The effects of the 'SLICK' allele of the prolactin receptor gene on the cold stress response of dairy heifers

---

A Dissertation

submitted in partial fulfilment

of the requirements for the Degree of

Bachelor of Agricultural Science

at

Lincoln University

by

Luke Andrew Robb

Lincoln University

2023

Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science.

The effects of the 'SLICK' allele of the prolactin receptor gene on the cold stress response of dairy heifers

by

Luke Andrew Robb

Animal welfare concerns such as heat stress is becoming a more pressing issue under the increasing variability in climatic events in the temperate New Zealand dairy industry. Assessment of heat stress and management strategies are key areas of research, for which the SLICK allele is a potentital genetic management strategy for heat stress in New Zealand. However the interaction between this allele and cold stress is largely unknown. This research was undertaken to investigate the effects of the SLICK allele on the cold stress response of dairy heifers. Three groups of heifers representing the national herd genetics (Control) or, SLICK, and Non-SLICK, were investigated over 29 days between 13th June and 11th July in an outside grazing trial to identify any behavioural, or physiological responses to periods of natural cold stress. Control and SLICK calves (n= 6 and 7 respectively) wore SensOor accelerometers to monitor behaviour, and rectal temperatures were recorded on potential cold stress days. The frequency of cold stress days, which were determined as wind chill temperature falling below 0°C (dry) or 15.5°C (wet), were 7 days out of 29. SLICK heifers had 5% lower liveweight than control or non SLICK (221.9 vs 233.9kg, P = 0.62). However, there was no difference between treatments for liveweight gain, rectal temperature, rumination, active, or high active time. SLICK calves spent more time inactive compared with control calves (466 vs 426 minutes/day, $p < 0.008$). An interaction between treatment and date for eating time (minutes/day, $p < 0.05$) showed that at high pasture allocation SLICK spent less time eating than control calves, but similar eating time under restricted pasture allocation. Differences in coat characteristics suggested SLICK heifers had an improved insulative effect compared with Non-SLICK heifers. SLICK heifers had a greater mean fibre diameter (36 vs 30, $p < 0.005$), medulation percentage (28.18 vs 22.3 %, $p = 0.047$), and greater percentage of coarse edge fibres (21.8 ± 1.79% vs 14.0 ± 0.90%, P = 0.005). Overall, the results suggest that under mild levels of cold stress conditions there are small differences in response to cold stress between heifers carrying the SLICK allele compared to heifers which do not

carry the SLICK allele. Control heifers appeared to respond to cold stress through increased activity and increased eating time, whereas SLICK heifers appeared to favour inactivity and increasing insulative coat to conserve energy. However without strong temperature, or growth differences it suggests that the cold challenege is not strong enough to observe any different welfare concerns in SLICK carrier heifers.

Keywords: SLICK, cold-stress, animal welfare, rectal temperatures, behaviour, fibre.

Acknowledgements

Thank you to all people who have assisted and supported this honours dissertation, with particular thanks to the following contributors:

To Jon Hickford and Racheal Bryant, who supported me as supervisors for this dissertation. I deeply appreciate all the feedback, discussions, encouragement, and time you dedicated towards me as a student and this project. Your passions and expertise are admirable and I am extremely thankful for all you have taught me and will take this with me into the next stage of my career.

To the Livestock Improvement Company, for the financial assistance and ongoing support for the SLICK research. Also, to the staff and faculty of Agriculture and Life Science for helping fund this, and your constant elevation of students, thank you.

Paige, Jess, Ben, Abhi, Diego, Lucas and Aline for your assistance caring day to day for the heifers during the trial, and enduring the cold early morning to help take animal measurements. Your technical support planning, during, and after the trial made the data collection process stress-free and very enjoyable helping to drive my passion for this project and the industry.

Special thanks to Don and Pastoral Measurements Limited, for taking the time to show me FibreScan and giving cattle hair sample analysis a go. I am pleased with the results we gathered and feel they added a unique aspect to my dissertation.

To my friends and fellow honours students, thank you for your comradery the whole way through undergraduate life, and sharing the drive to persist and achieve. A special thanks to flatmate Sarah and close friend Sam for their sharing of ideas and support as we completed this honours year.

To my Mum, Grandma, Grandad, my brothers, Samuel and Benjamin, and partner Andrea. Thank you for always having my back by supporting this passion of mine in everyway possible. The encouragement through the highs and lows have allowed me to push boundries and achieve so much. This is as much your achievement as it is mine. I know your love and support will continue beyond now into the next stage of the adventure.

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

In New Zealand pastoral dairy production represents the country's largest exporter, earning over $18.6 billion in revenue in 2021 (The Treasury NZ, 2022). Despite traditionally being a temperate climate, which has underpinned the competitive ability of the country in terms of pasture production, New Zealand agriculture is vulnerable to climate change. From an animal welfare perspective, heat stress is becoming more of a prevalent issue in temperate climates such as New Zealand as the climate changes globally. The intensity and frequency of conditions causing heat stress in our outdoor agricultural systems is increasing (Carvajal *et al.*, 2021) and science forecasters anticipate the number of 'hot' days to increase in New Zealand by 40-100% by 2040, and this could be up to 300% by 2090 (Ministry for the Environment, 2016). This will pose a significant health and welfare concern for many farmers.

Solutions to reduce the effect of heat stress in livestock include cooling techniques such as sprinklers, building or planting shelter in fields, and breeding for heat tolerance are employed as mitigation strategies to reduce the negative welfare, and economic aspects associated with heat stress (Lees *et al.,* 2019). In 2014, the New Zealand dairy breeding and genetics company Livestock Improvement Corporation (LIC), introduced a genetic variant known as the SLICK allele (derived from tropical Senepol cattle), into a Holstein-Friesian dairy herd to ascertain its relevance in the temperate climate New Zealand dairy industry. The SLICK allele is caused by a single base pair deletion in the gene that codes for THI) prolactin receptor (*PRLR*) and originates in Senepol cattle (a cross between *Bos taurus* and *Bos indicus* breeds) that have superior performance in tropical regions, compared to conventional Holstein, Angus, and other breeds (Donkersloot *et al.,* 2014).The initial assessments of this breed under New Zealand conditions by LIC, revealed a 0.5-1.0°C difference in rumen temperature between SLICK heifers and a control group at temperature-humidity index (THI) values above 70 (Donkersloot *et al.,* 2014). Whilst the SLICK cows performed worse than their conventional cow counterparts in producing on average approximately 82% of the accumulated milk volume, LIC attributed this to the lower genetic merit of the SLICK population.

This initial research conducted by LIC suggested that the presence of this allele in the New Zealand dairy population could provide an effective tool in a dairy system to help reduce the negative effect of heat stress in our temperate outdoor production system, but that there were factors that needed further investigation. The first has been touched on already, being the dilution of New Zealand conventional dairy genetics, which needs to be refined for cows to produce at least at the same level

as current standards under 'normal' climatic conditions. Other production factors, such as growth also need to be investigated to determine the influence on calf and heifer management. Additionally, the welfare value of this genotype and the influence of this genotype in conditions other than heat stress need to be investigated due to the New Zealand climate being temperate rather than tropical. These all will help to determine the true value of this allele to the New Zealand dairy production system.

The main welfare concern with the SLICK allele is the potential welfare risk associated with cold stress. Many of the phenotypes associated with the SLICK allele, such as cattle having short coats, improved sweating ability, and lower rumen temperatures; are traits that may predispose cattle to being vulnerable to cold stress. The insulative ability of cattle coats in reducing wind speed and the cooling effect of rain, metabolic activity and eating habits, and the effects of shelter, all influence how a cow feels the cold and how their body will react to cold challenges (Cox *et al*., 2016). The short, sleek coat of SLICK cattle has been described, but little is known if SLICK carrier cattle can or need to adapt their coat as seasons change, the eating and metabolic changes in SLICK cattle in response to cold challenge, and their shelter-seeking and cold mitigating behavioural adaptations have also not been characterised leaving large gaps in our current understanding of how SLICK cattle will respond to cold challenges in temperate climates.

The purpose of this honour's dissertation is to address some of the knowledge gaps in how individuals carrying the bovine SLICK allele interact with the environment, specifically in relation to cold challenges. It is hypothesised that the characteristics such as having short and light coats, and increased sweating ability that provides an advantage at raising the upper critical temperature for these animals, will provide a disadvantage during times of cold stress. To achieve this, key performance characteristics such as liveweight, growth rate, and coat analysis will be used to judge a heifer's performance and adaptability to cold conditions. Behavioural responses such as rumination activity, eating, and physical activity will be assessed as heifers with and without the SLICK allele are exposed to cold challenges in the field. Additionally, body temperature responses assessed using rectal temperatures, and thermal imaging analysis to ascertain heifer responses in cold conditions. The measurement of these performance, behavioural, and temperature traits should help to develop an understanding of how the SLICK phenotype influences the welfare of cattle carrying this novel allele in the New Zealand climate.

