity climate. The 71 heifer calves were born between November 2019 and March 2020. Birth weights (P = 0.08), weaning weights (P = 0.7) and average daily gains (P = 0.8) were similar between slick and non-slick calves. Nulliparous heifers were evaluated for their age at first breeding, pregnancy success to first service, overall pregnancy success, number of services per conception, age at calving, calving success, and pregnancy length. Slick and non-slick heifers had similar reproductive performance across all parameters. In conclusion, Holstein heifers inheriting the SLICK1 allele in a low humidity climate had similar preweaning growth and reproductive performance compared to Holstein half-sisters that did not carry the allele.

INTRODUCTION

Heat stress is a well-known challenge for the dairy industry as seen by the US dairy industry losses of approximately $1.3 billion annually when adjusted to the 2021 US dollar (St. Pierre et al., 2003). Heat stress occurs when the ability of an animal to dissipate heat is exceeded by its metabolic heat load combined with the external heat load. To measure the external heat load, a temperature humidity index (THI) is often used instead of ambient temperature alone as THI also accounts for the influence of humidity. While a large body of research on heat stress exists for

mature cows, a growing area of interest is the detrimental impacts of heat stress on young stock (i.e. calves and growing heifers). For lactating cows, THI thresholds for heat stress range from 68-72 depending on the measured physiological response (Ravagnolo et al., 2000; Zimbelman et al., 2009; Pinto et al., 2020). Conversely, a THI threshold of 77 was formerly assumed for dairy heifers 0-1 yr old (St-Pierre et al., 2003) due to their lower metabolic heat load and surface area to mass ratio. More recently, as research into young stock grows, this THI threshold for preweaned calves has dropped to 65-69 (Dado-Senn et al., 2020b).

When environmental conditions rise above the THI thresholds, heat stressed calves will reduce their milk and dry matter intake to decrease their metabolic heat production (Lopez et al., 2018; Dado-Senn et al., 2022). In turn, these calves generally have a lower average daily gain and weaning weight (Bateman et al., 2012; Lopez et al., 2018). The effects of reduced growth may go beyond just smaller size, as lower weight at breeding has been associated with greater services per conception and older age at first calving (Wathes et al., 2008; Costa et al., 2021).

Selection for genes that are advantageous for thermotolerance offers a more permanent tool to help mitigate the detrimental effects of heat stress. One such promising option is the SLICK1 allele at the prolactin receptor (*PRLR*) gene which has a dominant mode of action and results in a short, sleek hair coat called slick. Research in tropical and sub-tropical climates under heat stressed conditions has shown that lactating cows carrying a slick mutation have lower internal temperature and have a less dramatic drop in milk yield compared to lactating cows with normal hair (Olson et al., 2003; Dikmen et al., 2014). Recent research from our group showed that pre- and postweaned slick Holstein calves raised in Florida maintained lower rectal temperatures compared to non-slick half-sisters (Carmickle et al., 2022); moreover, preweaned slick heifer calves in California maintained a more stable body temperature as THI increased throughout the day (Carmickle et al.,

unpublished). The SLICK1 allele is the result of a single cystine deletion yielding a frameshift mutation that causes a premature stop codon in the prolactin receptor gene that creates an intracellular truncation of the resulting protein (Littlejohn et al., 2014).

Few studies have evaluated the body weight and growth of animals carrying the slick mutation. In beef crossbred slick and non-slick calves raised in Florida, no difference between slick and non-slick calves was observed (Olson et al., 2003); similarly, slick-haired Holstein heifer calves in Puerto Rico had similar weekly body weights during the first 8 weeks of life compared to normal-haired calves (Colón-Rodríguez et al., 2020).

Due to the importance of PRL in reproduction (Shibaya et al., 2006; Bachelot et al., 2007; Binart et al., 2010) and with this mutation occurring in the *PRLR* gene, there is potential for the SLICK1 allele to have deleterious pleiotropic effects on reproductive performance of cattle. To our knowledge, the only data available for slick cattle on this topic come from a study evaluating calving interval in slick and wild-type Puerto Rican Holstein cows. That study found that the slick cows had a 1.64-month shorter calving interval than wild-type cows, although it did not report the age at first calving or lactation number of the animals (Ortiz-Uriarte et al., 2020). Accordingly, Sosa et al. (2021) found that inheritance of the SLICK1 allele did not compromise the competence of the embryo to develop to term. Although these findings are indicative of appropriate reproductive function in animals carrying the slick mutation, there are still no data on reproductive efficiency leading to first calving.

Our objectives were to evaluate slick and non-slick half-sibling Holstein heifers born and raised during the cool season in dairy farms located in the Central Valley of California for birth weight, weaning weight, and preweaning average daily gain. We then followed these calves to

63

breeding age and evaluated their reproductive performance. We hypothesized that calves would have comparable birth weight, and weight gain. Moreover, we hypothesized that inheritance of the SLICK1 allele would not result in reduced reproductive performance in nulliparous Holstein heifers.

MATERIALS AND METHODS

Collection of environmental data

Environmental data was obtained retrospectively from the National Weather Service website (https://www.weather.gov/wrh/Climate?wfo=sto). For farms 2 and 3, the closest point of data recording was the Hanford Municipal Airport (36.3143 °N, 119.6282 °W), whereas the closest point for farms 1 and 4 was the Stockton Metropolitan Airport (37.8969 °N, 121.2515 °W).

Animals

The study was conducted on four commercial dairies in central California (farm 1 – Escalon, 37.7974° N, 120.9966° W; farm 2 – Hanford, 36.3275° N, 119.6457° W; farm 3 – Corcoran, 36.0980° N, 119.5604° 92W; farm 4 – Modesto, 37.7092ºN, 121.1299ºW). This study utilized cattle from another study reported by our group (Carmickle et al., 2022). Two Holstein bulls heterozygous for the dominant SLICK1 allele were bred to lactating Holstein cows via artificial insemination with frozen-thawed semen. Semen from sire A (genetic lifetime net merit +320) was X-sorted and semen from sire B (genetic lifetime net merit +592) was conventional. Calves were born during California's cool season between November 2019 and March 2020 and only the females were evaluated in this study. Heifers were genotyped via the Clarified Plus test (Zoetis, Parsippany-Troy Hills, NJ) and classified as slick (heterozygous for the SLICK1 allele)

or non-slick (wild-type, not carrying the SLICK1 allele). Table 1 outlines the number of animals that participated in the various stages of the project.

Growth Measurements

Individual birth weights were collected within 24h of parturition using a digital hanging scale (SF-918, KlauDirect, China). Individual weaning weight was measured with a portable cart scale (Caf-Cart, Raytec, Ephrata, PA) within one week of weaning according to calf ranch protocols. Weaning age in days was recorded and used to calculate the average daily gain. Due to limitations for collecting individual weaning weights, farm 4 did not participate in this portion of the study.

Reproduction Measurements

Heifer breeding was performed according to farm protocols. Farm 1 only employed estrus detection and artificial insemination without the use of any hormones for synchronization of estrus and ovulation. Farms 2, 3 and 4 utilized 2 doses of prostaglandin F2-alpha (Lutalyse, Zoetis, Kalamazoo, MI) 14 d apart and performed visual estrus detection and artificial insemination. Farm 2 continued this protocol until detection of pregnancy or until the heifer reached 535 d of age before marking as "do not breed". On farm 3, heifers were bred by artificial insemination three times and then placed in a pen with bulls for natural mating. On farm 4, heifers were subjected to the Ovsynch protocol if no estrus was detected after the initial two injections of prostaglandin F2-alpha 14 d apart.

Heifers were bred between January and September 2021. Data were obtained via backups of the Dairy Comp 305 software (Valley Agricultural Software, Tulare, CA) retrieved remotely every 2 weeks. The data included age at first breeding, number of times bred, pregnancy success

to first breeding, pregnancy success 60 d after breeding, calving success and pregnancy length of the pregnant heifers.

Statistical Analysis

Statistical analysis was performed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Cary, NC). For analysis of birth weight, the model included the fixed effects of genotype, sire, farm, gestation length, lactation number and milk yield of the dam, and the interactions between genotype and sire and genotype and farm. For analysis of weaning weight and average daily gain between birth and weaning, the model included the fixed effects of genotype, farm, sire, and birth weight, and the interactions between genotype and sire and genotype and farm. Calf was considered random.

The models for analysis of reproductive performance included the fixed effects of genotype and farm with animal considered a random effect. Interactions between genotype and farm was initially included but removed from the final models due to lack of significance. Response variables included overall pregnancy success, pregnancy success to first service, age at first breeding, services per conception, calving success of pregnant heifers, pregnancy length, and age at calving. In all analyses, effects associated with P-values ≤ 0.05 were considered significant. Results are expressed as least squares means $\pm$ standard deviations of the means.

RESULTS

Environmental conditions during data generation

Between November 2019 and March 2020, the average temperature ranged from 8.9-13.1ºC for farms 2 and 3 and from 9.9-13.5ºC for farms 1 and 4. For farms 2 and 3, the monthly

average temperature during the breeding period began at 9.3ºC in January 2021 and steadily rose to 29.7ºC in July 2021 before dropping to 24.8ºC in September. During the same period, farms 1 and 4 had monthly average temperatures beginning at 10.3ºC in January, rising to 25.5ºC in July, and dropping to 22.4ºC in September.

Characteristics of dams, birth and weaning weights, and average daily gain

The dams that gave birth to either slick or non-slick heifer calves had similar pregnancy length ($P = 0.8$), lactation number ($P = 0.9$), and 305 d milk yield equivalents ($P = 0.3$). Slick and non-slick calves had similar birth weights (40.0 ± 0.9 vs 38.0 ± 1.0 kg, respectively; $P = 0.08$; Figure 2.1A) and weaning weight (80.6 ± 2.1 vs 79.3 ± 2.4, respectively; $P = 0.7$; Figure 2.1B). Weaning age was similar between genotypes ($P = 0.9$); slick calves were weaned at 64.7 d and non-slick calves at 64.5 d. Accordingly, there was no difference in the average daily gain between birth and weaning of slick (0.63 kg/d) and non-slick (0.64 kg/d) calves ($P = 0.8$; Figure 2.1C). Farm had an effect on all parameters measured due to different practices in raising the calves. However, there was no interaction between farm and genotype.

Reproductive Performance

No difference was observed in overall pregnancy success between slick (95.3%) and non-slick (99.8%) heifers ($P = 0.16$). Slick heifers had a numerically greater (10%) pregnancy success to first service compared to non-slick heifers (53.2 ± 6.4 versus 42.8 ± 5.9%, respectively); however, this difference was not statistically significant ($P = 0.22$). Calving success was not different ($P = 0.25$) between pregnant slick and non-slick heifers (86.9 ± 3.9 and 94.3 ± 3.5%, respectively). There was no difference in services per conception ($P = 0.52$) between slick (1.9) and non-slick heifers (2.0). Age at first breeding ($P = 0.64$) and age at calving ($P = 0.32$) did not

differ between genotypes, and pregnancy length was similar between pregnant slick (279.3 d) and non-slick (276.1 d) heifers ($P = 0.13$) (Figure 2.2A-G).

DISCUSSION

This study is the first to report on the birth weight and preweaning phase of growth of Holstein calves carrying the SLICK1 allele and their reproductive performance. We found that birth weights, weaning weights, and average daily gains between birth and weaning were similar between the genotypes. The slick and non-slick heifers also had comparable reproductive performance. We recognize that the number of animals evaluated for reproductive endpoints, particularly pregnancy and calving success and number of services per conception, did not provide sufficient power for a definitive conclusion on whether there could be a beneficial effect of this mutation on reproduction; however, the main goal of this evaluation was to assess whether the inheritance of the SLICK1 allele would not be detrimental to reproductive performance. The present data suggest that Holstein heifers carrying this mutation have similar reproductive efficiency as non-slick Holsteins.

Prior studies have demonstrated the superior thermoregulatory ability of lactating Holstein cows carrying the slick hair mutation through reduced internal body temperatures and a less dramatic reduction in milk yield during environmental conditions consistent with heat stress (Olson et al., 2003; Dikmen et al., 2008, 2014). More recently, our group demonstrated that Holstein calves and heifers maintained lower rectal temperatures when carrying the SLICK1 allele in a subtropical climate (Carmickle et al., 2022). This SLICK1 allele is the result of a single nucleotide deletion (ss1067289408; chr20:39136558GC > G) in the prolactin receptor gene (*PRLR*) yielding a premature stop codon (p.Leu462*) thereby shortening the intracellular portion of the

PRLR protein to an intermediate length, which results in the characteristic short, sleek hair coat (Littlejohn et al., 2014). While a total of six slick mutations have been identified (Porto-Neto et al., 2018; Flórez Murillo et al., 2021), this study only utilized animals carrying the SLICK1 allele.

A report by Olson et al. (2003) evaluated the effects of inheritance of the slick mutation on weaning weights of Senepol-Hereford crossbred calves in Venezuela and found no difference in weaning weights between the two hair coat types. More recently, Colón-Rodríguez and colleagues (2020) reported on the weekly weight gain of Puerto Rican Holstein female calves during their first 8 weeks of life from August to November of 2019 and did not find any differences between the slick-haired and wild-type calves. Our results are consistent with the results of Olson et al. (2003) such that no difference in weaning weights was observed between slick-haired and normal-haired calves. Similarly, our birth weight findings align with those of Colón-Rodríguez et al. (2020). They reported comparable initial weights of 44.58 ± 8.15 and 45.58 ± 8.35 kg for the approximately 4 day-old non-slick and slick calves, respectively. Some differences between studies, however, should be noted. In the present study, birth weight measurements were taken within the first 24 h of life while the initial weights captured by Colón-Rodríguez et al. (2020) were taken at approximately 4 days of age. Secondly, these Puerto Rican Holsteins may have an alternative slick mutation besides the SLICK1 allele carried by our slick heifer calves due to previous crossbreeding with non-Senepol criollo breeds (e.g. Carora that can carry SLICK1, 2, 5 or 6; Flórez Murillo et al., 2021). Moreover, the calves in our study were born between November and March while the study by Colón-Rodríguez et al. (2020) measured weights of calves between August and November. The two months prior to birth, when approximately 60% of the birth weight is gained (Bauman and Currie, 1980), were primarily during the summer for the study by Colón-Rodríguez et al. (2020) while the last two months *in utero* for the calves in our study were primarily

during fall or winter which has characteristically lower THI than summer months. The higher THI in Puerto Rico – due to humidity or month of birth – may result in increased maternal heat stress thereby impacting the growth of the developing fetus (Monteiro et al., 2016; Laporta et al., 2017; Dado-Senn et al., 2020a). Further research evaluating the factors influencing birth weight and preweaning growth of slick Holstein cattle will provide a clearer understanding of the impact of inheriting the slick hair mutation for pre and postnatal growth.

Due to the critical role of prolactin in reproductive processes, it is important to evaluate the reproductive performance of nulliparous heifers to ensure that inheritance of the SLICK1 allele has no major deleterious effects on reproductive ability leading to first calving. Evidence thus far indicates that the slick heifers and cows do not have impaired reproductive function. To our knowledge, the only reproductive performance data that exist for cattle carrying any slick mutation is for calving interval, for which slick Puerto Rican Holsteins had an average calving interval of 14.42 ± 0.13 months and wild-type cows an average of 16.06 ± 0.08 months (Ortiz-Uriarte et al., 2020). In that study, age at first breeding and age at first calving were not reported; however, those could be important confounding factors if the slick animals were bred or calved at an older age, therefore being potentially more developed and better able to resume cyclicity and become pregnant again. In our study, the slick and non-slick heifers had comparable ages at breeding and first calving, and we did not identify any negative effects of presence of the SLICK1 allele in the variables evaluated. A follow up study to evaluate reproductive performance during a shorter interval under heat stress conditions would be able to answer the question of whether this allele provides an advantage for reproductive function.

CONCLUSION

Inheritance of the SLICK1 allele in Holstein heifers born in the cool season in California yielded similar birth weights, weaning weights, and average daily gains between birth and weaning. There was no significant effect of presence of the SLICK1 allele on reproductive performance in nulliparous heifers. Overall, our results indicate no significant effects of inheriting the SLICK1 allele on the preweaned growth and reproductive performance of Holstein heifers raised in dairy farms located in the Central Valley of California as compared to non-slick half-sisters.

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Table 1. Number of slick (SLK) and non-slick (NON) half-sisters evaluated for growth, breeding, pregnancy, and calving parameters.

| Genotype	Sire	Growth	Breeding	Pregnancy	Calving
SLK	A	23	40	35	28
	B	12	23	22	19
NON	A	26	53	48	45
	B	10	25	25	23
Total		71	141	130	115
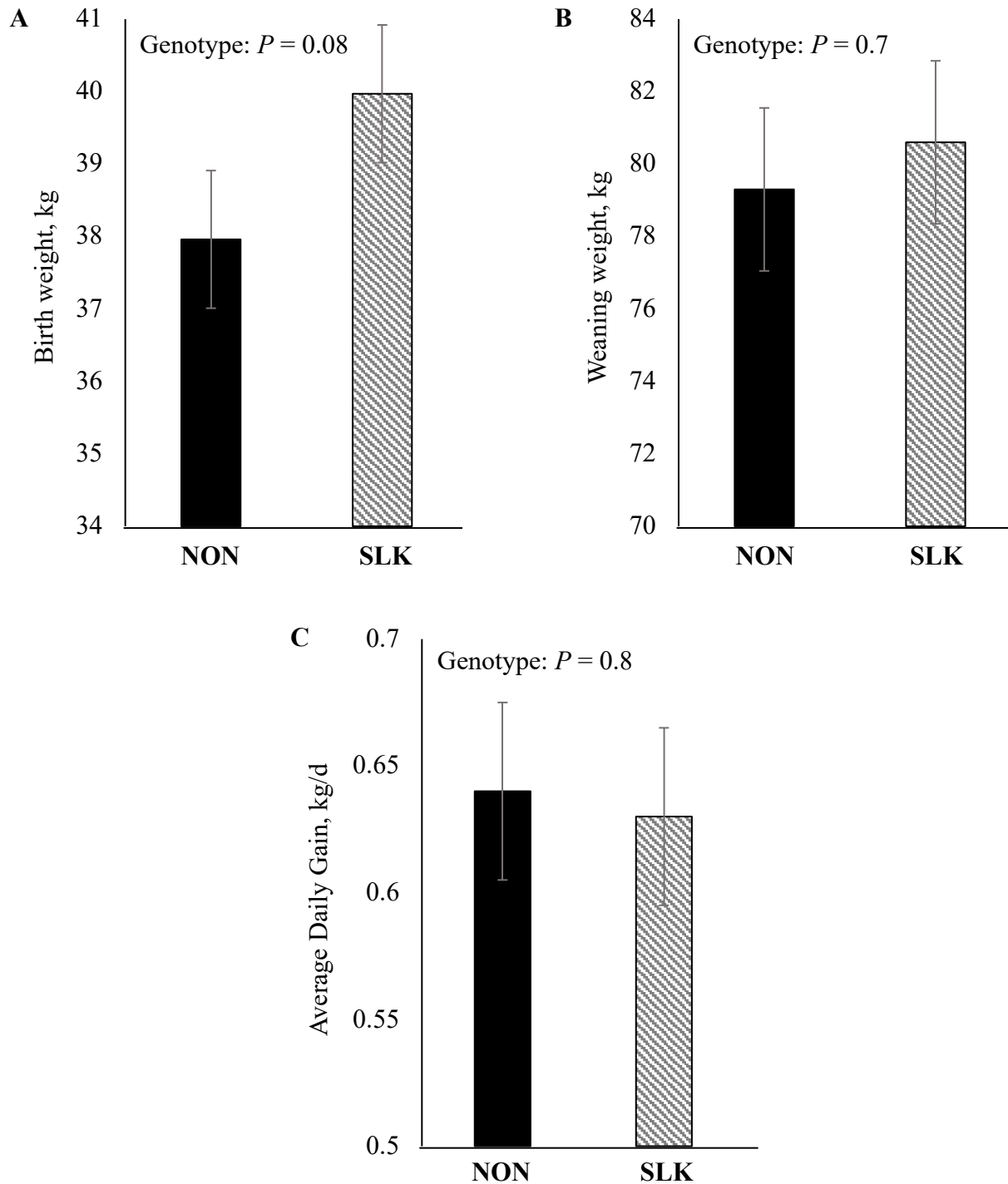
Figure 2.1. Growth parameters of slick (SLK; striped) and non-slick (NON; black) heifer calves.

A) Birth weight, B) weaning weight, C) average daily gain. Effects associated with *P*-values ≤

0.05 were considered significant. Values expressed as least square means ± standard deviation of
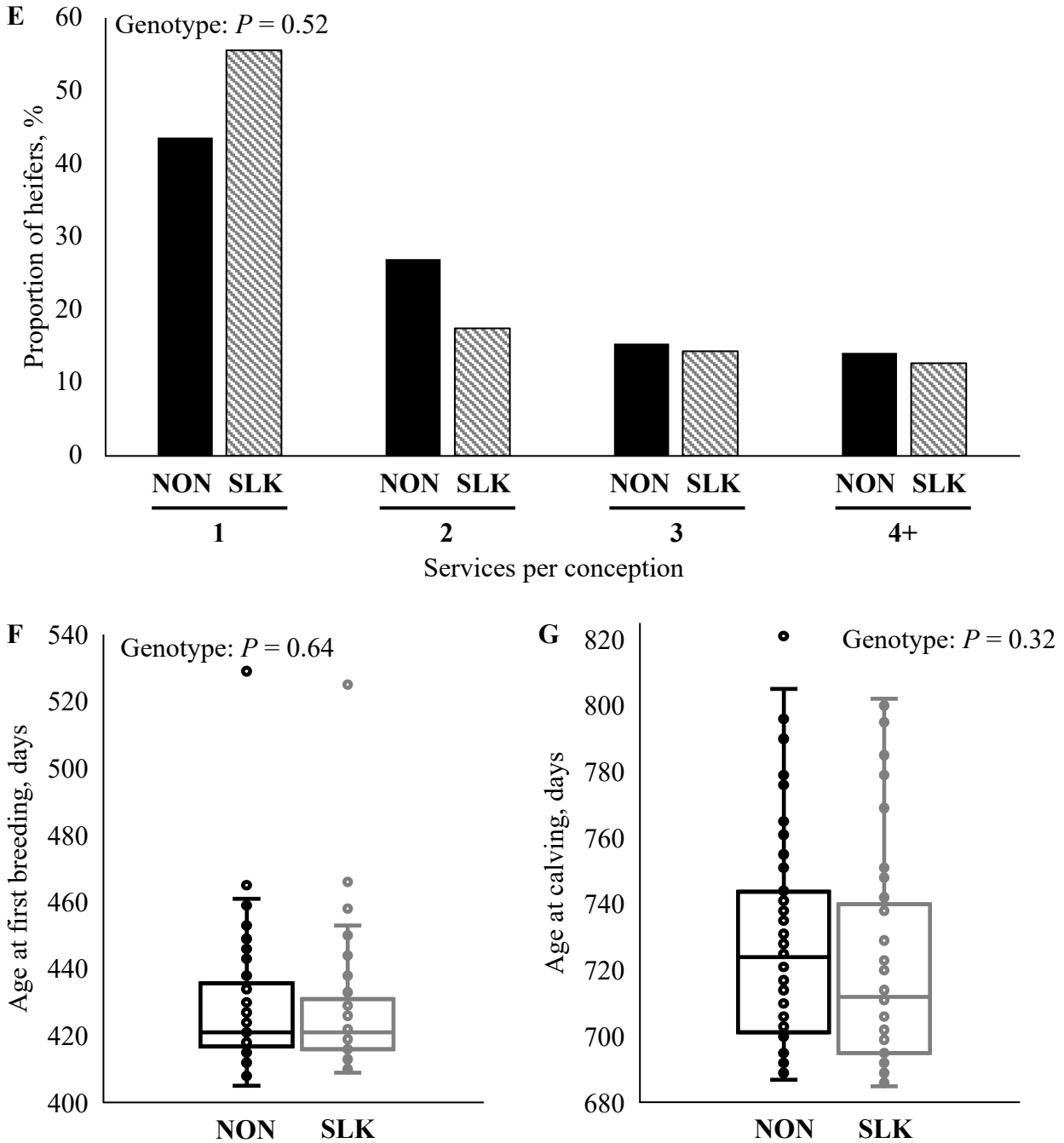
the means.
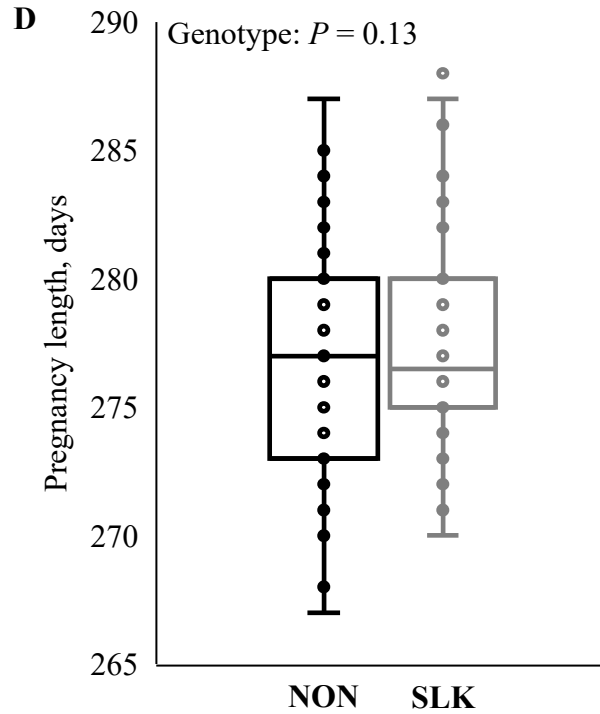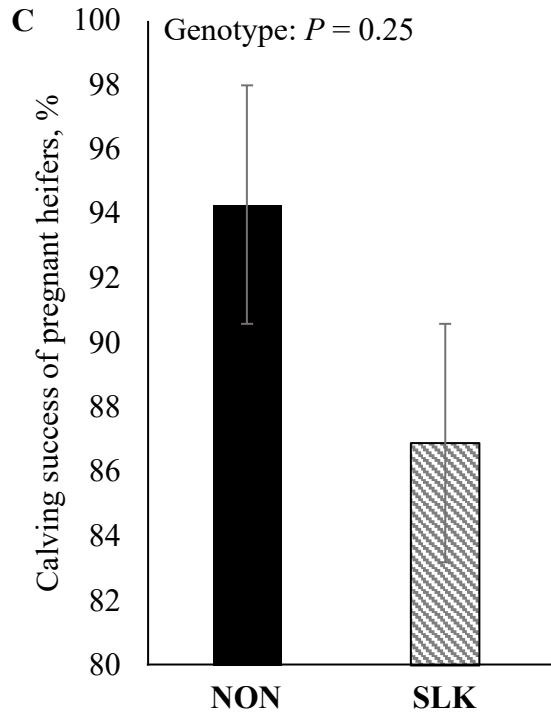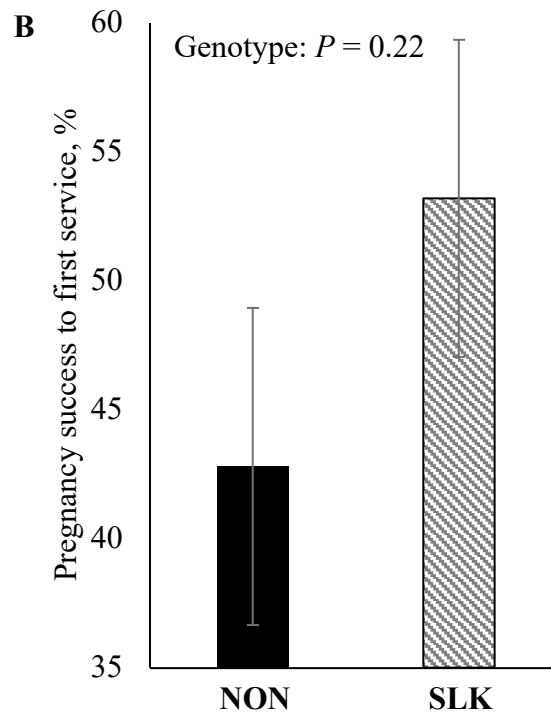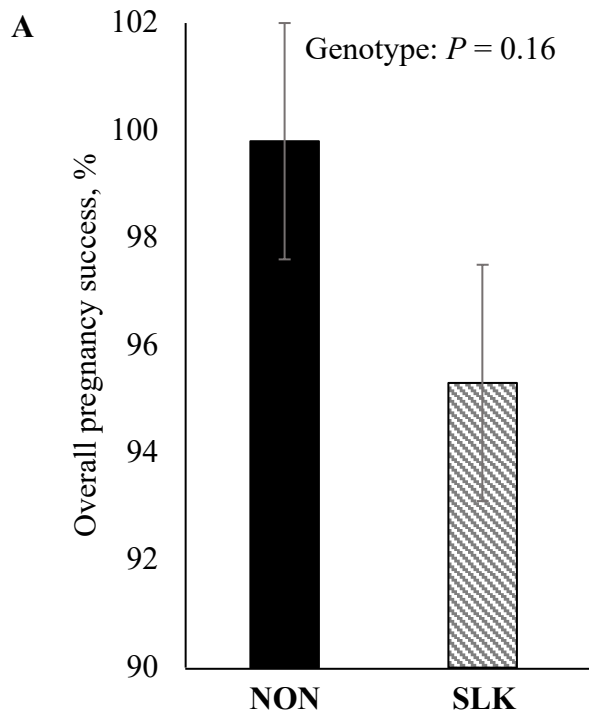
Figure 2.2. Reproductive performance of slick (SLK; grey, striped) and non-slick (NON; black) heifers. A) Overall pregnancy success, B) pregnancy success to first service, C) calving success of pregnant heifers, D) pregnancy length, E) services per conception, F) age at first breeding, and G) age at calving. Effects associated with *P*-values ≤ 0.05 were considered significant. Values expressed as least square mean ± standard deviation of the mean.

Chapter 3: Physiological responses of Holstein calves and heifers carrying the SLICK1 allele to heat stress on California and Florida dairy farms

ABSTRACT

Inheritance of the SLICK1 allele of the prolactin receptor gene improves thermotolerance of lactating Holstein cows under humid heat stress conditions. The aim of this study was to investigate whether pre- and postweaned Holstein heifers carrying the SLICK1 allele would show physiological responses indicative of higher tolerance to heat stress in high and low humidity climates. A total of 101 heifer calves of two age groups heterozygous for the SLICK1 allele and 103 wild-type half-siblings were evaluated during July 2020 in three dairy farms in central California and two in south Florida. Dry bulb temperature and relative humidity data were recorded during evaluation and used to calculate the temperature-humidity index (THI). Physiological measurements were obtained between 1600 and 1900 h in California, and 1200 and 1400 h in Florida and included rectal temperature, respiration rate, skin temperature and sweating rate. Data were analyzed via Generalized Linear Mixed Models including the main effects of genotype, state, group, sire, farm within state, and interactions; THI was included as a covariate. The correlations between THI and dependent variables was analyzed via linear regression. The average 24-h THI was higher in Florida compared to California (90 vs. 72, respectively); the main driver of the higher THI in Florida was the high relative humidity (average 85.6% in Florida vs. 36.7% in California). In Florida, the rectal temperature of slick calves was 0.4 ºC lower than non-slick calves (39.5 ± 0.1 vs 39.9 ± 0.1 °C); no differences were detected between slick and non-slick calves in California. Regardless of genotype, heifer calves in Florida had higher respiration rate, higher rectal and skin

temperatures, and lower sweating rate than in California. This study is the first to evaluate physiological responses of calves carrying the SLICK1 allele under heat stress conditions in different climates. Our findings demonstrate that the presence of this allele is associated with lower rectal temperatures in pre- and postweaned Holstein females. According to the physiological parameters evaluated, calves raised in Florida appeared to be under more severe heat stress; in those conditions, the SLICK1 allele was advantageous to confer thermotolerance as evidenced by lower rectal temperature in slick animals.