Chapter 2

Literature Review

2.1 Introduction

'Global Warming,' 'Climate Change' and 'Climate Crisis' are not unfamiliar phrases worldwide with increasing frequency of adverse weather events such as floods, wildfires, heat waves and out of season weather increasing the challenges in the agricultural sector. The issue of the steady rise of global temperatures from the 1850's to now presents concerns to not just feed supply, but also to animal welfare. Analysis of global temperature trends by Rahmstorf *et al.* (2017) highlights an increase in temperature anomalies since the 1980's. The average rise in temperature since the third changepoint around 1970 is becoming more of an issue for farmers, as the climate in regions all over the world change and become more unpredictable. Figure 1 below illustrates these temperature anomalies and is based on the analysis of multiple global climate data sets.

Material removed due to copyright compliance

Figure 1. Global temperature trends from 1850-2015, according to changepoint analysis from NOAA, GISTEMP, Berkeley, Cowtan&Way and HadCRUT data sets (From Rahmstorf et al., (2017); Fig.2).

These changes in temperature are expected to have a wide range of implications for animal production systems as described in a review by Nardone *et al.* (2010). These authors highlighted that the impact of temperature is expected to affect animal health and welfare by exposing animals to

longer periods of stress during longer periods of hot and cold weather, feed restrictions due to harder pasture and crop growth seasons, and through a shift in pathogen and disease loadings. Changes in hormone production, such as low luteinising hormone levels in hot climates, can drop conception by 20-27% in cattle, and with pigs, it has been reported that there is a 5-6 fold higher death rate in sows when giving birth in temperatures above 33°C. Milk production in dairy cattle can decrease by 0.26 kg/day for every unit of temperature-humidity index (THI) above 70 as a consequence of a 0.23 kg/day decrease in dry matter intake. The concentration of milk fats and proteins also changes with changing THI.

Temperature challenges are not however a new concept to livestock production and several mitigations can be adopted by farmers to manage climate risk. These mitigations include making improvements in infrastructure and shelter and by selecting superior animal genetics. For example, Zebu cattle (*Bos indicus*) and hybrid Brahman cattle (*Bos indicus/Taurus*) are used in Australia and India for their ability to maintain performance in sub-tropical regions.

To illustrate the benefit of this, a feedlot study in Australia compared 17 genotypes of cattle with varying levels of heat load index (HLI) relative to their panting score. Panting score is used as an indicator of heat stress and Gaughan *et al.* (2009) used this to show that in extreme HLI conditions (>96), a greater number of *Bos taurus* and *Bos taurus*-cross cattle had a panting score greater than Brahman and Brahman-cross cattle ($P < 0.001$). However, the sub-tropical breeds are poorer producers than the *Bos taurus* breeds and hence are less popular in temperate climates, but there is variation in heat-tolerance among breeds within a given cattle species. For example, Senepol cattle have been bred by crossing Red Poll and N'Dama cattle, then selecting for parasite and heat-tolerance traits and production traits (Kava *et al.*, 2021). This breeding and selection gave the Senepol cattle physiological advantage through increased saculiform gland formation and adaptive coats for heat dissipation and absorption of intense radiation (Kava *et al.,* 2021).

Historically, temperate countries like New Zealand have been able to focus on production and fertility traits using a small range of breeds. However, changes in weather patterns and a social climate that is pushing for improved animal welfare, has created the need for re-evaluation of the approaches used in cattle breeding. Given this challenge, in 2014 New Zealand's largest dairy breeding company, LIC, introduced 'SLICK' (a novel allele of *PRLR)* into the national dairy herd, with the aim of providing a genetic solution for New Zealand farmers to combat the issue of heat stress in cows. While Donkersloot *et al.* (2014) reported a 0.5 to 1.0 ^{o}C lower rumen temperature in SLICK cows compared to non-SLICK cows when exposed to heat stress, the change in heat-tolerance may

compromise those animals in cold environments, swapping the heat-tolerance welfare issue for that of a cold stress issue.

The purpose of this literature review is to better understand some of the questions that have been raised around the introgression of sub-tropical genetics into a temperate climate production system. It will investigate the mechanisms of thermo-tolerance in dairy cattle, their specific responses to both hot and cold stress, and the natural adaptation to extreme conditions, with a specific focus on young stock.

2.2 Thermoregulation in dairy cattle

The following information in this section is, unless otherwise stated is adapted from the textbook '*Heat Stress and Animal Productivity'* (Aggarwal and Upadhyay (2013).

Thermoregulation is the process by which homeotherms (organisms which maintain their own body temperature at a constant level) maintain their body temperature at a constant level through balancing heat gained through metabolism with heat gained or lost to the ambient environment (Sepehr Foroushani & Amon, 2022 and Council, 2023). It has been reported that the average rectal temperature for dairy cattle is 38.6°C, with a range from 38.0 to 39.3°C (Andersson & Jonasson 1993). Any temperature outside of this range would suggest that the environment is overcoming any thermoregulatory mechanisms employed by the animal, and that the cattle will need to employ voluntary and involuntary responses to their environment.

Heat is produced in dairy cattle from metabolic activity in the body, as well as from fermentation processes in the rumen. They aim to maintain a heat flow from their bodies to the surrounding environment. In lactating dairy cattle metabolic activity is high, and this causes much more heat to be produced than needed, meaning heat dissipation mechanisms need to be activated. This includes increasing blood flow to the skin and extremities so that excess heat can be exchanged through conduction and convection to the environment. If the excess body heat persists, sweating and panting occurs, such that through evaporation the surface of the skin can be cooled using the animals body heat to evaporate off water and dissipate the heat. Sweating accounts for 70-85% of evaporative heat loss, with panting making up the rest. Other measures of thermoregulation in cattle include seeking shaded areas, as well as decreasing feed intake rate to slow down the production of heat from rumination.

The comfortable temperature range (or thermoneutral zone) is the range in which the animal does not need to expend any extra energy maintaining homeostasis for temperature. For lactating dairy cattle, the range is much smaller than for other cattle due to their abnormally high metabolic output, meaning their heat load is often much more than other cattle face. An accepted environmental

temperature range for production dairy cattle is between 5°C and 25°C. Five degrees Celsius is considered the lower critical temperature (LCT), with a 1% increase in energy intake required for every degree below the LCT to account for the drop in temperature. The most common behaviour change at lower environmental temperatures is an increase in feed intake. Twenty-five degrees Celsius is considered the upper critical temperature (UTC), and when temperatures exceed 25°C non-evaporative heat loss via conductive, convective, and radiative mechanisms become less effective and cattle will turn to more effective evaporative cooling. This is summarised in Figure 2 below, which illustrates the changes in heat production and core body temperature as cattle transition above and below their upper and lower critical temperature.

Material removed due to copyright compliance

Figure 2. Representation of thermoneutral zone showing upper and lower critical temperature, zones of metabolic regulation and the relationship between ambient temperature and heat stress in mammals (From Silanikove & Darcan (2015); Fig. 2).

Ambient temperature does not convey what the animal feels with respect to temperature. This is where the concept of 'Effective Ambient Temperature' (EAT) becomes important. EAT is an example of an index that is employed to describe what influences how an animal will feel and respond to the environment. It combines factors such as the effect of thermal radiation, humidity, air movement, animal contact surfaces, and precipitation to provide an EAT value that shows the effect of the environment on the animal (Council, 2023). An EAT value can be broken down into two classes of variable: climatic conditions and phenotypic differences.

2.2.1 Climatic conditions effecting thermal stress

There are several climatic variables that effect how an animal feels the temperature. Wind velocity can affect the level of evaporative cooling that is able to occur during hot weather; with wind moving warm air away from an animal's body, thus increasing the cooling ability. However, during cold conditions calves, and heifers especially, are vulnerable to cold wind taking heat away from them, which is known as wind chill.

Humidity (concentration of moisture in the air) affects the efficiency of the evaporative measures of heat dissipation, which can have a negative effect on heat dissipation in hot climates. However, this can be an advantage in cold climates as a more humid air makes it less likely for frosting to occur, and thus it decreases evaporative heat loss from calves and heifers.