INTRODUCTION

The negative impact of heat stress on health, production, and reproduction of dairy cattle is well recognized. Adjusting estimates to 2021 US dollars, heat stress costs the US dairy industry an approximate $1.3 billion each year due to losses associated with reproductive failure, increased disease incidence, and decreased milk production (St-Pierre et al., 2003). Heat stress ensues when the combined heat load from external forces and basic metabolic functions exceeds the animal's ability to dissipate heat. Milk yield and dry matter intake are associated with increased heat production in lactating dairy cows (Igono et al., 1992). Lactating dairy cows are more susceptible to heat stress compared to dry cows and growing heifers due to a higher metabolic rate associated with milk production and lower ratio of body surface area to body mass (West, 2003). Although it has been generally accepted that young stock (i.e., calves and growing heifers) are less susceptible to heat stress compared to lactating cows (St-Pierre et al., 2003; Kovács et al., 2018), it is becoming clear that exposure to high environmental temperature and/or humidity can have long term consequences to the health and productive life of young animals (Wang et al., 2020; Laporta, 2021). The environmental conditions that preweaned Holstein calves are typically exposed to in areas of

high temperatures lead to physiological responses consistent with heat stress and affect their health and welfare (Kovács et al., 2018; Dado-Senn et al., 2020a,b).

Genetic selection for increased heat tolerance can provide a long-term solution to ameliorate the effects of heat stress in cattle. The introduction of the SLICK1 allele into the Holstein breed has resulted in cows that are more tolerant to heat stress. The SLICK1 allele is a single nucleotide polymorphism in the prolactin receptor (*PRLR*) gene which results in a premature stop codon in the cytoplasmic tail of the receptor (Littlejohn et al., 2014). Other point mutations have been identified that result in shortening of the PRLR (Porto-Neto et al., 2018; Flórez Murillo et al., 2021), and the effects of these mutations in improving thermotolerance are also being investigated. In contrast to the inheritance of a missense mutation in the prolactin (*PRL*) gene, which causes a thick, long hair coat and lactation failure (Littlejohn et al., 2014), the SLICK1 mutation of the *PRLR* is associated with a short, fine hair coat phenotype and no detectable lactation defects (Dikmen et al., 2008; Dikmen et al., 2014). Indeed, animals with mutations in *PRLR* have been reported to have higher milk yield in the summer (Olson et al., 2003; Dikmen et al., 2014). These mutations act in a dominant fashion, and therefore heterozygous animals typically present the slick phenotype.

Lactating Holsteins carrying the SLICK1 allele maintained vaginal temperature approximately 0.6 °C lower compared to their non-slick half-siblings and unrelated counterparts in summer (Dikmen et al., 2008, 2014); moreover, slick cows had similar milk yield when calving in the summer and winter, whereas wild-type cows had lower milk yield when calving in the summer (Dikmen et al., 2014). The increased thermotolerance of slick Holsteins has been attributed, at least in part, to increased sweating rate due to the shorter hair coat, leading to a superior ability to dissipate heat (Dikmen et al., 2008). Effects on sweating rate have not always

been observed, however (Dikmen et al., 2014). The benefits of the *PRLR* mutation have also been demonstrated in beef calves under high temperature conditions, where slick calves maintained an average rectal temperature 0.5 °C lower than their wild-type counterparts (Olson et al., 2003).

Studies evaluating lactating dairy cows carrying slick mutations have been limited to a relatively small number of animals and to the high temperature, high humidity conditions found in sub-tropical Venezuela (Olson et al., 2003), Florida (Dikmen et al., 2008, 2014) and Puerto Rico (Ortiz-Colón et al., 2018). It remains to be determined whether presence of the SLICK1 allele in high temperature and low humidity would confer an advantage to withstand heat stress. To date, moreover, there are no studies evaluating the response of SLICK1 carrier Holstein calves to heat stress. Therefore, the present study had two main objectives: to investigate whether presence of the SLICK1 allele would be advantageous to preweaned and postweaned Holstein calves under heat stress; and to evaluate if the potential effects of carrying the mutation would hold in both humid and dry heat conditions.

MATERIALS AND METHODS

Animals and Treatments

All experimental procedures involving animals were approved by the University of California Davis Institutional Animal Care and Use Committee protocol # 20919. The experiment was conducted in five commercial dairies: three in central California (Corcoran, 36.0980° N, 119.5604° W; Escalon, 37.7974° N, 120.9966° W; Hanford, 36.3275° N, 119.6457° W) and two located in south Florida (Okeechobee, 27.2439° N, 80.8298° W). Lactating Holstein cows were bred by artificial insemination with frozen-thawed semen from two registered Holstein sires heterozygous for the SLICK1 allele. Semen from sire A was X-sorted and semen from sire B was

conventional. Inseminations were performed to yield two groups of calves: group 1 calves were born between November 2019 and March 2020, and group 2 calves between May and July 2020. Heifer calves were genotyped via Clarifide Plus test after the experiment (Zoetis, Parsippany-Troy Hills, NJ) and classified as slick (carrying the SLICK1 allele) or non-slick (half-siblings not carrying the SLICK1 allele). Therefore, at the time of data collection, the research team did not have information about the genotypes. Female calves were included in the cross-sectional study performed in July, 2020 to test the physiological responses to environmental conditions associated with heat stress. At the time of testing, the median age of group 1 calves (postweaned) in California was 195 days (range: 108 to 244 days of age); the median age for group 2 calves (preweaned) was 23 (range: 4 to 48 days of age). In Florida, the median age of group 1 calves (postweaned) was 242 days (range: 159 to 265 days of age); the median age for group 2 calves (preweaned) was 29 days (range: 10 to 55 days of age). None of the calves in group 2 were weaned at the time of testing. Group 1 calves were kept in dry lots with shade structures and were brought to a feeding area containing headlocks (California) or kept in pasture pens and brought to a chute (Florida) for testing. Group 2 calves were kept in individual wood hutches outside (2 locations) or individual pens inside a barn and in wood hutches outside (1 location) in California; in Florida, group 2 calves were kept in individual wire hutches with shade cloth.

Environmental Measurements

The MX2301A HOBO Temperature/relative humidity data logger (Onset, Bourne, MA) was used to record environmental data and set to record every 15 min with a sampling duration of 1 min. The data logger was attached to a pole inside the pen in the dry lots, a pole amongst the stalls inside the barn (1 location where some of the calves were inside a barn), or between hutches. In all locations, data loggers were installed approximately 140 cm above the ground, at least 15

min prior to the beginning of data collection. In California, the data loggers were kept in the pens for at least 24 h; at approximately mid-day, the instrument was rotated to the opposite side of the pole in order to keep it out of direct sunlight. In Florida, data loggers were turned off approximately 15 min after all data collection was completed. To complement the environmental data from Florida, we combined the data obtained via data logger during animal evaluation with data recorded by the Florida Automated Weather Network (https://fawn.ifas.ufl.edu/) to create 24-h temperature and humidity distribution during the days of the experiment. The environmental data were used to calculate the temperature-humidity index (THI) according to the following equation:

$$THI = T_{db} - [0.55 - (0.55 \times RH/100)] \times (T_{db} - 58)$$

where T_{db} is dry bulb temperature in °F and RH is relative humidity expressed as a percentage according to Allen et al. (2015). The THI was then matched to the closest time point within a 15 min interval to the physiological measurement of each animal.

Physiological Measurements

Physiological measurements were obtained from slick and non-slick animals during the period of the day encompassing the highest THI (between 1630 and 1900 h in California and 1200 and 1400 h in Florida; Fig. 1A). All measurements were taken in Florida before any rain events on that day.

Before the start of the experiment, the research team was trained on how to obtain each measurement. A rectal thermometer equipped with a 4-inch angled probe (AG-102 Animal Thermometer, AG-Medix, LLC, Mukwonago, WI) was used to measure rectal temperature by fully inserting and holding the probe against the rectal wall. Hair clippers (Oster Turbo 360 Clipper, Oster Professional Products, McMinnville, TN) were used to remove the hair in an area of

approximately 5 x 5 cm at a point caudal to the shoulder blade on the right side of the animal. Respiration rate was measured in breaths per minute (BPM) by counting flank movements for one minute prior to obtaining the other physiological measurements. The skin temperature was measured using an infrared thermometer (IR270, FLIR Systems, Inc., Wilsonville, OR) with emissivity set to 0.98. To keep a consistent distance from the skin, a ruler was taped to the left side of the thermometer such that the opening for the thermometer was 25 cm away from the skin surface when the tip of the ruler was touching the animal. For the clipped skin measurement, the laser dot was pointed at the top of the square of clipped hair and the ruler was placed on the left side of the clipped square. For the unclipped measurement, the laser was placed at approximately the same level as the top of the clipped square but immediately to the side, to measure an area containing hair. Sweating rate was recorded using a SWL4 Vapometer (Delfin Technologies Ltd., Kuopio, Finland) in both clipped and adjacent unclipped areas by holding the instrument against the skin for approximately 10-20 sec until a sound signal indicated that the reading was complete.

Measurement schedule

California

Postweaned heifers were in dry lot pens with shade and were restrained in headlocks during feeding. Animals were released as their measurements were completed. Preweaned calves had their measurements taken in their individual hutches or pens.

Florida

Postweaned heifers were brought from their pasture pens in the morning to a holding pen and were given a minimum of 2 h to settle before physiological measurements were taken. Animals had *ad libitum* access to water and partial shade in the holding pens. To obtain the physiological

measurements, the animals were brought up to a chute. Preweaned calves were tested in their individual hutches.

Statistical analysis

Analyses were performed via Generalized Linear Mixed Models using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Cary, NC). Animal was the experimental unit and was considered random. Models included the main effects of genotype, state, group, sire, farm within state, and the interactions between genotype and state, genotype and group, state and group, and genotype, state, and group. The THI was included as a covariate. The variation in THI between states was analyzed via Generalized Linear Models using the GLM procedure of SAS.

Dependent variables were rectal temperature, respiration rate, surface temperature and sweating rate in clipped and unclipped skin. When THI had a significant effect, a regression analysis was performed between THI and the response variable using the REG procedure of SAS.

A second analysis was performed to evaluate the effects of clipping the hair on skin temperature and sweating rate. Animal was the experimental unit and was considered random. The model included the main effects of genotype, state, sire, farm within state, hair clipping (yes/no), and the interactions between genotype and state, genotype and hair clipping, and clipping and state.

The data are reported as least squares means ± standard error of the mean. Effects associated with P-values ≤ 0.05 were considered significant. Tendencies were associated with $0.05 < P < 0.1$.

RESULTS

Environmental measurements

The 24-h mean THI in California during the testing period was 72 ± 0.2 (range 61-82), whereas in Florida it was 90 ± 0.2 (range 83-100; $P < 0.0001$). Figure 3.1A depicts the 24-h variation of THI during testing days in both states; the boxes indicate the window of sample collection. The average temperature, relative humidity, and THI during the time of collection in California and Florida are depicted in Figure 3.1B-C. The THI was consistently higher in Florida compared to California, and in California, the animals experienced several hours of THI below 68 overnight. In Florida, the THI remained above 80 for the entire study period.

Physiological responses to heat stress

Table 1 depicts the number of animals per genotype and group that were evaluated in each state. The rectal temperature of slick animals was 39.3 ± 0.1 °C whereas for non-slick animals it was 39.5 ± 0.1 °C ($P = 0.01$). This difference was only observed in Florida, where postweaned slick heifers had 0.31 °C ($P < 0.05$) and preweaned calves had 0.55 °C ($P < 0.001$) lower rectal temperature compared to their non-slick counterparts. In California, rectal temperature was not different between slick and non-slick animals ($P = 0.8$; Fig. 2A). Regardless of genotype, California animals had lower rectal temperature than Florida animals (39.2 ± 0.04 °C vs. 39.7 ± 0.1 °C; $P < 0.001$). Overall, postweaned heifers had higher rectal temperature than preweaned calves (39.7 ± 0.05 °C vs. 39.1 ± 0.09 °C; $P < 0.0001$). Again, this effect was due to differences observed in Florida, where postweaned heifers had higher rectal temperature than preweaned calves; in California, no difference was detected (Figure 3.2A).

Genotype did not affect respiration rate (Figure 3.2B). Overall, postweaned heifers had higher respiration rate compared to preweaned calves (78 ± 2 vs. 66 ± 4 BPM; $P < 0.01$). Postweaned heifers in Florida had the highest respiration rate, whereas in California there was no difference between age groups (Figure 3.2B).

The temperature in the clipped skin was not affected by genotype, state, or group (Figure 3.2C). We observed an interaction between group and state: preweaned calves had higher clipped skin temperature than postweaned heifers in California, whereas this relationship was inverse in Florida ($P < 0.01$; Figure 3.2C). Skin temperature in unclipped areas was not affected by genotype, state, or group (Figure 3.2D).

There was no effect of genotype on sweating rate regardless of presence of hair (Figure 3.2E,F). Overall, California heifers sweated more than Florida heifers in clipped (139.0 ± 6.0 vs. 63.6 ± 18.7 g/m²h; $P < 0.001$) and unclipped skin (113.5 ± 5.9 vs. 70.3 ± 17.4 g/m²h; $P < 0.05$). Postweaned heifers sweated more than preweaned calves in both clipped and unclipped skin ($P < 0.01$ – Figure 3.2E,F).

Regardless of genotype, daughters of sire A had higher respiration rate (77 ± 3 vs. 68 ± 3 BPM; $P < 0.01$) and clipped skin temperature (35.1 ± 0.2 vs. 34.7 ± 0.2 °C; $P < 0.05$) compared to daughters of sire B. Moreover, the sweating rate in clipped skin of daughters of sire A was lower than that of daughters of sire B (90.7 ± 9.1 and 115.7 ± 10.4 g/m²h; $P < 0.01$). There were no interactions between genotype and sire.

When analyzing the effect of hair clipping on skin temperature and sweating rate, we observed that for preweaned calves in California there were tendencies for the interaction of genotype by hair clipping on the sweating rate ($P = 0.09$) and skin temperature ($P = 0.09$). Slick calves showed little change in sweating rate (84.7 ± 10.8 vs. 99.2 ± 10.8 g/m²h for unclipped and clipped skin, respectively) and skin temperature (34.59 ± 0.2 vs. 34.62 ± 0.2 °C for unclipped and clipped skin, respectively), whereas non-slick calves had higher sweating rate (109.2 ± 14.3 vs. 143.7 ± 14.3 g/m²h in unclipped and clipped skin, respectively) and higher skin temperature in clipped skin (34.67 ± 0.2 vs. 34.94 ± 0.2 °C in unclipped and clipped skin, respectively). We did

not observe differences in postweaned heifers in California or in either pre- or postweaned heifers in Florida.

Effects of THI on physiological responses

The THI at the time of measurement tended to affect rectal temperature ($P = 0.06$), and affected unclipped skin temperature ($P = 0.03$) and clipped skin sweating rate ($P = 0.02$). Therefore, we examined the effect of THI in each of these variables in slick and non-slick animals of both age groups combined. Slick animals kept a more stable rectal temperature as THI increased, whereas non-slick animals experienced an increase in temperature in response to increasing THI (Figure 3.3A). Response to THI for unclipped skin temperature and clipped skin sweating rate was similar for slick and non-slick animals (Figure 3.3B,C).