Precipitation, as rain or snow, decreases the thermal insulation ability of an animal, meaning they will feel colder, and the intensity of solar radiation (including the effect of shade, cloud cover, etc.) will influence the amount of heat load, having a 3°C to 5°C influence above or below the actual temperature, depending on the intensity of the exposure.

Any combination of these climatic conditions can influence how cattle feel the temperature, and both positively and negatively, and the effects are also dependent on an animal's age and other factors that may be affecting its health and mobility.

2.2.2 Phenotypic differences

Passive measures of heat dissipation like conduction, convection, and radiation, are dependent on the surface area to bodyweight ratio of an animal and its coat. The surface area to bodyweight ratio dictates that at a lower surface area to bodyweight ratio (i.e., more surface area to less bodyweight) an animal will struggle to maintain heat, as there is more surface area to lose heat from, compared to body mass that might create heat. This means a cows age as well as breed will have an influence on how well they gain or lose heat, which also affects how cattle should be managed to mitigate heat and cold stress factors.

The second phenotypic difference is the lustre, colour, and density of the coat. This influences the radiant heat loss from a cow. The shine and colour of the coat affect how much radiative heat the animal absorbs that will influence its temperature from an external source. Darker and duller coats absorb more heat, whereas lighter and shiny coats reflect heat more effectively. The thickness of the coat, which depends on the number of fibre follicles has an insulative effect. In hot climates, having a thin coat means there is more contact with the surrounding air to dissipate heat and in cold climates a thicker coat provides less contact with the surrounding air, decreasing the heat dissipation as well as trapping more heated air around the skin to help maintain temperature. Age and season can affect how much the coat influences the cow's ability to dissipate heat, as coats change with the season as well as when an animal grows.

2.2.3 Predictors of hot and cold stress

Council's (2023) description of the EAT highlights that animal-specific thermal indexes are the best measures of the influence of the environment on an animal's thermoregulatory ability.

The creation of temperature-humidity indexes (THIs) is an example of a method used to combine two climatic factors, temperature, and humidity, to determine the heat stress load being applied to cattle. The main advantage of a THI index is its ease of use on farm and scientifically as it only requires easily obtained values from meteorological data (Gatti *et al*., 2019). THI values 70 and below are considered comfortable, 75-78 stressful, and 78 plus is extremely stressful (Mostafa *et al*., 2018). Mostafa *et al.* (2018) goes on to explain that most iterations of the THI will display slightly different THI thresholds for comfort, stress, and extreme stress which can make accurate interpretation of the influence of temperature and humidity on heat stress difficult to ascertain. A THI value succeeds at using climate factors of the EAT to predict the animal response with strong correlations between increasing THI and respiration rate, sweating, other heat dissipation behaviours and rectal temperature (Mostafa *et al*., 2018).

An alternative heat stress index to THI, designed specifically for extensively grazed dairy cattle, is called the grazing Heat Load Index (HLI) which was developed to integrate wind and solar radiation climate variables into a heat stress index (Bryant *et al.,* 2022). This study revealed the modelled grazing HLI had a stronger correlation between daily predicted and actual respiration rate at R^2 value of 0.53 compared with other HLIs, which ranged from 0.13 to 0.36 R^2 values. This index had a better integration of animal observations of respiration rate, a known indicator of thermal load on cattle, and the relative environmental factors. This is demonstrated with an increase of 4.21 breaths per minute for every 1°C ambient temperature increase, 5.89 breath per minute increase per one megajoule/m^2 increase in solar radiation, and a 1.70 decrease in respiration rate per 1m/s increase in wind speed. The reported critical grazing HLI value was 70, which saw exponential increases in respiration rate above this value. Like the THI, grazing HLI is not a standardised measurement, meaning caution is needed to directly compare index values. It does, however, demonstrate that the combination of many different climate and animal factors can be used to model the influence of heat stress on cattle, and thus provide more specific measures for dairy cattle.

Wind Chill Temperature (WCT) is the most common measure used to predict the influence of environmental temperatures and cold stress in cattle. The WCT measures temperature and wind speed in cold conditions as being the most influential factors in making animals feel cold. This index does not set values at which animals may be stressed, but instead aims to give a more realistic assessment of what the animal or human body would be responding to. The WCT is then compared

to a critical temperature table, which combines seasonal coat, and precipitation factors to determine what the lower critical temperature would be for that day, based on environmental conditions.

These types of measures aim to better explain how an animal will respond to the environment, and to help give researchers and farmers a better idea of how cattle or any other animal, will respond to the conditions to help mitigate the welfare issues associated with thermal stress, account for the production losses and describe behavioural changes.

2.3 Heat stress

Heat stress occurs when the environmental temperature exceeds the upper critical temperature threshold of the thermoneutral zone. When dairy cattle are heat-stressed, adaptations in intake and behaviour must occur for the individual to thermoregulate.

2.3.1 Feed and water intake

Several studies have investigated the impact of variation in ambient and body temperature on the feed intake of cattle. Generally, as temperature increases, intake declines. For example, Hahn (1999; Figure 3) measured the response of steers (n = 8) to increasing environmental temperature (from 10°C to 30°C) in a climate chamber (right axis) over a period of 8 days. The results showed that as chamber and tympanic temperature increases, and daily feed intake (in kg dry matter; KgDM) declines.

Material removed due to copyright compliance

Figure 3. The response of steers (measured as tympanic temperature; left axis, over a period of 8 days (x-axis) to increasing environmental temperature (ranging from 10° to 30° Celsius) (From Hahn (1999).

The relationship between a temperature-humidity index and dry matter intake (DMI) has also been shown to be strongly negatively correlated (R = -0.63) in Jersey cows, reducing the DMI for multiparous cows by 22% and the DMI for primiparous cows by 6% (Farooq *et al*, 2010). The authors suggested that the main influence on this difference is the effect of variation in body size and the metabolic rate of the cows. The decrease in DMI under heat stress conditions is caused by the cooling centre of the hypothalamus, the control centre for all homeostatic functions in mammals. It stimulates the medial satiety centre in the hypothalamus to reduce appetite and therefore lower DMI (Yadav *et al*., 2013). At the same time, rumination time is decreased, pushing food through the rumen digestion system quicker to lower the amount of metabolic heat produced and to lower the internal body temperature in response to higher environmental heat (Yadav *et al*., 2013).

Diurnal patterns of grazing can also be affected by heat stress and the thermal load displayed in dairy cattle. (Carvalho *et al*., 2021) explained that animals graze intensively in the early morning, and late afternoon to avoid grazing and producing large volumes of heat during the hottest part of the day. If the environment changes temperature during these times, or close to these times, animals will modify their behaviour, which aligns with the findings of Hahn (1999) and Farooq *et al.* (2010) above. (Carvalho *et al.,* 2021) further explained that if night-time conditions do not allow for enough heat dissipation from cattle to the environment, morning rumination and eating behaviour will be reduced, and hypothalamic control will not increase appetite until the risk of heat stress is reduced, regardless of what might be considered the normal grazing pattern.

Increasing temperature-humidity index is also associated with an increase in water intake in cows, ranging from 4.8 to 9.8% (water intake as percentage of bodyweight) (Farooq et al, 2010). Mishra's (2021) review on the behavioural and physiological responses of cattle to heat stress reported a 19% increase in water intake between lactating cows with no shade, compared to shaded cows. (Roman-Ponce *et al,* 1981) reinforced this observation, describing a 30% increase in water intake in heat-stressed bulls, compared to non-heat stressed bulls (Meyerhoeffer et *al,* 1985). The increase in water intake is likely caused by an increase in water demand because of increased sweating during heat stress conditions. Sweating utilises evaporative-type cooling, which increases the water and electrolyte demand of cattle as water is partitioned into sweat. Drinking more water and increasing electrolyte consumption are both vital factors at maintaining this method of cooling (Shalit *et al*., 1991).

Reduced feed intake, changes in grazing time, and increased water intake are substantial consequences of heat stress observed in cattle, but cattle behavioural adaptations will influence the degree to which these consequences are observe as will be discussed below.

2.3.2 Cattle behaviour when heat challenged

Behavioural responses to heat stress are an important mechanism for thermoregulation, and some breeds have a more significant behavioural change to heat stress, than others.

Allen *et al.* (2015) illustrated that when a cow's core body temperature reached 38.9°C, there was a 50% likelihood that a cow would be standing, which was explained as a sign of lack of comfort. Similarly, Tucker *et al.* (2008) reported an increase of 10% (from 13.8 to 15.3 hours per day) in cattle standing time for every 15% increase in heat load. When the cattle in the Tucker *et al.* (2008) study were standing, their core body temperature was 0.2°C higher ($p = 0.004$) than cattle standing under shade that provided a 99% reduction in solar radiation.

Respiration rate is an indicator of panting activity, which is a major part of cattle's immediate behavioural responses to heat stress. Panting can account for 30% of total heat dissipation (Idris *et al.,* 2021) and their review described an increased respiration rate of 4.3 breaths per minute for every degree Celsius above the threshold temperature of 21.3°C, and which had the potential to go up to a total of 200 breaths per minute during large heat loads. This behaviour is particularly important when the humidity is high, as the evaporative heat loss from skin reduces due to a decreased vapour pressure gradient which reduces the ability of water (in the form of sweat) to move into the air taking heat with it. Panting can continue to provide a cooling benefit beyond that of evaporative skin cooling, as the hot air inhaled by animals is able to be warmed to the internal body temperature, which removes that heat when air is expired (Sparke *et al*., 2001).

Zebu cattle, originating in tropical regions, are adapted to warmer climates by having an increased number of skin folds, and enlarged ears, dewlaps, naval flaps, and vulvas to increase the surface area to volume ratio (Brody, 1956). Brody (1956) explains that this adaptation is enhanced by preferential blood flow to these enlarged areas, which act as radiators of heat for these animals. Referred to as the vasomotor dilating and contracting device, hormonal control of heat stress detection alters the blood flow to capillaries in the upper surface of the skin to maintain skin temperature. When coupled with increased sweating, it allows for evaporative cooling helping to cool blood temperature, which is then circulated back into the core to lower the body temperature of cattle. Aside from the size of skin folds and peripheral structures, coat density affects the rate at which heat exchange can occur. Noting the difference between Bonsma's European-evolved shorthorn cattle, which had coat samples weighing 300 g compared with Zebu cattle which had a weight of 10 g (collected from the same sized area) revealed that denser hair provides an insulative effect by trapping more air around the skin surface, thus reducing the effectiveness of the evaporative cooling effect.

Voluntary behavioural adaptations such as standing and panting, as well as involuntary adaptations such as increased predentary blood flow to peripheral organs, allow cattle to better manage heat stress. As effective as these behaviours can be, there is the potential that these behaviours can affect cattle performance, as will be described below.

2.3.3 Cattle performance during temperature challenges

A measure of the success of a dairy farm is the quantity and quality of milk and the fertility of the animals. Several studies have demonstrated a drop in milk yield associated with heat stress.

Milk yield is influenced by heat stress, with seasons of increased THI value yielding less milk than seasons with lower THI values. For example, Chanda *et al.* (2017) revealed an average decrease in milk yield (litres/day) of 14% and 23% in two groups of Holstein-Friesian-cross cows between May-June (hot period) compared to December-February (cold period) in Bangladesh ($p < 0.05$). Milk fat, protein and lactose were also significantly less (%) in the hot months compared to the cold months ($p < 0.05$). The difference in milk yield between the hot and cold months was attributed to the decrease in intake rate during the hot months, with animals focusing on spending energy cooling, rather than on milk production. This has a flow on effect, with it not only lowering the milk composition (content of fat, protein, and lactose), but also by decreasing the rate at which fat, protein, and lactose is synthesised. Similar findings were obtained in a comparison of Holstein and Jersey dairy cattle in Mississippi, which revealed that both breeds had a decrease in milk production and milk composition during times of heat stress, compared to times with no heat stress. The Holstein cattle had a larger influence from heat stress than their Jersey counterparts (Smith *et al.,* 2013). The results are summarised in Table 1 below.

Table 1. Milk yield, milk fat, and milk protein composition of Holstein and Jersey cattle during conditions of non-heat stress (HS-) and heat stress (HS+) conditions (Adapted from Smith et al. (2013); Table 1)

	Holstein			**Jersey**			***P*-value**		
Item	**#HS-**	**HS+**	**SE**	**HS-**	**HS+**	**SE**	**THI**	**Breed**	**Breed x THI**
n	580	526	-	140	129	-	-	-	-
Milk Yield (kg/d)	35.6^{a}	34.2^{b}	0.14	25.9^{c}	26.6^{d}	0.26	0.04	<0.01	<0.01
Fat (%)	3.3^{a}	3.7^{b}	0.02	4.6^{c}	4.6^{c}	0.02	0.02	<0.01	0.04
Protein (%)	3.2^{a}	3.1^{b}	0.01	3.6^{c}	3.5^{d}	0.01	0.01	<0.01	0.84

$^{a-d}$ Values within rows that are significantly different ($p < 0.05$) have different superscripts.
#HS- at THI <68 and HS+ at THI >68.

Smith *et al.* (2013) also linked the reduction in milk yield to a reduction in feed intake, thus decreasing the proportion of energy available for milk synthesis. The difference in biotype was related to the size differences between Holstein and Jersey cows, which see Jersey cows having a

lower body weight to surface ratio allowing more efficient evaporative cooling from increased skin surface area per unit of bodyweight, compared to Holstein animals.

One study modelling the economic impact of production loss from dairy cattle due to the influence of heat stress compared the losses in grazed versus total mixed ration systems in Brazil and New Zealand (Table 2). This table was adapted from Thornton et al. (2022) and the study revealed that New Zealand farmers in arid or semi-arid areas can expect to see a 1.5% decrease in milk production from the baseline production reported in 2005. For New Zealand, the reduction in milk production appears negligible when compared to a country like Brazil, which stands to lose anywhere between 6.8% to 57.7% of its milk production because of heat stress (Thornton et al., 2022).

Table 2. Comparison of milk production loss from 2005 baseline production in different regions climatically of Brazil and New Zealand

	Grazed/Pasture Based			**Mixed/Total Mixed Ration**		
	Arid-Semiarid	***Humid-subhumid***	***Temperate***	***Arid-Semiarid***	***Humid-subhumid***	***Temperate***
BRAZIL						
Reduction (%)	7.0	17.8	9.6	57.7	11.3	6.8
Baseline (Kg/day)	0.7	3.6	9.9	0.9	6.3	16.3
NEW ZEALAND						
Reduction (%)	1.5	1.1	0.8	1.3	1.1	0.7
Baseline (Kg/day)	8.6	17.7	23.6	9.1	17.9	22.0

This modelling confirms that there are at times sizeable differences in the potential losses from different countries and climates across the globe, as well as different effects when using grazed pasture production systems, compared to total mixed ration approaches. The reduction in milk yield demonstrates the potential threat to dairy businesses (and the wider economies of many countries) globally, which for New Zealand could amount to $330-million loss from the dairy industry based on 2022 export value of $22 billion (Statista, 2023). This study likely underestimates the losses from a New Zealand system, due to its methods for collecting data. The country was split into regions based on their climate type (arid, temperate, etc.) and production (with changes averaged from 2001-2010) was assumed to be evenly distributed across the country. Our dairy system is very region-specific and has changed since the early 2000's, so the effect of heat stress is likely more than reported. Therefore, whilst providing a perspective into New Zealand dairy farming's quantifiable losses because of heat stress, this study should be viewed conservatively.

Fertility is a vital production trait as it allows cows to become pregnant, without which they cannot produce milk. Heat stress has been shown to have a negative effect on cow fertility caused by the reduction in feed intake. De Rensis & Scaramuzzi (2003) explained the changes in Luteinising Hormone (LH) levels in heat stress areas, suggesting that the stress contributed to a 20-30% decrease

in fertility when comparing summer and winter conception rates, and therefore decreased follicular growth and development (De Rensis & Scaramuzzi, 2003). Their review also explained how heat stress conditions could prolong periods of negative energy balance, by affecting plasma concentrations of insulin and glucose, and thus reproduction and post-partum anoestrus, with the suggestion that if exposure to heat stress conditions was prolonged it could also alter the hormonal profile of the lactating animal. The ability for lactating animals to efficiently loose and gain weight throughout the dairy season is a pivotal aspect of pastoral farming, and if a cow's ability to lose and gain weight is potentially affected by heat stress, then this is a key area of production which will need to be better quantified in future study.

This following section of the literature highlights the economic and welfare issues that have led to the investigation of potential solutions to heat stress, like the introduction of heat tolerant genetics to New Zealand. The next stage in the process of making this a commercially available product is to fully understand how this SLICK allele of *PRLR* will operate in a New Zealand climate.

2.4 Cold stress

Cattle thermoregulatory behaviours have been described above, and it has been revealed that responses to cold stress involve changes in feed intake, production, and behaviour. In this section these changes will be further explored and quantified.