DISCUSSION

The present study had two main goals: to evaluate whether the presence of the SLICK1 allele would be advantageous to Holstein preweaned calves and postweaned heifers under heat stress; and to evaluate if the potential effects of carrying the mutation would hold in both humid and dry heat conditions. We found that in Florida, pre- and postweaned animals carrying the SLICK1 allele had lower body temperature compared to their wild-type half-siblings. No differences due to genotype in body temperature or the other parameters evaluated were detected in pre- or postweaned animals raised in California.

The dominant SLICK1 allele was originally described in the Senepol breed in the Caribbean Island of St. Croix. The resulting phenotype is a short and sleek hair coat, visually similar to many *Bos indicus* breeds (Olson et al., 2003). Later, the slick locus was mapped to bovine chromosome 20 (Mariasegaram et al., 2007; Flori et al., 2012), and finally the mutation

was found to be due to a loss of a cytosine in exon 10 of the prolactin receptor gene (*PRLR*) that causes a premature stop codon and the loss of 120 amino acids in the C-terminus of the resulting protein (Littlejohn et al., 2014). Additional mutations in the *PRLR* (named SLICK2-6) have been found in Limonero, Carora and other breeds of cattle that also yield the slick phenotype (Porto-Neto et al., 2018; Flórez Murillo et al., 2021). The mutation explored in this study is the one described by Littlejohn et al. (2014) which has been notated as SLICK1 (Porto-Neto et al., 2018). Studies of lactating Holsteins and Holstein crosses have demonstrated that, compared to wild-type cattle, cows carrying the SLICK1 allele are able to maintain milk yield and lower body temperature under tropical and sub-tropical heat stress conditions (Olson et al., 2003; Dikmen et al., 2008, 2014). Recently, Landaeta-Hernandez et al. (2021) examined the physiological responses of slick Criollo Limonero (likely carrying one of the other SLICK mutations) 1-year-old heifers to heat stress in sub-tropical conditions and found that slick heifers maintained lower body temperature and respiration rate compared to non-slick heifers. To our knowledge, the present study is the first to evaluate the mutation in young (less than one year old) Holsteins, including preweaned calves; our data show that presence of the SLICK1 allele confers distinguishable short hair and is associated with lower body temperature in sub-tropical heat.

The average THI experienced by the animals during data collection in California was 72 (range 61-80), whereas in Florida it was 90 (range 83-100), exceeding heat stress thresholds on a daily basis in both states (Dado-Senn et al., 2020a). Our data indicate that both preweaned calves and postweaned heifers experienced hyperthermia in both states, confirming that these animals were experiencing some degree of heat stress. To our knowledge, all previous studies looking at physiological responses to heat stress of animals carrying the SLICK1 allele have been conducted in sub-tropical or tropical climates. These climates are characterized by high temperatures and high

humidity during the day that persist overnight. Conversely, California has a Mediterranean climate, characterized by high temperatures and low humidity during the day, often with a decrease in temperature accompanied by an increase in humidity overnight. An evaluation of the drivers and the distribution of THI in each location confirmed that California had higher air temperature and lower relative humidity compared to Florida. Another important difference between the two states was that in Florida, the animals experienced several more hours of more severe heat stress each day, and no relief at night. Dado-Senn et al. (2020a) showed that exposure to chronic heat stress (i.e., no or short period of relief during the night) decreased the threshold at which preweaned Holstein calves begin changing their physiological parameters in response to heat stress. Perhaps the heat stress conditions in Florida were sufficiently severe to trigger the physiological responses observed, allowing the differences between the slick and wild-type animals to affect thermoregulation.

Cattle dissipate excess heat load via evaporative heat loss by increasing sweating and respiration rates, and the efficiency of these processes is largely dependent on the humidity of the air (Dos Santos et al., 2021). The high humidity climate found in Florida compromises evaporative heat loss (Berman, 2006), whereas in California's low humidity climate, the water vapor easily leaves the hair coat, contributing to cooling (Gebremedhin and Wu, 2001). The faster the process of evaporative heat loss or water vapor moving from skin to air, the faster the animal can reduce its heat load (Gebremedhin et al., 2008). Accordingly, we observed lower rectal temperature, respiration rate, skin temperature, and higher sweating rate in animals raised in California. Although rectal temperature was at or above the threshold for hyperthermia in both states, postweaned heifers tested in Florida had significantly higher rectal temperature and respiratory rate compared to preweaned calves and to both age groups in California. Conversely, sweating rate

was highest in the postweaned heifers raised in California, likely resulting in the lower skin temperature compared to Florida. Clearly, the animals raised in California were better able to dissipate heat via sweating.

The finding that sweating rate was not different between genotypes is inconsistent with previous reports that lactating slick Holsteins in Florida had higher sweating rate than non-slick counterparts (Dikmen et al., 2008; 2014); however, the results of previous reports are also inconsistent. In the first report, Dikmen et al. (2008) found that the sweating rate of slick cows was greater when the hair was present; however, removal of the hair abolished the difference, leading the authors to conclude that the difference observed was due to the shorter hair of the slick animals resulting in less humid air trapped within the hair. In a second report, Dikmen et al. (2014) did not find large differences in sweating rate between slick cows and their non-slick relatives. Landaeta-Hernandez et al. (2011), examining Criollo Limonero cows, found that animals carrying the slick mutation (likely the SLICK2 or SLICK3 allele) had larger sweat glands compared to wild-type herd mates, although the number of sweat glands was not different between the groups. Littlejohn et al. (2014), however, did not find a difference in the number of sweat glands or hair follicles of cows carrying the SLICK1 allele. We did not evaluate the characteristics of the skin of our animals, and therefore cannot make inferences about whether there is a physical difference in sweat gland maturity and function compared to lactating cows, or if the lack of differences could be caused by a lower ability of young calves to regulate sweat in response to heat.

Analysis of the effect of hair clipping on sweating rate and skin temperature revealed that there was no difference in sweating rate or skin temperature of preweaned slick calves in California before or after hair clipping, whereas non-slick calves had higher sweating rate and skin temperature when the hair was removed. Although this finding confirms the report by Dikmen et

al. (2008) in lactating Holsteins that the hair of slick animals does not impact these parameters, it contradicts that report in the present finding that overall, slick calves sweated less than non-slick calves. Evaluation of our animals as they become lactating cows would be important to confirm or refute previous reports and shed more light into the relationship between the SLICK1 allele and evaporative heat loss by sweating.

One potential explanation for the lower body temperature found in Florida in the absence of differences in sweating rate could be that the slick animals had lower metabolic rate. This was suggested by Dikmen et al. (2008), however, analysis of milk yield demonstrated that slick cows produced the same or greater amount of milk (observed during summer; Dikmen et al., 2014), therefore refuting the hypothesis of a lower metabolic rate. Increased heat loss via conduction and convection is the most likely explanation to the differences observed.

Young cattle have been generally regarded as more resistant than lactating cows to heat stress. Recent data, however, show that preweaned calves can begin showing signs of heat stress at a THI of 65-69 (Dado-Senn et al., 2020a), i.e., substantially lower than the previously estimated value of 77 (St. Pierre et al., 2003), and similar to the value of 68 established for lactating cows (Zimbelman et al., 2009). In a recent study, Young et al. (2020) showed that the temperature of preweaned Holstein calves surpassed the 39 °C mark at a THI of 70. As more research is performed to evaluate how young cattle respond to excessive heat, it is becoming evident that heat stress can have long term negative consequences to future health and performance (reviewed in Laporta, 2021). It is therefore critical that we continue to focus on the development of sustainable strategies to improve the adaptation of *Bos taurus* cattle to heat stress.

CONCLUSION

This study demonstrated that presence of the SLICK1 allele results in lower body temperature in young Holstein cattle exposed to the sub-tropical heat conditions found in Florida, whereas no differences were observed in the Mediterranean heat conditions found in California. Presence of the SLICK1 allele seems particularly important when high temperature-humidity index is driven by high humidity, and the effects of the allele on the physiological responses to heat stress can be detected in Holstein calves as early as the preweaned stage of life.

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Table 2. Number of slick (SLK) and non-slick (NON) half-siblings evaluated in Group 1 (postweaned heifers) and Group 2 (preweaned calves) in California and Florida during July of 2020.

State	Group 1			Group 2			TOTAL		
	SLK	NON	Total	SLK	NON	Total	SLK	NON	Total
California	50	54	104	30	20	50	80	74	154
Florida	13	20	33	8	9	17	21	29	50
Total	63	74	137	38	29	67	101	103	204
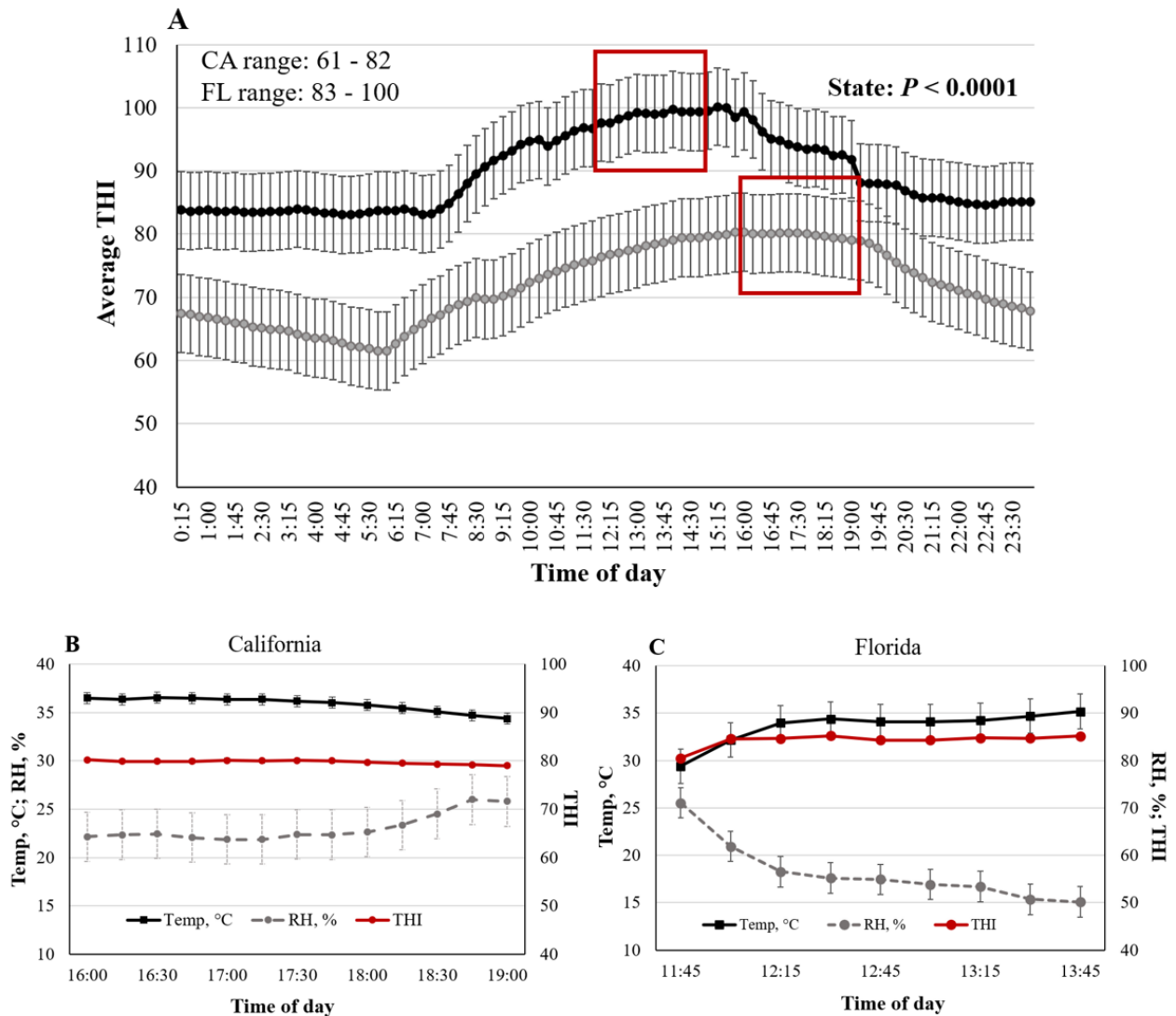
Figure 3.1. Dry bulb temperature (°C), relative humidity (% RH) and temperature-humidity index (THI) during the experiment in California (CA) and Florida (FL). A) The average of the 24 h-THI during the days of physiological measurements in slick and non-slick Holstein calves in California (gray circles) was 72 and in Florida (black circles), 90. The box in each line represents the period of the day when measurements were taken. B,C) Dry bulb temperature, relative humidity and THI in California and Florida during the period when physiological measurements were taken. Values expressed as least square mean ± standard deviation of the mean.
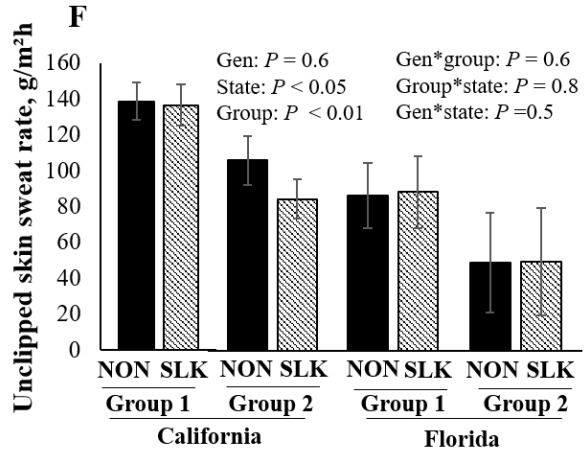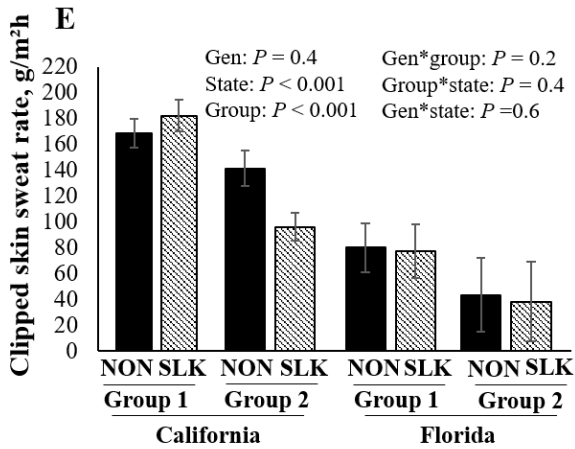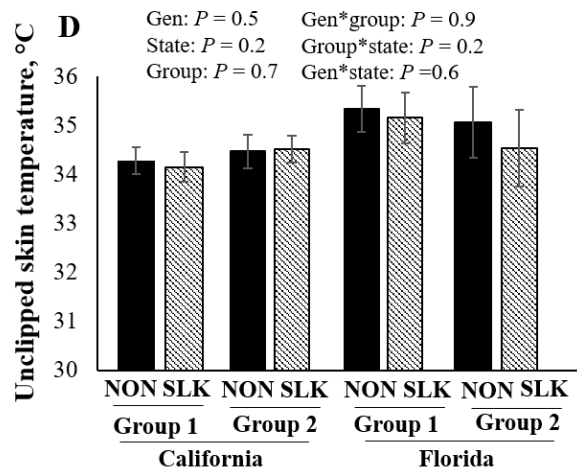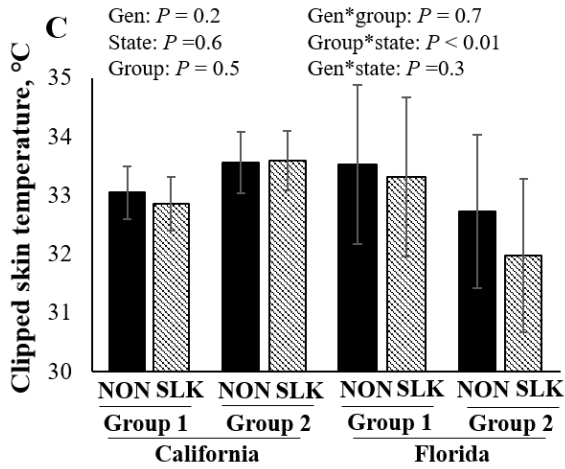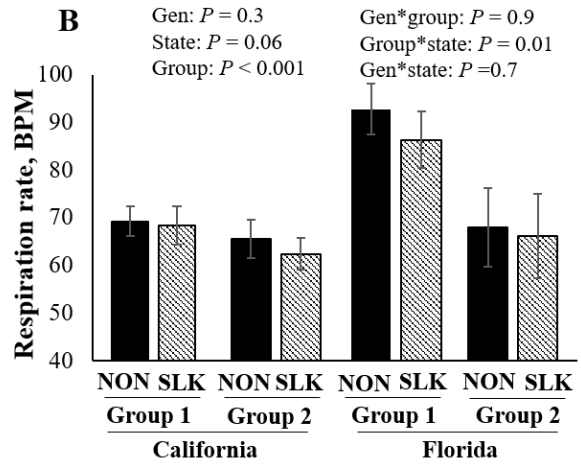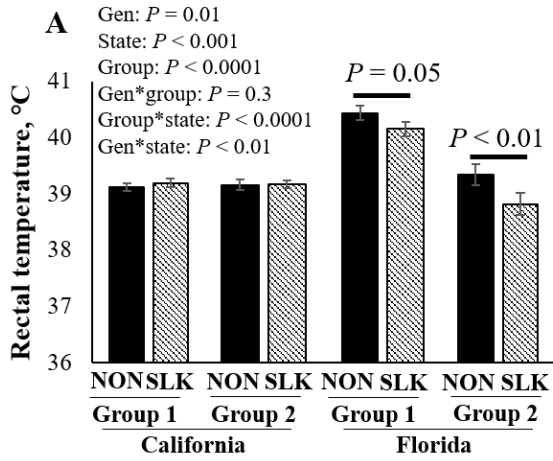
Figure 3.2. Physiological responses to heat stress in preweaned and postweaned Holstein calves carrying the SLICK1 allele. Effects on A) rectal temperature; B) respiration rate; C) skin temperature without hair (clipped); D) skin temperature with hair (unclipped); E) sweating rate without hair (clipped); F) sweating rate with hair (unclipped). SLK = animals carrying the SLICK1 allele; NON = half-sisters not carrying the allele; group 1 = Holstein heifers 108-265 days old; group 2 = Holstein calves 4-55 days old. Gen = genotype (SLICK1, wild-type). Where present, tendencies ($P < 0.1$) and significant differences ($P \leq 0.05$) are noted. Values expressed as least square means ± standard deviation of the means.
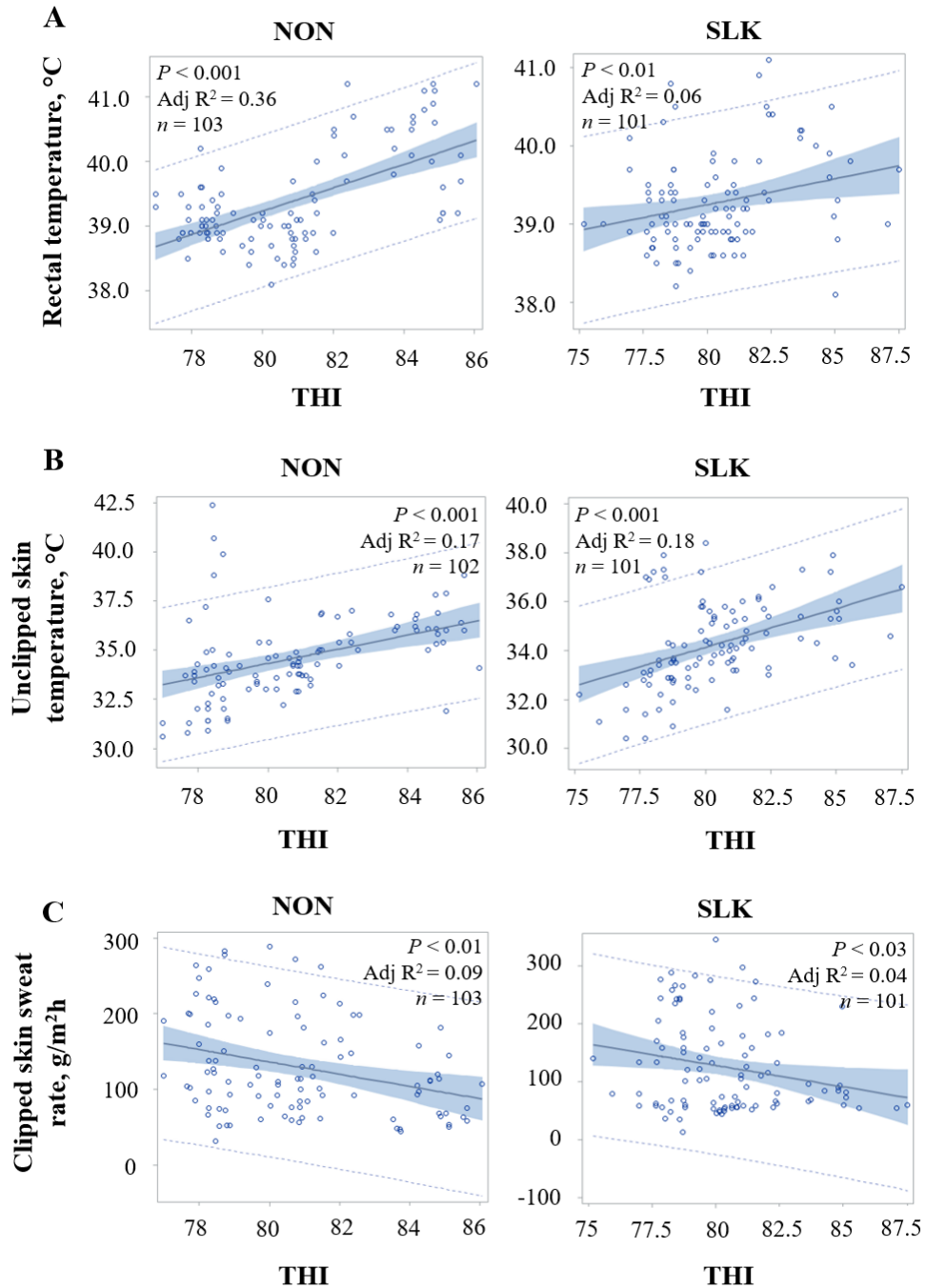
Figure 3.3. Effects of temperature-humidity index (THI) on A) rectal temperature; B) surface temperature in unclipped skin; and C) sweating rate in clipped skin. SLK = animals carrying the SLICK1 allele; NON = half-siblings not carrying the mutation. State (California and Florida) and age group (pre- or postweaned heifers) are combined within each genotype. Adj R^2 = adjusted R^2.