2.4.1 Feed intake during cold stress challenge

The physiological mechanism of heat production in cattle primarily comes from metabolism, which means that under conditions of cold stress their feed intake increases to meet demand. A study of 49 Hereford steers in a feedlot system kept either indoors or outdoors, observed an 18% higher estimated fasting heat production for outdoor steers ($p < 0.05$), and a corresponding 14% decrease ($p < 0.01$) in the efficiency of metabolizable energy for maintenance for outdoor steers, when compared to indoor steers. This resulted in an increase of 41% in maintenance requirements for outdoor steers compared with the maintenance requirements for indoor steers (Delfino & Mathison, *1991*). Carcass evaluations to quantify the net energy gain (NE_g) through protein deposition in this study, revealed that these increased maintenance costs did not correlate to carcass gains. The NE_g was 1.29 Mcal/kg of feed for indoor kept steers, compared to 0.76 Mcal/kg feed for outdoor kept steers. This led to the conclusions that the reduced energy efficiency in the cold weather, was due to the increased maintenance cost, and the greater proportion of the diet being retained as protein.

The temperature of feed can also influence the amount of metabolic heat cattle produce, which will not only decrease their feed efficiency, but also influence their lower critical temperature. Nicol & Young, (*1990*) compared heat production in cattle fed 15 kg of turnips at different temperatures and

noted increasing metabolic heat production as the temperature of the feed declined. This corresponded with an estimated increase in lower critical temperature of +13.9°C at -8°C ambient environment temperature (from a base of 38.5°C). This suggests that cattle consuming cold food in a cold climate have a higher energy requirement to meet metabolic heat requirements, and this has a direct effect on the minimum temperature the cattle can function at. Nicol & Young (1990) went on to explain that this is caused by the body being required to partition more energy to heat production, due to exposure to cold climates, reinforcing the findings of Delfino & Mathison (1991); as well as needing to warm the cooling rumen because of ingesting cold food, thus reducing body heat and stimulating heat producing pathways.

These two pieces of literature demonstrate the relationship between cold weather and feed intake, due to the need to produce metabolic heat to maintain homeostasis. Nicol & Young (1990) suggested that factors like feed temperature can magnify this effect by requiring heat to warm up cold or frozen feeds. This becomes relevant when considering cattle feeding in persistent snowy climates, where feed may become frozen, and therefore demonstrating how external factors also have an influence on cattle feeding behaviours in cold climates.

2.4.2 Behavioural adaptations to cold stress

A range of behavioural changes have been described in outside kept feedlot steers in cold weather (Gonyou *et al*., 1979). Changes in respiration rate increased with feeding level ($p < 0.05$) and temperature ($p < 0.01$), increased rumination time ($p < 0.01$), increased time spent lying down, and decreased grooming time ($p < 0.05$); when comparing outside feedlot steers to inside feedlot steers. Average rectal temperatures were 0.2°C higher in the outdoor steers compared with the indoor steers ($p < 0.05$), with a strong relationship to the temperature. Whilst not reporting any strong correlations, Gonyou *et al.* (1979) observed weak correlations between these observations and the season, suggesting that as the winter developed, the steers became more adapted to the cold temperatures. This would suggest that there is not only a behavioural adaptation to cold temperatures, but that the level of adaptation is dictated by seasonal variation in an animal's physiology.

Similar results were found in a New Zealand study that revealed that cattle spent more time standing when outside and were five times more likely to do so with their heads down, when compared to when they were inside a barn (Tucker *et al.,* 2006). Additionally, cattle would spend more time with their head in feeding troughs, often without increasing intake if the trough had a cover that protected their head from wind and rain. Like Gonyou *et al.* (1979) researchers observed a decrease in time spent lying during the trial. However, the conclusions were not the same. Tucker *et al.* (2006) cited literature that demonstrated cattle would increase lying-down time if the surface they are lying

on is not wet or uncomfortable, with the former increasing the thermal conductivity of the surface and thus drawing more heat away from the body. This was reinforced by observed increases in cortisol and reduced growth hormone levels, which indicate that the cattle felt deprived of comfortable areas to lie down. This reduced their lying time to 4 out of every 24 hours, which was below the sited average of 12 to 13 hours per day, which is considered normal for dairy cattle. This is an example of the many factors that influence the cattle behaviour undertaken to avoid the cold.

Interestingly the same preferential blood flow mechanism responsible for increasing heat dissipation from the periphery organs of an animal's body, can also be responsible for warming up peripheral organs such as eyes, ears, and other hairless structures (Brody, 1956). Vascular feedback or multi-channel counter-current heat exchangers use preferential blood flow to warm up parts of an animal's body at most risk of cold, with this allowing the survival of tissues at extreme temperatures. This increased blood flow also allows for less blood to be pumped to other areas of skin by prioritising exposed skin, thus decreasing the total heat loss from an animal's body. Vasoconstriction and piloerection also increase the natural insulative effect around an animal's body. These mechanisms work in tandem to pull blood away from the upper surface of skin, reducing the ability of the environment to exchange the heat from the skin to the air, and through the contraction of arrector pili muscles, which can double the depth of the coat. This allows air to be trapped between the hair and skin, thus creating a warm layer around the animal (Shephard & Maloney, 2023), and reducing the total heat loss from radiation. Variables such as coat length, ambient temperature, and precipitation will all change the efficacy of these mechanisms reducing the total amount of insulative effect, the ability of hair to move, and the degree at which air is warmed around the animal. These variables are known, but largely not quantified to the degree of influence they have on the insulative ability of the individual.

Finally, shivering is an involuntary animal response to cold stress. Shivering occurs when muscles contract and expand simultaneously, without the purpose of creating directional movement (Shephard & Maloney, 2023). It is usually a last resort response from muscles responding to the decline in metabolic heat and subsequent drop in body temperature. Due to its intensive energy demand, prolonged periods of shivering are not possible. Young animals are especially at risk due to their high surface area to body ratio, and low insulative status with low levels of subcutaneous fat and short coats. Newborn calves do not ruminate and cannot use a shivering response and so utilise specialised fat reserves known as brown adipose tissue which breaks down ATP into heat (Shephard & Maloney, 2023). The shivering response in cattle again highlights the various factors that determine how and when an animal may start to shiver; based on age, coat status, and fat reserves.

SLICK calves are characterised by their light coats, and high surface area available to radiate heat. This could predispose them to lose more heat during winter months. Conversely, being a tropical animal, they can employ several types of heat exchange to increase heat conservation. This highlights the need to understand what variables are interacting together in a SLICK animal's response to cold challenges, and whether these can be used to predict the expected changes in production over this time.

2.4.3 Milk Production in response to cold stress

Cold stress has been demonstrated to decrease milk yield in cattle by 1 to 2 kg per day , with a strong correlation with wind chill measurements in housed cattle in Poland (Angrecka & Herbut, 2015). The link between air temperature and wind speed/air velocity allowed researchers to quantify the average daily wind chill temperature between trial groups of high (1), medium (2), and low (3) producing cows. By measuring cattle production against the daily average wind chill temperature, they successfully determined the strong negative correlation between wind chill temperature and milk production (Figure 4). The wind chill temperatures are quantified for each barn zone, and are represented by WCT1, WCT2 and WCT3. The milk yield for each group is represented by the bars on the graph, M1, M2 and M3. Milk yields for group 1 (M1 + WCT1) stayed relatively high, which was expected due to their average wind chill temperature being higher. However, this group observed a relationship between milk yield and WTC (r= 0.72). Group 2 (M2 & WCT2) saw the strongest correlation (r = 0.89), and group 3 (M3 & WCT3) saw a correlation of 0.62. It was concluded that the average milk yield of a group, no matter their base production status, was significantly affected by the average wind chill temperature, with strong correlations between the intensity of the wind chill and the milk production for barn-housed cattle.

Material removed due to copyright compliance

Figure 4. Milk yield affected by daily average wind chill across high, medium, and low production cattle. WCT1 is high, WTC2 is medium, and WTC3 is low wind chill temperature.

2.5 Adaption to extremes

2.5.1 Hot climates

Cattle have adapted to hot climates, with Zebu, Senepol, and Brahman cattle being three of the more highly studied breeds because of their adaptation for greater heat tolerance. All three breeds can be considered hybrids of *Bos taurus* and *Bos indicus* cattle, but that have traits developed through years of selection from both cattle species. It is the *Bos indicus* traits that give superior heat-tolerance ability to these breeds. Most phenotypes that have been characterised are related to the skin and hair providing better cooling, colour, and density, which provides the ability to better accommodate heat, but with physiological differences also suspected, but not well understood.