Chapter 4: Physiological responses of pre- and postweaned Holstein heifers carrying the SLICK1 allele to heat stress on California dairy farms

ABSTRACT

Under heat stress conditions, lactating Holstein cows that carry the SLICK1 allele of the prolactin receptor gene have improved thermotolerance in high humidity climates. The objective of this study was to evaluate the physiological responses of pre- and postweaned Holstein heifers carrying the SLICK1 allele in a characteristically low humidity hot climate to assess whether the responses were consistent with increased thermotolerance. A total of 142 postweaned and 80 preweaned slick, non-slick half-sisters, or genetically unrelated control Holstein heifer calves were evaluated on three dairy farms located in central California in July 2020. Physiological measurements were collected from each animal in both age groups in the morning and afternoon. An additional midday data collection was obtained for the preweaned heifer calves. Measurements included rectal temperature, respiration rate, skin temperature, and sweating rate. The temperature humidity index (THI) ranged from 61-80 with a daily average of 72 ± 0.2 across all days of data collection. Inheritance of the SLICK1 allele had little effect on physiological responses for both pre- and postweaned heifers; however, regression analysis to evaluate the correlation between rectal temperature and THI during the window of the day when THI was highest revealed a weaker correlation between those parameters in slick calves (Group 1 Adj. $R^2 = 0.01$; Group 2 Adj. $R^2 = -0.04$) compared to non-slick (Group 1 Adj. $R^2 = 0.12$; Group 2 Adj. $R^2 = 0.19$) and control calves (Group 1 Adj. $R^2 = 0.10$; Group 2 Adj. $R^2 = 0.07$). These findings suggest that slick heifer calves may be able to maintain a more constant body temperature as the THI increases, compared to the non-slick and control calves. Preweaned calves had lower physiological measurements in the morning compared to postweaned heifers, yet the physiological measurements of preweaned

calves taken in afternoon were comparable or even slightly higher than the measurments of the postweaned heifers. In California, inheritance of the SLICK1 allele allowed heifers to maintain a more consistent body temperature as THI rose; moreover, regardless of genotype, preweaned calves were more responsive to the increase in THI throughout the day compared to postweaned heifers.

INTRODUCTION

It is well established that heat stress negatively impacts dairy cattle welfare and production. The United States dairy industry loses approximately $1.3 billion annually due to heat stress costs resulting from reproductive failure, increased disease incidence, and decreased milk production when adjusting from 2003 to 2021 US dollar (St-Pierre et al., 2003). Heat stress occurs when the accumulation of external heat load and basic metabolic functions is greater than the animal's ability to dissipate the combined heat. Heat dissipation in cattle mainly occurs via evaporative heat loss in the form of increased respiratory and sweating rates (Berman, 2004; Hansen, 2004). While extensive research exists for effects of heat stress on mature cows (e.g. decline in milk yield and reproductive performance), fewer studies have been performed on young stock in part due to their lower metabolic heat load and higher surface area to mass ratio (West, 2003).

Like mature cows, exposure to high temperatures impacts the welfare and production of young stock. Preweaned calves have increased rectal temperature (RT), respiration rate (RR), skin temperature (ST), heart rate, and decreased milk intake in high temperature conditions (Kóvacs et al., 2018; Young et al., 2020; Dado-Senn et al., 2022). Previously, heifers between 0 and 1 years old were estimated to be heat stressed above a temperature humidity index (THI) of 77 (St-Pierre et al., 2003); however, recent studies report elevated physiologic parameters indicating heat stress at THIs as low as 65-70 for preweaned calves (Dado-Senn et al., 2020a; Young et al., 2020). Both

pre- and postweaned heifers are reported to have reduced dry matter intake under heat stress (Nonaka et al., 2007; Wang et al., 2020), which has been associated with lower weaning weights and average daily gains (Bateman et al., 2012; López et al., 2018; Wang et al., 2020) and may result in lighter weights at breeding age which is associated with decreased reproductive efficiency (Wathes et al., 2008; Costa et al., 2021).

While most methods of mitigating heat stress for young stock are focused on environmental conditions (Spain and Spears, 1996; Moghaddam et al., 2009; Kovács et al., 2018), genetic selection for greater heat tolerance is a long-term strategy to ease the damaging effects of heat stress. Introgression of the SLICK1 allele from Senepol cattle to Holstein cattle via crossbreeding produced lactating Holstein cows with increased heat tolerance in tropical and sub-tropical climates (Olson et al., 2003; Dikmen et al., 2008, 2014). The SLICK1 allele is a dominant mutation in the prolactin receptor (*PRLR*) gene resulting from a single nucleotide polymorphism yielding a frameshift that creates a nonsense mutation, truncating the intracellular portion of the receptor (Littlejohn et al., 2014). Additional slick mutations of the *PRLR* gene that yield a truncated PRLR have been identified in other thermotolerant breeds, but their exact function and impact are a topic of further investigation (Porto-Neto, et al., 2018; Flórez Murillo et al., 2021). The slick phenotype – occurring in both SLICK1 heterozygotes and homozygotes due to the dominance of the mutation – presents as a short, sleek hair coat with no apparent negative pleiotropic effect on lactation (Dikmen et al., 2014). In addition to having lower vaginal temperatures than non-slick half-siblings and unrelated cohort cows, slick Holsteins cows may also have improved evaporative heat loss via increased sweating rate, although the results on sweating rate between studies are mixed (Dikmen et al., 2008, 2014).

In a study by Olson et al. (2003), beef calves carrying the SLICK1 allele presented with beneficial phenotypes: short hair coat and a lower average rectal temperature than their wild-type counterparts. Landaeta-Hernández and colleagues (2021) reported lower rectal temperatures and respiration rates in slick-haired versus normal-haired non-pregnant Criollo Limonero heifers (approx. 410 d of age); however, it should be noted that Criollo Limonero likely carry different *PRLR* alleles (i.e., SLICK2/SLICK3) (Porto-Neto et al., 2018; Flórez Murillo et al., 2021). We recently compared the physiological responses to heat stress in SLICK1 and non-slick half-sister Holstein heifer calves raised in California and Florida and found that, during the period of the day of peak THI, slick calves in Florida maintained lower rectal temperatures than their non-slick counterparts, whereas no difference between genotypes was detected in California (Carmickle et al., 2022).

Studies evaluating the thermoregulatory abilities of dairy cattle carrying the SLICK1 allele have primarily focused on lactating Holsteins housed in tropical or subtropical locations characterized by high temperature and humidity. More detailed studies to determine whether the presence of the SLICK1 allele in high temperature and low humidity would present an advantage to withstand heat stress are critical to establish how useful this genetic mutation is in different dairy environments. Therefore, our objectives were to evaluate the physiological responses of pre- and postweaned Holstein heifers carrying the SLICK1 allele to heat stress in California. We expanded on our previous report by analyzing multiple datapoints per animal while also including data collected from genetically unrelated contemporary animals raised on the collaborating farms.

MATERIALS AND METHODS

Animals and Treatments

All experimental procedures involving animals were approved by the University of California Davis Institutional Animal Care and Use Committee protocol # 20919. The experiment was conducted in three commercial dairies in central California (Corcoran, 36.0980° N, 119.5604° W; Escalon, 37.7974° N, 120.9966° W; Hanford, 36.3275° N, 119.6457° W) as previously described (Carmickle et al., 2022). Briefly, lactating Holstein cows were inseminated with one of two Holstein sires heterozygous for the SLICK1 allele. Semen from sire A was X-sorted and semen from sire B was conventional. Inseminations led to the birth of groups of calves: group 1 calves were born between November 2019 and March 2020, and group 2 calves between May and July 2020. Heifer calves were genotyped via Clarifide Plus test (Zoetis, Parsippany-Troy Hills, NJ) and classified as carriers (slick) or non-carriers (non-slick) of the SLICK1 allele; therefore, slick and non-slick heifer calves were half-siblings (daughters of the same sires). Cohort females of the same age range as the study animals were randomly chosen and evaluated as a group of genetically-unrelated controls. They were classified as being non-slick based on their sires not carrying the SLICK1 allele (control). During 10 days in July of 2020, female calves were tested for physiological responses to heat stress. At the time of the experiment, Group 1 calves (postweaned) were between 107 and 244 days of age, and Group 2 calves (preweaned) were between 4 and 48 days of age. Group 1 calves were kept in dry lot pens with shade in 2 locations and in a free-stall barn in 1 location. Group 2 calves were kept in individual wood hutches in all 3 locations. In 1 location, some calves were kept inside a barn.

Environmental Measurements

Data loggers (MX2301A HOBO; Onset, Bourne, MA) were used to record the dry bulb temperature, relative humidity, and dew point in each location for 24 hours during the time of testing. Loggers were set to record a measurement every 15 min, with sampling duration of 1

minute. The data logger was attached to a pole inside the pen or between hutches at approximately 140 cm above the ground, at least 15 minutes prior to the beginning of data collection. The dry bulb temperature and relative humidity were used to calculate the temperature-humidity index (THI) according to the following equation:

$$THI = T_{db} - [0.55 - (0.55 \times RH/100)] \times (T_{db} - 58)$$

where T_{db} is dry bulb temperature in °F and RH is relative humidity expressed as a percentage according to Allen et al. (2015). The THI was then matched to the closest time point within a 15 min interval to the physiological measurement of each animal.

Physiological Measurements

Physiological measurements were obtained from slick, non-slick, and control animals in the morning between 900 and 1130 h, and afternoon between 1600 and 1900 h, in three body areas on the right side of each animal (Figure 4.1): immediately behind the shoulder blade, on the rump (midway between the hip and pin bones), and on the thigh (midway between the rump and the knee). In preweaned calves, a third timepoint was added between 1200 and 1400 h. Postweaned heifers were restrained in headlocks during feeding and released immediately upon completion of the measurements. Physiological measurements of preweaned calves were taken in their individual hutches. The research team was trained on how to obtain each measurement before the first testing day.

Respiration rate was measured in breaths per minute (BPM) by counting flank movements for one minute prior to obtaining the other physiological measurements. A rectal thermometer equipped with a 4-inch angled probe (AG-102 Animal Thermometer, AG-Medix, LLC, Mukwonago, WI) was used to measure rectal temperature by fully inserting and holding the probe

against the rectal wall. A hair clipper (Oster Turbo 360 Clipper, Oster Professional Products, McMinnville, TN) was used to clip the hair in an area of approximately 5 x 5 cm at the three locations on the body mentioned above. Clipped and unclipped skin temperature and sweating rate were recorded at each of the three locations. The skin temperature was measured using an infrared thermometer (IR270, FLIR Systems, Inc., Wilsonville, OR) with emissivity set to 0.98. To keep a consistent distance from the skin, a ruler was taped to the left side of the thermometer such that the opening for the thermometer was 25 cm away from the skin surface when the tip of the ruler was touching the animal. For the clipped skin measurement, the laser dot was pointed at the top of the square of clipped hair and the ruler was placed on the left side of the clipped square. For the unclipped measurement, the laser was placed at approximately the same level as the top of the clipped square but immediately to the side, to measure an area containing hair. Sweating rate was recorded using a SWL4 Vapometer (Delfin Technologies Ltd., Kuopio, Finland) in both clipped and adjacent unclipped areas by holding the instrument against the skin for approximately 10-20 sec until a sound signal indicated that the reading was complete. Due to the length of time required for the Vapometer to record sweating rate, sweating rate was not measured on preweaned calves during midday data collection. The average clipped and unclipped skin temperature and sweating rate was calculated from the shoulder, rump, and thigh measurements for statistical analysis.

Statistical analysis

All statistical analyses were performed using SAS software version 9.4. Data were analyzed by repeated measures analysis of variance using the Generalized Linear Models (GLM) procedure. The model included the main effects of genotype, group, farm, and the interactions between genotype and group, genotype and farm, and genotype and afternoon THI. The afternoon THI was included as a covariate. The dependent variables were rectal temperature (RT),

respiratory rate (RR), surface temperature in clipped (STC) and unclipped skin (STN), and sweating rate in clipped (SRC) and unclipped (SRN) skin. We also analyzed the effects of clipping the hair on skin temperature and sweating rate using the Generalized Linear Mixed Models (GLIMMIX) procedure. Animal was the experimental unit and was considered random. The model included the main effects of genotype, farm, hair clipping (yes/no), and the interaction between genotype and hair clipping. The afternoon THI was included in the model as a covariate. Tukey-Kramer was used as the post-hoc test when pairwise analyses were necessary. Finally, regression analyses were performed using the REG procedure to evaluate the correlation between rectal temperature and THI in the different measurement windows during the day.

The data are reported as least square means ± standard error of the mean. Effects associated with P-values ≤ 0.05 were considered significant. Orthogonal contrasts were pre-planned to evaluate differences between slick carriers and animals from non-slick genotypes.

RESULTS

The 24-hour average THI during the testing period was 72 ± 0.2 (range 61-80). The average THI was 75.1 ± 0.2 during the morning measurement, 79.1 ± 0.2 in the middle of the day, and 80.8 ± 0.2 in the afternoon measurement ($P < 0001$; the three measurements were different among each other). Figure 4.2 depicts the average 24-hour temperature, relative humidity and THI during the 10-day testing period, as well as the THI calculated in each farm location during the testing windows.

The analysis included 222 animals (Table 3). Of these, 142 animals belonged to group 1 (postweaned heifers, 107-244 days old) and 80 belonged to group 2 (preweaned calves, 4-48 days old). There were 80 slick, 81 non-slick, and 61 control animals in both groups combined.

Slick animals had similar rectal temperature compared to control animals in the morning (38.8 ± 0.04, 38.8 ± 0.05 and 38.7 ± 0.04 for control, non-slick and slick animals, respectively; P = 0.1) and afternoon (39.2 ± 0.05, 39.2 ± 0.05 and 39.1 ± 0.04 for control, non-slick and slick animals, respectively; Figure 4.3A). Rectal temperature (RT) was affected by age group ($P < 0.001$) and farm ($P = 0.01$) as the day progressed. In the morning, preweaned calves had 0.2 °C lower temperature compared to the postweaned heifers ($P < 0.01$). In the afternoon, the two groups were similar ($P = 0.26$; Figure 4.3A). Rectal temperature of the animals was different in the three farms in both morning ($P < 0.001$) and afternoon ($P < 0.001$) measurements. Farm 1 had cooler animals (38.6 ± 0.04 °C in the morning and 38.9 ± 0.05 °C in the afternoon), whereas farm 2 had the hottest animals (39.0 ± 0.04 °C in the morning and 39.5 ± 0.05 °C in the afternoon). On farm 3, animals had intermediary temperatures (38.8 ± 0.04 °C in the morning and 39.1 ± 0.04 °C in the afternoon) There were no interactions between farm and age group or between farm and genotype.

A regression analysis testing the correlation between rectal temperature and THI (Figure 4.5) revealed both pre- and postweaned slick animals to have a weaker correlation (Group 1 Adj. $R^2 = 0.01$; Group 2 Adj. $R^2 = -0.04$) compared to non-slick (Group 1 Adj. $R^2 = 0.12$; Group 2 Adj. $R^2 = 0.19$) and control calves (Group 1 Adj. $R^2 = 0.10$; Group 2 Adj. $R^2 = 0.07$).

There was no effect of genotype on respiration rate in the morning or afternoon. Time of measurement affected the respiration rate of pre- and postweaned animals differently ($P = 0.02$): during the morning measurements, preweaned calves had lower respiration rate compared to postweaned heifers (51.0 ± 2.0 and 63.5 ± 1.4 BPM, respectively; $P < 0.01$); preweaned calves had a greater increase in RR compared to postweaned heifers, and thus the two groups were similar in the afternoon (66.5 ± 2.5 and 71.3 ± 1.8 BPM respectively; $P = 0.17$) (Figure 4.3B).

Slick animals had similar skin temperature in clipped areas compared to non-slick and control animals in the morning (33.32 ± 0.2, 33.29 ± 0.2 and 33.19 ± 0.2 °C for control, non-slick and slick animals, respectively; contrast slick vs others: $P = 0.08$) and in the afternoon (34.46 ± 0.1, 34.23 ± 0.1 and 34.42 ± 0.1 °C for control, non-slick and slick animals, respectively; contrast slick vs others: $P = 0.7$) (Figure 4.3C). Preweaned calves had lower clipped skin temperature compared to postweaned heifers in the morning (32.90 ± 0.2 and 33.64 ± 0.2 °C, respectively; $P = 0.01$); in the afternoon there was also an age group effect, but this time due to preweaned calves having greater clipped skin temperature compared to postweaned calves (34.58 ± 0.1 and 34.17 ± 0.1 °C, respectively; $P = 0.02$) (Figure 4.3C). Unclipped skin temperature was not affected by genotype in the morning or afternoon (Figure 4.3D). Again, preweaned calves had lower unclipped skin temperature in the morning (31.76 ± 0.3 vs 32.87 ± 0.2 °C, respectively; $P < 0.01$), but in the afternoon, the skin temperature was similar between pre-and postweaned calves (33.94 ± 0.2 and 33.84 ± 0.1 °C, respectively; $P = 0.63$) (Figure 4.3D).

Sweating rate in clipped or unclipped areas was not affected by animal genotype. Age group had an effect on sweating rate. Preweaned calves sweated less than postweaned heifers in the morning and afternoon measurements in both clipped [53.56 ± 6.4 vs. 120.31 ± 4.5 g/m²h for pre- and postweaned animals in the morning and 84.10 ± 7.1 vs. 147.70 ± 5.0 g/m²h for pre- and postweaned animals in the afternoon ($P < 0.001$); Figure 4.3E] and unclipped skin areas [48.49 ± 5.2 vs. 93.51 ± 3.6 g/m²h for pre- and postweaned animals in the morning and 64.88 ± 5.4 vs. 100.01 ± 3.8 g/m²h for pre- and postweaned animals in the afternoon ($P < 0.001$); Figure 4.3F]. There was an effect of farm on clipped ($P < 0.01$) and unclipped ($P < 0.01$) sweating rate, but no interaction between farm and genotype.

When evaluating the effect of hair clipping on skin temperature and sweating rate, we observed no differences in postweaned heifers. In preweaned calves we observed that in the morning, clipping the hair resulted in increased skin temperature of all genotypes, although the difference was of lower magnitude in slick calves (33.0 ± 0.3 and 31.9 ± 0.2 °C for clipped and unclipped skin; $P < 0.01$) compared to both non-slick (33.4 ± 0.3 and $31.6 \pm 0.$ 2 °C for clipped and unclipped skin; $P < 0.001$) and control calves (33.2 ± 0.3 and 31.7 ± 0.2 °C for clipped and unclipped skin; $P < 0.001$) (Figure 4.4A). The same was observed in the afternoon: although all animals had greater skin temperature compared to the morning measurements, the increase in temperature observed in clipped versus unclipped skin was of lower magnitude in slick calves (34.9 ± 0.2 and 34.6 ± 0.1 °C; $P = 0.3$) compared to both non-slick (34.8 ± 0.2 and 34.2 ± 0.1 °C; $P = 0.01$) and control calves (34.8 ± 0.2 and 34.4 ± 0.1 °C $P = 0.03$) (Figure 4.4A). When evaluating sweating rate, preweaned slick animals had the lowest overall sweating rate (71.1 ± 6.1 gm2/h compared to 98.5 ± 7.8 and 75.3 ± 6.2 g/m²h in non-slick and control calves, respectively; contrast slick vs others: $P = 0.05$). No differences due to clipping were observed in the morning (Figure 4.4B); in the afternoon, slick calves had similar sweating rate in clipped and unclipped skin (78.4 ± 6.7 vs 68.8 ± 6.7 g/m²h, respectively; $P = 0.6$), whereas sweating rate was greater in the clipped skin of both non-slick (116.9 ± 8.5 vs 80.1 ± 8.5 gm2/h in clipped and unclipped skin; $P = 0.03$) and control calves (88.4 ± 6.8 vs 62.1 ± 6.8 gm2/h in clipped and unclipped skin; $P = 0.08$; Figure 4.4B).

DISCUSSION

The two objectives of this study were to assess the physiological responses of pre- and postweaned Holstein heifer calves to heat stress conditions in California's low humidity climate and to evaluate whether inheritance of the SLICK1 allele would alter the physiological responses

in a way to confer more tolerance to the heat. Our data indicate that, compared to preweaned calves, postweaned heifers are under a greater degree of heat load as seen by higher physiological measurements in the morning; furthermore, the preweaned calves may be more susceptible to the fluctuation in external heat load as seen by their lower physiological measurements in the morning yet comparable afternoon measurements to postweaned heifers. Inheritance of the SLICK1 allele did not alter the physiological responses to heat by cattle in either age group; however, presence of the SLICK1 allele was correlated with a more constant body temperature as THI increased, and with little changes in sweating rate and skin temperature in the presence of hair, compared to non-slick and control calves.

We have recently reported the physiological responses of Holstein pre-and postweaned heifer calves carrying the SLICK1 allele in heat stress conditions, which was evaluated in calves raised on farms in California's low humidity and Florida's high humidity climates (Carmickle et al., 2022). When looking at a single timepoint during the window of highest THI for the day, we observed that both pre- and postweaned slick heifer calves had lower rectal temperatures than their non-slick counterparts in Florida, while no difference was observed between slick and non-slick animals in California (Carmickle et al., 2022). In the present report, we took advantage of the collection of multiple timepoints from each animal, as well as the collection of physiological data from genetically unrelated control calves in each farm, to expand the evaluation of the performance of slick Holsteins under dry heat conditions. We found that inheriting the SLICK1 allele had little influence on the physiological responses of either age group to heat stress conditions which is consistent with our previous findings for California animals; however, by taking repeated measurements, we observed that slick calves better maintained their body temperature as THI increased, compared to calves that did not carry the mutation.

111

Olson and colleagues (2003) were the first to define the dominant slick hair mutation described in offspring of beef breeds crossbred with Senepol cattle, a *Bos taurus* dairy breed from central America. The short, sleek hair coat created by this mutation from the Senepol cattle is visually similar to the coat of a *Bos indicus* breed. These slick-haired crossbred calves had lower rectal temperature compared to the normal haired contemporaries. Additional studies corroborated the lower body temperature of slick animals compared to normal haired cattle in tropical and sub-tropical climates during periods of heat stress in criollo-breed heifers (Olson et al., 2003; Landaeta-Hernandez et al., 2021) and mature lactating Holstein cows (Dikmen et al., 2008, 2014).

The slick phenotype was first mapped to bovine chromosome 20 (Mariasegaram et al., 2007; Flori et al., 2012) and later the exact causative mutation – termed SLICK1 by Porto-Neto et al. (2018) – in Senepol cattle was located to exon 10 of the prolactin receptor gene (*PRLR*) as a deletion of a single cytosine (chr20;39136558GC>G) creating a downstream premature stop codon that causes the loss of 120 C-terminal amino acids thereby truncating the intracellular tail of the prolactin receptor protein (Littlejohn et al., 2014). Further research has reported additional mutations in the *PRLR* (named SLICK2-6) causing the slick hair phenotype in other *Bos taurus* breeds such as Carora and Criollo Limonero (Porto-Neto et al., 2018; Flórez Murillo et al., 2021). Today, there are several registered Holstein sires carrying the SLICK1 allele due to introgression of the allele into the breed through mating with Senepol – a breed to have only been reported to carry the SLICK1 allele.

In the present experiment, the postweaned group of animals had more consistent physiological measurements between the morning and afternoon as compared to the preweaned group. One possible explanation is that the preweaned animals were not as readily able to regulate their physiological responses to the increasing THI; as a result, their physiological parameters

would reflect more closely the variations that occur during the day. Another possibility is that the postweaned heifers were not as efficient at dissipating heat as during the night when the THI dropped. The preweaned animals have a larger surface area to mass ratio, which creates an advantage for heat loss. Therefore, preweaned calves would begin the day cooler compared to postweaned heifers.

One advantage of having a shorter hair coat is the increased ability of air to move across the skin thereby making evaporative heat loss more efficient compared to longer hair coats (Berman, 2005; Gebremedhin et al., 2008). We clipped a section of hair on the study animals as a way to evaluate the influence of the hair length. As expected, across all groups and genotypes, clipped areas had higher sweating rates compared to unclipped areas; however, the difference was consistently smaller in slick animals, indicating that presence of the hair in those animals did not seem to impair heat loss. Enhanced evaporative heat loss due to short hair is a promising theory to explain the lower rectal temperature observed in the slick-haired lactating cows from previous research, yet results for sweating rate in slick animals have been inconsistent. A study in Florida on sweating rate by Dikmen and colleagues (2008) first reported that the greater sweating rate of slick lactating Holstein cows compared to their non-slick counterparts was not observed in an adjacent clipped region on the shoulder; in turn, the authors concluded that the shorter hair coat of the slick cows resulted in greater evaporative abilities since less hot, humid air was trapped by the coat. However, a later study conducted by the same group in Florida (Dikmen et al., 2014) evaluated sweating rates of clipped and unclipped areas of the neck, loin, thigh, and rump in relatives that were either slick or non-slick lactating Holstein cows and reported no large difference between these cows. Similarly, we did not observe significant differences in sweating rate in pre- or postweaned heifer calves raised in Florida or California (Carmickle et al., 2022; present results).