As an example, in one study a group of Brahman cattle had an average 13.91 µm thinner epidermis than Angus cattle ($p < 0.05$), and the epidermis thickness increased with an increasing proportion of Angus genetics in the breed crosses (Mateescu *et al.,* 2023). This thinner epidermis gives purebred and high percentage Brahman cattle an advantage over purebred and high percent Angus cattle relative to heat dissipation ability. Coupled with this, the authors described wavier epidermis layers and larger sweat glands in the Brahman cattle compared to the Angus cattle. The wavier epidermis provided a larger surface area for heat dissipation, and the sweat glands provided better sweating ability, both vital for cooling of the skin. Figure 5 from Mateescu *et al.* (2023) reveals differences in the melanin content of the skin, with Brahman skin samples (B) having a larger amount of melanin pigment in the basal layers of the epidermis, compared to the Angus skin sample (A). This increased melanin was suggested to allow for chemical interactions with vitamin D3 decreasing UV light's ability to penetrate the skin, increasing UV light scattering and reflection and thus reducing heat load from the light. This may also induce thermoregulation responses in skin cells.

Material removed due to copyright compliance

Figure 5. Vertical skin section of Angus (A) and Brahman (B) heifers. Dark blue/black spots show melanin pigments in the basal layer of the epidermis (From Mateescu et al. (2023); Figure 5).

Additionally, crossbreeding using Senepol cattle has provided Holstein, Charolais and Angus cattle with a specific SLICK allele at the *SLICK* locus (now known to be *PRLR*) on bovine chromosome 20 (BTA20; Maxy Mariasegaram et al., 2007). This genetic variation presents itself in the prolactin receptor by causing a truncation of the C-terminal region involved in the JAK2/STAT5 activation that occurs during prolactin signalling (Sosa *et al.,* 2021). Cattle carrying the SLICK genotype have noticeably short coats with a distinct sleek look, fewer hair follicles and larger sweat glands, compared to those without the SLICK genotype (Huson *et al.,* 2014).

The influence of the SLICK allele becomes more apparent as it becomes better understood and giving it more relevance as a trait to be selected for to give cattle an adaptive advantage as the climate warms. This allele will be economically relevant to farmers who experience more consistent and frequent periods of heat stress, especially as the genotype of current cattle become more refined with the frequency of high productive traits being increased in SLICK populations. However, special consideration is needed as the phenotypes which provide the adaptive advantage to heat stress, may be the phenotypes that put cattle at risk of cold stress in temperate climates. Understanding the consequences relative to production, welfare, and the survival of individuals carrying the SLICK allele are vital next steps in determining the utility of this genetic variation in a temperate dairy climate, such as New Zealand.

2.5.2 Cold climates

Physiological differences in cattle will help different breeds tolerate colder climates better than other breeds. To better understand the mechanisms for cold stress, phenotypes that allow certain breeds to tolerate cold climates will be investigated and explained below.

Cattle hair characteristics

Cattle hair characteristics differ for breeds exposed to the cold. Peters & Slen (1964) demonstrated that a domestic cattle/bison hybrid displayed higher coat fibre density with samples taken from the mid-rib region, with an average of 13,714 fibres per square inch, compared to 4,533 fibre per square inch in Hereford cows. The fibres of the domestic/bison hybrid were much finer, and they had much longer guard hairs compared to the Hereford population. This suggests that the density and length of the fibres give Bison much better insulation in cold stress conditions, and that this will lower their lower critical temperature as their body heat dissipates more slowly. Webster (1973) described the insulation effect by revealing that as cattle coat depth increases, so too does the amount of external insulation it provides for the animal (see Figure 6). This figure shows a relatively linear relationship between the two parameters, and Webster described similarities between this work and that conducted in sheep.

Material removed due to copyright compliance

Figure 6: Relationship between coat depth and external insulation (From Webster (1973); Figure 10.2).

In the southeastern United States, Hayman, and Nay (1960) evaluated the changes in cattle summer and winter coats for *Bos taurus, Bos indicus* and crosses of the two species. They observed seasonal trends for shorter and lighter coats in the summer and heavier winter coats, but also noted the variation in different breeds. *Bos indicus* consistently had shorter and lighter coats, as well as having greater hair diameter in the summer compared to the winter. Additionally, their winter coat appeared to have a double layer of shorter hairs mixed with long hairs, whereas *Bos taurus* cattle grew a more uniform coat. Cross-bred cattle also appeared to resemble their *Bos indicus* origin in the summer and their *Bos taurus* origin in the winter. This suggests that there is an environmental factor dictating the expression of coat genes, and which can vary seasonally.

Gaps in our knowledge exist around the influence of cattle with summer type coats, and from having novel heat tolerant alleles such as SLICK, when exposed to cold temperatures. Whilst Hayman and Nay (1960) suggested that cross-bred cattle have the potential to express hybrid coats to allow for tolerance of cold stress, how this is affected by the presence of the SLICK allele is unknown.

Cattle bodyweight to surface area ratio

When understanding how an animal loses heat, it is no coincidence that the size of an animal effects the amount of heat it loses and therefore its vulnerability to cold stress. Berman (2003) analysed predictive equations for heat loss in terms of surface area and body weight in cattle. He tried to justify which equation is a better predictor for production loss. However, an animal grows so too

does its surface area and therefore also the amount of loss that can be observed. Under 100 kg the heat loss is similar, but as animal's body weight increase so too does the thermal loss from the skin and production losses (Berman, 2003). This shows the relationship between body weight and surface area, that the larger and heavier animal loses more heat compared to the smaller and lighter animals and therefore is more prone to cold stress.

This idea is reinforced by a study conducted to investigate the effect of shelter and body condition on the behaviour of cattle during the winter. This research revealed that cattle spent more time lying down when cold, and that animals that had lower body condition (and therefore less thermal insulation) spent more time lying down and had decreased intake rates (Tucker et al, 2006). The rationale behind these findings were that when cattle were cold, they changed their behaviour to reduce the surface area exposed to wind and rain to attempt to reduce their heat loss. Interestingly, this research also revealed that the influence of surface area may be in part countered by higher body condition scores and the effect of body fat as insulation.

The influence of bodyweight to surface area ratio will become more of an issue post-weaning for young stock as they begin to graze and change through maturity. Smaller and faster maturing animals will be quicker to build up insulation (in the form of fat) therefore reducing their exposure to high surface area to body weight ratios influencing their heat loss, whereas larger and slower maturing animals may be more disposed to loosing heat as younger animals. This can also be coupled with the influence of hair characteristics, with animals genetically disposed to lighter coats for heat loss being further disposed to heat loss.

Bodyweight to surface area ratio is a key adaptive advantage for both heat stress and cold-tolerance with larger biotypes having the advantage in cold climates, and smaller biotypes having the advantage in hot climates. However due to thermal-tolerance being a combination of compounding factors rather than one single trait, knowledge of how the SLICK allele cattle will adapt to cold climates is largely unknown. Their advantages in the summer dispose them to cold stress, but factors such as growth, increased ability to eat, behaviours to seek shelter, or grow a winter type coat are not known, leading to the need to investigate these specific adaptations for the SLICK genotype.

2.6 Conclusion

Thermo-tolerance and maintaining thermo-regulation in cattle is complex. It includes many factors that interact with each other for the animal to respond to thermal challenges. Much of the evidence discussed in this literature review highlights the different mechanisms, such as sweating, decreased appetite, and genetic adaptations which dispose breeds such as Brahman, Zebu, and Senepol to better adapt to hot climates; as well as the implications of these adaptations in terms of behaviour,

and production. Conversly coat characteristics, and physiological mechanisms of shivering and piloerection influence the genetic and behavioural adaptation of breeds like Bison and Yak to colder climates. The tension between adaptation to hot versus cold climates has highlighted the overarching issue about the introduction of the SLICK allele into the temperate New Zealand dairy system, which suggests superior heat-tolerance ability, but maybe at the cost of cold-tolerance. Better understanding of the various SLICK phenotypes is required so that their specific response to cold challenges can be quantified in a temperate climate dairy system.

Chapter 3

Materials and Methods

3.1 Experimental site and design

This trial was conducted with the approval of the Lincoln University Animal Ethics Committee (Approval AEC2023-17) over a four-week (29-day) period from 13th June to 11th July at the Lincoln University Research Dairy Farm (LURDF) (Figure 7). The experiment was a factorial design with three treatments and 7-10 animal replicates per treatment.

1. Control heifers: Heifers not mated to SLICK bulls, and do not carry the SLICK allele. Used as a negative control. (n = 10).

2. SLICK heifers: Heifers identified to be carrying at least one copy of the SLICK allele by DNA typing. (n = 10).

3. Non-SLICK heifers: Heifers mates to SLICK carrier bulls, which have been genetically identified by DNA typing to not carry the SLICK allele. Used as a positive control. (n = 10).

Figure 7. Map of trial site location. The yellow box indicates the paddock the heifers grazed, and the pink box indicates the location of the temporary yard (Source Google Earth).

3.2 Heifer management

Management of the heifers, and their day-to-day welfare and feeding, was undertaken by farm staff. The heifers were allocated a 15 m x 75 m (28.12m^2/heifer/day) block of mixed ryegrass/clover pasture each day over the 29-day period with a break-line and back-wire being moved across a larger paddock. They were also allocated 2.5 kg/heifer/day supplementary feed (silage) each day. Heifers were moved daily, early in the morning, or after the frost broke and were retained to their allocated block using temporary fencing, with silage fed out up approximately 3 days in advance. All the heifers had access to fresh water.

On weighing days, the heifers were walked from their allocated block to the LURDF milking shed where permanent scales are located but temporary yards were established in a paddock adjacent to the feed b lock, to minimise the movement required to take rectal temperature readings (Figure 8).

Figure 8. The temporary yards set up adjacent to the heifer feeding block.

3.3 Climate and weather measurements

Weather conditions for the duration of the trial (13th June to 11th July 2023) and historically for the same period (defined as the last 10 years, 2012-2022) were collected from the NIWA CliFlo database, station ID 17603.

These data, including average daily temperature and average daily relative humidity measurements, were used to calculate a temperature-humidity index (THI) as follows:

$$THI = 0.8T + (RH * (T - 14.4)) + 46.4$$

With this THI calculation, T is temperature and RH is relative humidity (from Bryant *et al.,* 2007).

Average, minimum (T_{min}), and maximum (T_{max}) temperature, total rainfall and average relative humidity was also retrieved from CliFlo database station (17603) for the 16th, 19th, 28th and 30th of June, and the 3rd, 4th, and 6th of July (coinciding with the days when the rectal temperatures were collected). This data was retrieved for the hours of midnight to 8.00 a.m. and THU values calculated for these days using the above equation.

3.4 Feed measurments

3.4.1 Pasture

Each week pasture mass was ascertained using a rising plate meter, with pasture samples for dry matter determination being harvested to ground using electrical shears, from 0.2m^2 quadrats for the seven days in front of where the heifers would be grazing next. This same method was used to ascertain the post-grazing pasture mass for the seven days of grazing prior (behind the back wire). Six quadrats were collected for pre-grazing mass, and five for post-grazing mass (approximately one quadrat per daily allocated area), for a total of 11 quadrats each week. The collected quadrat pasture samples were washed then oven-dried at 60°C for 48 hours, before their dry weights were recorded.

Botanical samples were also harvested by collecting handful sized samples, cut to ground level every 10-paces along the pasture walk transect. These samples were mixed and separated into 50 g sub-samples. After oven-drying at 60°C for 48 hours the dry weights were recorded, and each section of pasture composition (ryegrass, clover, dead material, and lotus) found in botanical analysis were expressed as a percentage of dry weight.

3.4.2 Supplement

Along with pasture, the heifers were fed silage over the duration of the trial. The dry matter percentage for the silage fed out was collected by weighing all the material applied in 1 m^2 quadrats and taking 100 g samples from each quadrat, for a total of 6 quadrats per week. The measurements were only collected on two out of the four sampling days due to the silage not having been fed out at the time sampling was taken. Samples were weighed into approximately 50 g fresh weight samples, oven dried at 60°C for 48 hours, and the dry weights recorded. Post-grazing silage utilisation was visually assessed, and the silage was deemed to be consumed at a 95% utilisation rate.

3.4.3 Intake

Apparent intake was calculated using a calibration method. Plate meter data (clicks) collected for each sample, along with the dry weights, allowed analysis of each quadrat to determine pre- and post-grazing pasture mass. Sample weights expressed in grams of DM were multiplied by 50 to convert pasture mass into kg DM/ha (based on using a 0.2 m^2 quadrat size). The average post-grazing

mass can be subtracted from the average pre-grazing mass then divided by the area per heifer (0.002812 ha/heifer/day) to give the apparent dry matter intake per heifer per day.

Apparent silage intake was calculated by multiplying the 2.5 kg of silage offered per heifer per day by the total average dry matter percentage values from supplement sampling. Based on the assumption of 95% utilisation, this value then gave the apparent silage intake per heifer per day. Total apparent intake is a sum of apparent pasture intake and apparent silage intake.

3.5 Animal measurements

3.5.1 Growth

Live weight was measured on four occasions using Tru-test (Datamars) scales. The dates upon which liveweight were recorded were the 19th of May (used as a baseline/initial weight), 14th of June, 27th of June and 11th of July. Heifer growth rate was calculated by subtracting the heifer weight from their previous weighing and dividing this by the time interval (days) between weighing.

3.5.2 Behaviour

Cow Manager sensor ear tags were used to monitor activity in the three groups of heifers: Control (n = 5), SLICK (n = 6), and Non-SLICK (n = 3). These tags were attached to existing NAIT ear tags (Figure 9) on the 14th of June. The numbers of animals in each group were based on previous allocation of the sensors and at the time this author was not aware that they had permission to use more tags on animals to balance the trial. This error was not noticed until the post-collection analysis, and the Non-SLICK heifer data was excluded from analysis because of low sample size. Activity data was collated for a 14-day period between the 22nd of June and the 5th of July (inclusive). The tags categorise movement into five non-overlapping activity categories: eating, ruminating, not-active, active, and high-active (Cow Manager, 2023).

Figure 9. Heifer with Cow Manager tag installed on NAIT tag (left ear from observation of reader, see yellow circle).

3.5.3 Cold stress assessment

Cold stress potential was calculated using a wind chill temperature (WCT) equation sourced from Webster *et al* 2008, which originally comes from Environment Canada (the Canadian Government weather and environment agency), and was as follows:

$$W = 13.12 + 0.6215 * T - 11.37 * V^{0.16} + 0.3965 * T * MAX(1, V)^{0.16}$$

Where W is Wind Chill, T is the air temperature measured in degrees Celsius and V is the wind speed in kilometres per hour.

Using this equation and long-range weather forecasts, the likely daily wind chill temperature could be calculated in advance. This enabled prediction of when cold days might occur and thus enabled the collection of rectal temperature measurements under a cold challenge. Specifically, the output value from this equation was compared to the critical temperatures for cattle described in Table 3 (highlighted yellow for wet or winter dry), and if the temperature was below, or within 2 degrees of the critical temperature rectal temperatures were collected from the heifers in the morning (between 8-9 am) of that day.

Table 3. Lower critical temperatures for cattle in different seasons (Adapted from SDSU, 2020).

Coat	Critical Temperature (°C)
Summer or Wet	15.5
Autumn - Dry	7.2
Winter- Dry	0
Heavy Winter - Dry	-7.2

Rectal Temperatures were collected from all 27 heifers, on days that were determined to be cold using the above criteria. The temperatures were measured using a rectal thermometer (Liberty Health Products Digital Thermometer), held in the rectum of the heifer until the thermometer reached a stable reading indicated by a beeping sound.

Figure 10: Rectal temperature being collected from heifer on cold stress morning in the temporary yards.

In addition to rectal temperatures, a HIKMICRO handheld thermography E1L camera was used to take thermal images of the heifers on the 4th and 6th of July to determine variation in body temperature. These images were taken when the heifers were restrained in the crush and included images of their back (4th of July), rump and eye (6th of July). The camera was set up with an emissivity value of 0.97 and measurement range of -20°C to ~150°C. They were used at a one metre distance. The images taken were analysed using the HIKMICRO Analyser software, where target regions were chosen to derive maximum, minimum, and average temperatures readings (in degrees Celsius).

3.5.4 Hair sample collection

Hair samples were collected from all SLICK and Non-SLICK heifers at the commencement of the trial. Small electronic clippers (Codos, WithPet CP-6800) were used to collect a sample by shaving 5 cm x 5 cm patch from the right side over the 13th rib of the heifers. The blade used was a number zero blade allowing fibre to be cut as close to the skin surface as possible. Each cut was undertaken in a single stroke with fibre from any part stroke discarded.

The hair samples were tested using a laser-based fibre analysis platform (Fibrescan) provided by Pastoral Measurements Limited (Timaru, New Zealand), and individual fibres measured to ascertain mean fibre diameter (MFD), fibre curvature, staple length, and the standard deviation of these measurements.