Our studies extend previous findings from lactating Holsteins to young growing Holstein calves and overall do not point to a clear difference in sweating rate as a cause for lower body temperature, although at this point it cannot be ruled out that more subtle differences in sweat gland structure or function could be playing a role. Sosa et al. (accepted to JDS, 2022) found no difference in sweat gland abundance between heterozygous SLICK1 heifers and wild-type heifers; however, they did report that slick-haired heifers had twice as much forkhead transcription factor A1 (FOXA1) – a protein required for secretory function of the sweat gland – in the sweat gland epithelia compared to wild-type heifers.

While genotype did not appear to have an effect on sweating rate, preweaned calves clearly sweated less than postweaned heifers. This could be due to postweaned animals being more heat-stressed or to preweaned animals being less able to regulate their physiological responses to rising environmental conditions. Another possibility could lie in an immaturity of the sweat gland function or development for the preweaned calves.

Using a model based on beef cattle, St-Pierre et al. (2003) estimated a THI threshold of 77 for heat stress in dairy cattle between 0 and 1 year of age. Recent studies, however, have demonstrated the susceptibility of preweaned calves to heat stress at a significantly lower THI (Dado-Senn et al., 2020a,b; Young et al., 2020). Dado-Senn et al. (2020a) observed signs of heat stress in preweaned calves at THIs that ranged from 65 to 69; furthermore, at a THI of 70, Young et al. (2020) reported internal temperatures greater than 39 ºC for preweaned Holstein calves. These THI thresholds are vastly lower than the one previously estimated and much closer to the THI threshold of 68 that has been established for lactating dairy cattle (Zimbelman et al., 2009). The heat stress experienced by these young calves can be detrimental to their immediate well-being, while also having long-lasting and, potentially even generational, negative effects (Laporta,

2021). Further research expanding upon the effects of heat stress – both short and long term – on young dairy cattle is warranted for improvement of welfare and production of animals in the dairy industry.

CONCLUSION

This study demonstrated that preweaned Holstein calves carrying the SLICK1 allele maintained a more constant body temperature as THI increased throughout the day in California's low humidity heat conditions; however, when evaluating the physiological parameters in the morning and afternoon, the presence of the SLICK1 allele did not alter the physiological responses of either preweaned or postweaned Holstein females. Preweaned calves had lower physiological measurements during the morning but may have a greater reaction to rising THI due to their comparable or even slightly higher measurements than the postweaned calves in the afternoon.

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Table 3. The number of animals evaluated for physiological responses to heat stress in both Group 1 (postweaned) and Group 2 (preweaned). CON: unrelated control, NON: non-slick, SLK: slick.

Genotype	Group 1	Group 2	Total
CON	36	25	61
NON	56	25	81
SLK	50	30	80
Total	142	80	222

Figure 4.1. (A) Preweaning aged Holstein heifer calves with a slick heifer calf in the background and a non-slick heifer calf in the foreground. (B) Postweaning aged Holstein heifer showing the location of the data collection of unclipped (the location of the number) and clipped (the clipped square adjacent to the number) skin temperature and sweating rate.
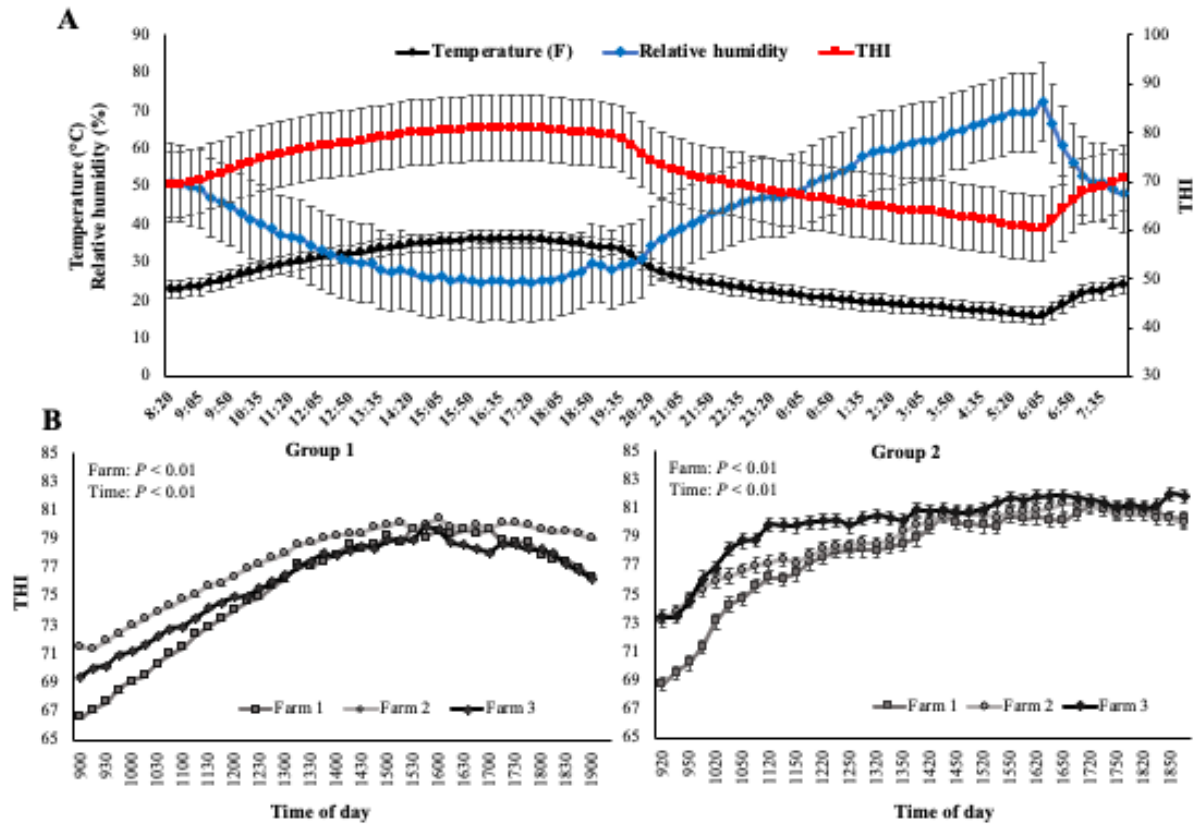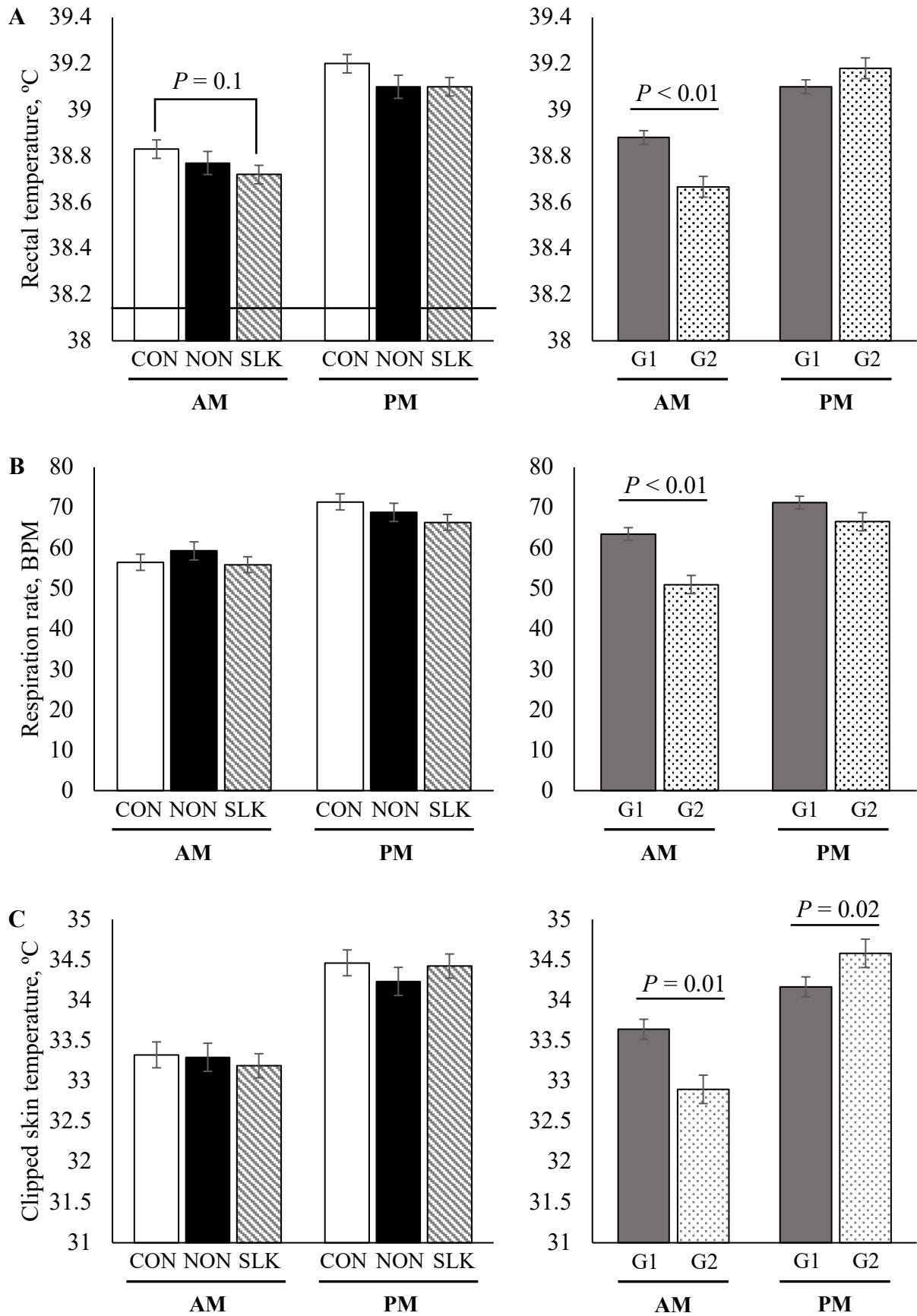
Figure 4.2. A) The average of the 24 h dry bulb temperature (ºF), relative humidity (% RH), and temperature humidity index (THI) during the days of physiological measurement collection. B) For Group 1 and Group 2, the THI measurements from each day of data collection for each farm.
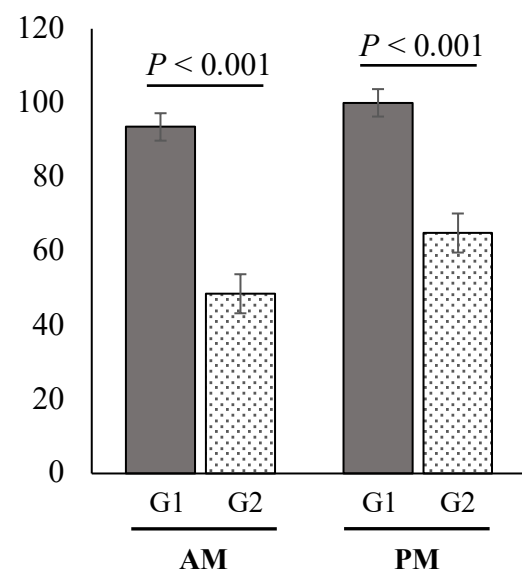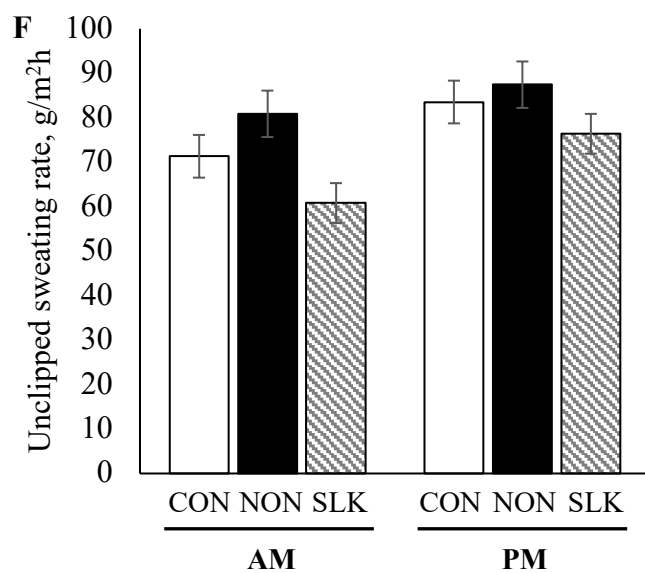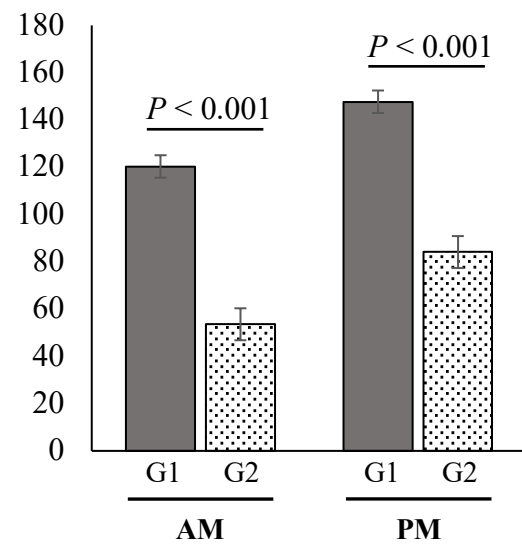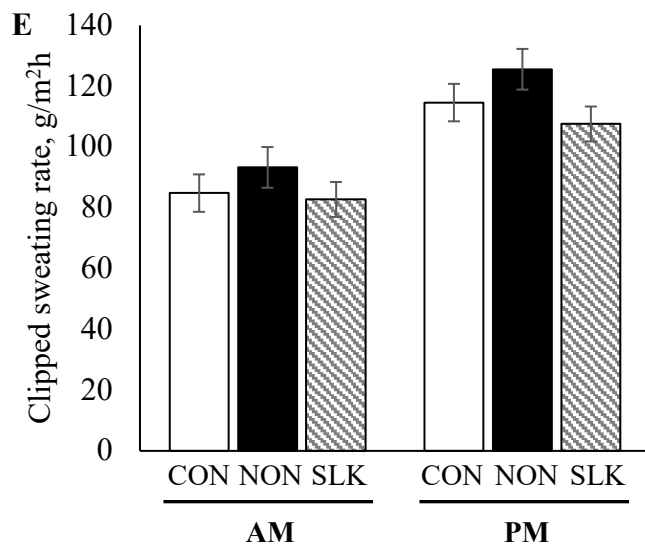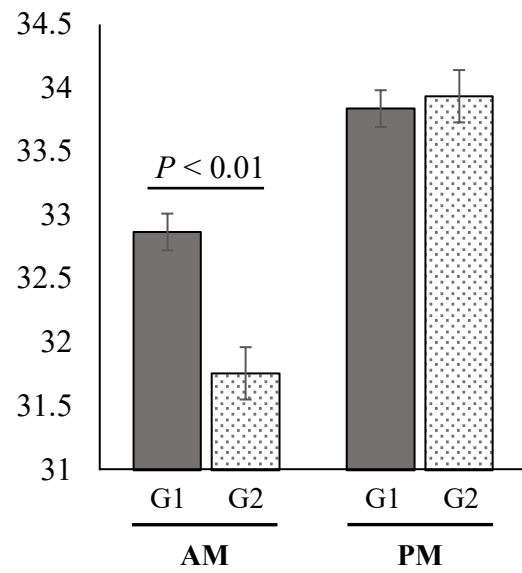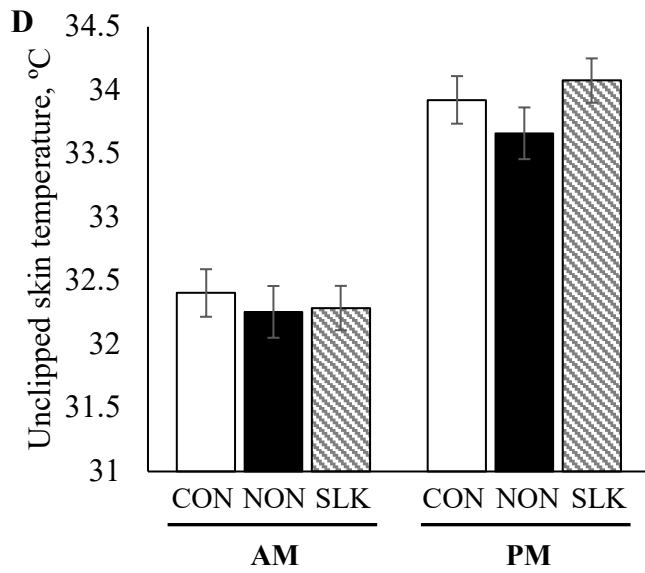
Figure 4.3. Physiological responses to heat stress during the morning and afternoon data collections in pre-weaned and post-weaned Holstein calves carrying the SLICK1 mutation. Effects on A) rectal temperature (a solid black line indicating the rectal temperature threshold); B) respiration rate; C) skin temperature clipped; D) skin temperature unclipped; E) sweating rate clipped; F) sweating rate unclipped. Animals carrying the SLICK1 mutation (SLK), half-sisters not carrying the mutation (NON), and unrelated cohort control (CON) were evaluated for both post-weaned (Group 1; G1) and pre-weaned (Group 2; G2) heifers. Where present, tendencies ($P \leq 0.1$) and significant differences ($P \leq 0.05$) are noted. Values expressed as least square means $\pm$ standard deviation of the means.
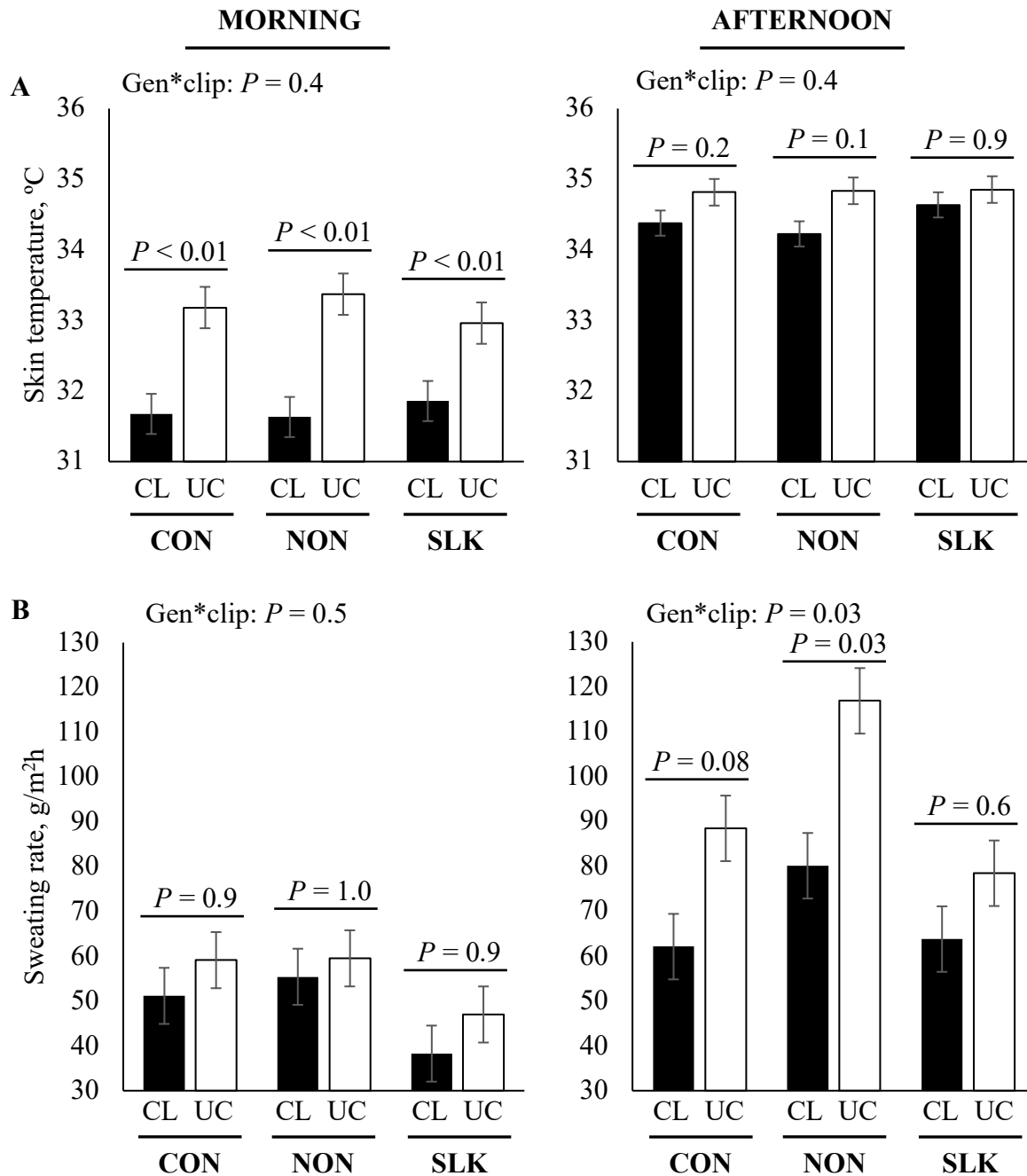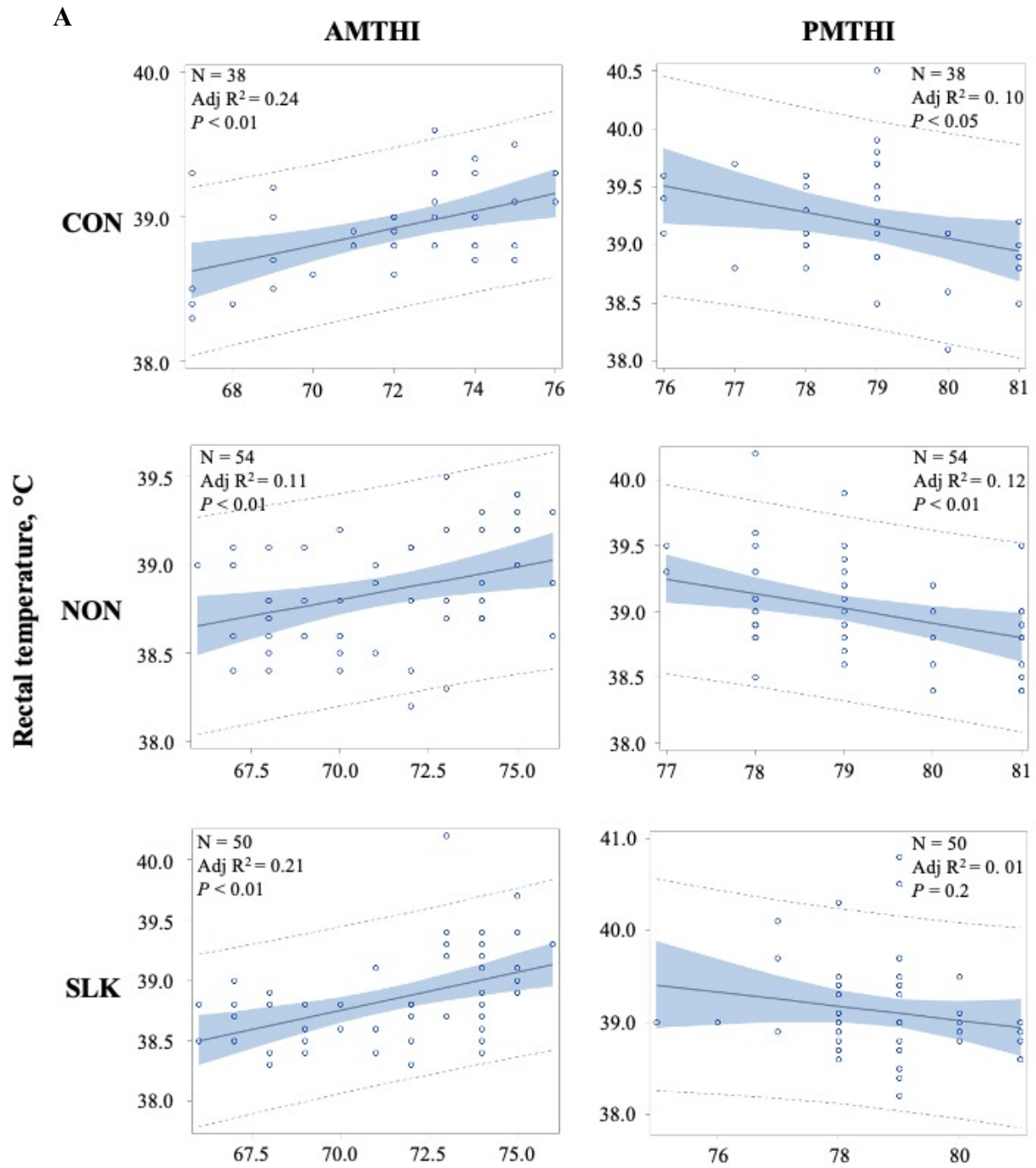
Figure 4.4. Effect of clipping the hair on A) skin temperature and B) sweating rate in slick (SLK), half-sister non-slick (NON), and unrelated cohort control (CON) heifers from Group 2. Significant effects were considered with $P \leq 0.05$ and tendencies were associated with $0.05 < P \leq 0.1$. Values expressed as least square means $\pm$ standard deviation of the means. Gen: genotype (slick, non-slick), clip: clipping status [clipped (CL), unclipped (UC)].

A

AMTHI   PMTHI

CON

NON

SLK

Rectal temperature, °C

N = 38
Adj R^2 = 0.24
$P < 0.01$

N = 38
Adj R^2 = 0. 10
$P < 0.05$

N = 54
Adj R^2 = 0.11
$P < 0.01$

N = 54
Adj R^2 = 0. 12
$P < 0.01$

N = 50
Adj R^2 = 0.21
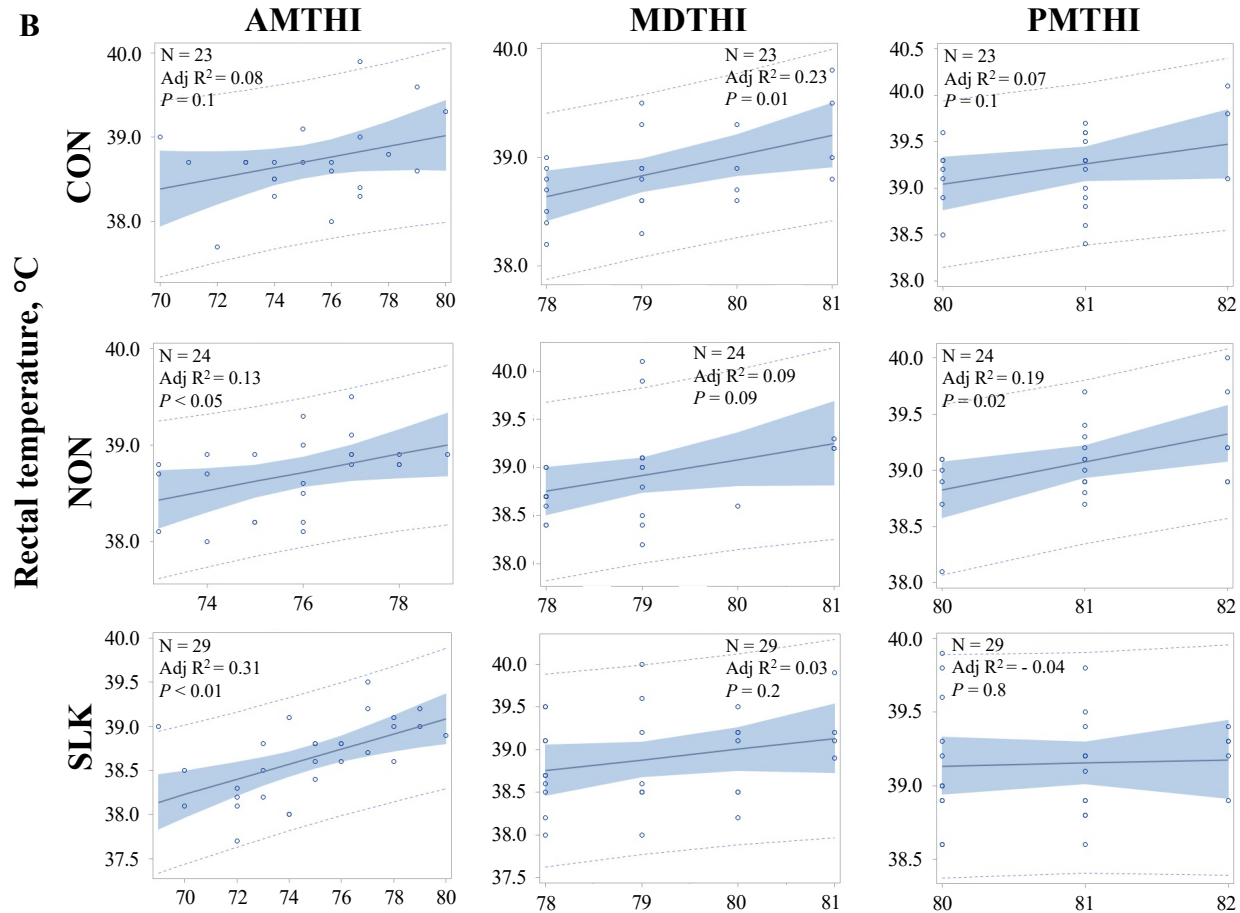$P < 0.01$

N = 50
Adj R^2 = 0. 01
$P = 0.2$

Figure 4.5. Effects of temperature humidity index (THI) on rectal temperature for slick (SLK), non-slick half-sister (NON), and unrelated cohort control (CON) heifer calves in A) Group 1 and B) Group 2. Significant effects were considered with $P \le 0.05$ and tendencies were associated with $0.05 < P \le 0.1$. Adj R^2 = adjusted R^2.

Conclusion

Inheritance of the SLICK1 allele by Holstein heifers was shown to result in a tendency for higher birth weights in California, increased thermotolerance as seen by lower rectal temperatures in Florida, and more consistent body temperatures with rising THI throughout the day in California preweaned calves when compared to non-slick half-sisters. We found that the calves in Florida appeared to experience more severe heat stress primarily driven by the high levels of humidity compared to those in California. In California, the preweaned calves may be more responsive to the rising daily THI when compared to the postweaned heifers who maintained more consistent physiological measurements throughout the day.

The research on thermotolerance of slick cattle has focused primarily in lactating dairy cows. This project was the first to investigate and report on the preweaning phase of slick Holstein calves in a low humidity climate, the physiological responses to heat stress of slick calves and heifers in both high and low humidity climate, and the reproductive performance of slick heifers. While this project provided novel data, additional research is required to further elucidate the effects of inheritance of the SLICK1 allele on the thermoregulatory ability in cattle in low humidity climates as well as its effect on reproductive performance. Research into genes, especially thermotolerant mutations such as the SLICK1 allele, is vital to increasing productivity, welfare, and profitability of dairy cattle through genetic selection that will provide long-term, heritable progress to the dairy industry.