3.6 Statistical analysis

The collected data was analysed using GenStat Version 22 (64-bit). Animal groups (Control, SLICK, Non-SLICK) are defined as experimental units. Data collected from the animals such as rectal temperature, growth, and behaviour were used as repeated measures, and day was used as random effect. Hair samples, pasture, silage, and pasture composition were all considered fixed terms, with day or week considered random. Repeated measures ANOVA tests were conducted on rectal temperature, growth, and behaviour data. One-way ANOVAs were used on hair sample data, with Fishers unprotected least significant difference tests used to compare the ANOVA predictions. A significant difference was accepted at $p < 0.05$.

Initial liveweight and growth BVs were considered as co-variates for growth, and rectal temperature measurements. Upon analysis, measured co-variates were deemed insignificant ($p > 0.05$) and therefore removed from the analysis.

Chapter 4

Results

4.1 Climate

Temperature humidity index (THI) value and rainfall data summarised for the 29 days the heifer trial was conducted in 2023 (13th June to 11th July) are presented in Figure 11. THI ranged between 41.6 and 56.92 and was an average of 31% below 70, the THI value at which heat stress is expected to occur. The average 2023 THI value was 5% higher than the 10-year average.

Cumulative rainfall over the period was 64.40 mm and was 13% more than the 10-year average of 55.55 mm for this period. The rainfall frequency appears to be less than the 10-year average, with 15 wet days compared to 29 wet days.

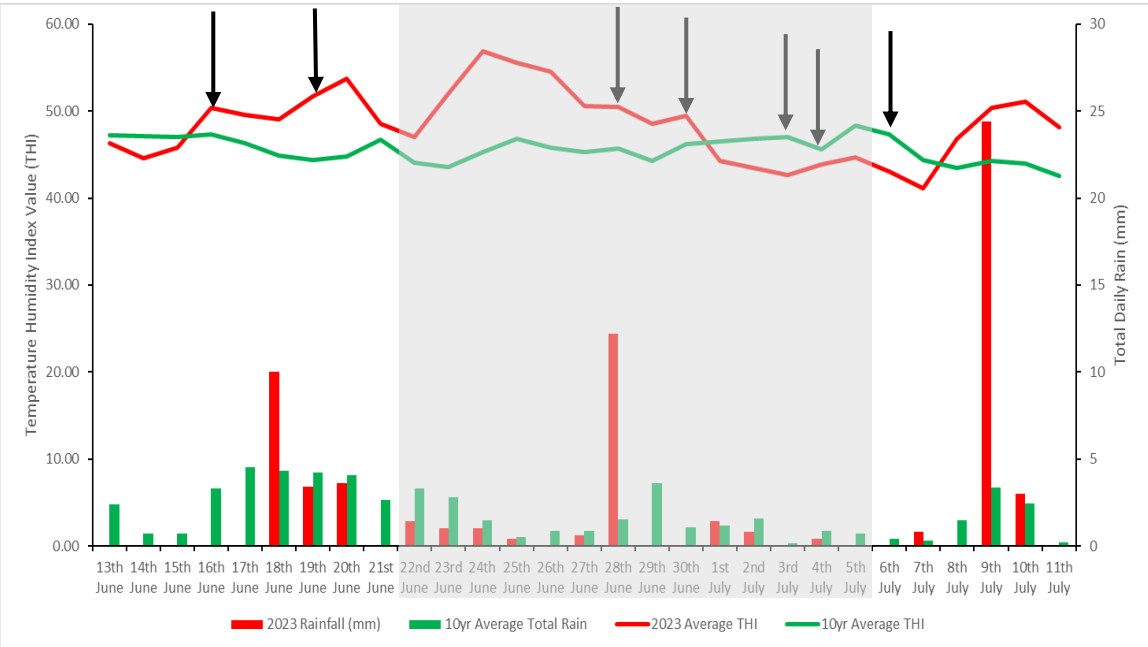

Figure 11. Year 2023 and 10-year average (2012-2022) temperature humidity index and rainfall during the heifer trial period 13th June to 11th July. The lines (left axis) indicate the daily temperature humidity index (THI) value for 2023(red) and the 10-year daily average (green) as bar graphs. The right axis indicated total daily rainfall (mm) for 2023 (red) and the 10-year daily average (green) as line graphs. The black arrows indicate the days the rectal temperature measurements were taken. The grey area represents the period of sensor behaviour measurement long and short-term data retrieved from the National Climate Database Station 17603.

Table 4 lists the T_{max} and T_{min} (°C), average temperature, total rainfall, average windspeed, (THI) values, and wind chill temperature for the mornings when rectal temperature data were collected from 0000hrs to 0800hrs each day. Three of the seven measurement days occurred when the

minimum temperature was less than or equal to zero, with only one of these three days (30th June) having a wind chill temperature less than or equal to zero.

Table 4. T_{max} and T_{min} (°C), average temperature (°), total rainfall (mm), average windspeed (km/hr), average relative humidity (%), THI values, and wind chill temperature (°C; for the days, that rectal temperatures were recorded) between the hours of midnight and 8 am.

	16th June (RT1)	19th June (RT2)	28th June (RT3)	30th June (RT4)	3rd July (RT5)	4th July (RT6)	6th July (RT7)
T_{max} (°C)	8.1	4.2	9.2	2.9	6.4	7.7	8.1
T_{min} (°C)	-0.4	0	6.5	-0.5	1.5	1.3	0.9
Average temperature (°C)	4.3	2.1	7.7	0.7	3.8	3.9	6.0
Total rainfall (mm)[1]	0	10	0.6	0	0.8	0	0
Average windspeed (km/hr)	11.08	5.14	15.93	6.9	9.8	7.8	9.5
Average relative humidity (%)	95.5	96.3	96.3	96.6	93.8	92.5	89.4
Temperature humidity index (THI)	40.17	36.38	46.11	33.47	39.56	40.11	43.87
Wind chill temperature (°C)	1.6	0.7	5.0	-1.5	1.3	2.0	4.0

[1]Total rainfall for the 24hr period prior to the day of measurement.

4.2 Pasture analysis

Average pre graze pasture mass was 2316 kgDM/ha with 86% utilisation (Table 5). Pasture mass was lower during the second half of the observation period. As there was no change in allocation area this resulted in a 22% decrease in apparent intake. The total average apparent intake (pasture + silage) is 6.6 kgDM/heifer/day over this period. Silage utilisation was high at 95% and intake of silage accounted for 15% of the total intake.

Table 5. Apparent intake from pasture analysis data for cold stress trial period 13th of June to 11th of July 2023.

	Mean (± standard error)	14th – 27th June (± standard error)	28th June – 11th July (± standard error)
Pre-graze pasture mass (KgDM/ha)	2316 ± 118.2	2546.7 ± 194.8	2076 ± 69.86
Post-graze pasture mass (KgDM/ha)	313 ± 43.0	285.2 ± 61.61	368 ± 63.03
Allocated area (m^2/heifer/day)	28.12	28.12	28.12
Apparent intake (KgDM/heifer/day)	5.6	6.4	4.8
Silage offered (KgDM/heifer)	1.075 ± 0.039	1.075 ± 0.039	1.075 ± 0.039
Utilisation (%)	95	95	95
Apparent silage intake (KgDM/heifer)	1.02	1.02	1.02
Total apparent intake (KgDM/heifer)	6.62	7.42	5.82

4.3 Liveweight and growth rate

Liveweight increased over time ($p < 0.001$), but there was no interaction between heifer group and time ($p = 0.119$) or between heifer treatments ($p = 0.624$) (Figure 12). The total average liveweight at

the 19th of May was 216 ± 5.5 kg, 13th of June 228 ± 5.7 kg, 27th of June 235 ± 5.6 kg and 11th of July 239 ± 6.0 kg, with a 9% increase in liveweight for all heifers from the 19th of May to the 11th of July.

The total average liveweight gain was 0.40 kg/day (Figure 13). Growth rate period 1 is for the 25-day period between the 19th of May and the 13th of June, before the start of the trial (to give an indication of pretrial growth rate). Growth Rate period 2 and period 3 are the 14-days between the 13th of June and the 27th of June, and the 27th of June and 11th of July, respectively.

There is no difference in growth rate for periods one and two between the treatments (p = 0.571 and p = 0.383 respectively). The average growth rate was 0.53 kg/d across all treatments when pasture allocation was high. However, when pasture allocation declined after the 27th of June, growth rate dropped to 0.31 kg/day across all treatments. SLICK heifers had an 82% higher growth rate in growth rate period 3 of 0.48kg/day, compared to control heifers at 0.09 kg/day (p = 0.036).

The co-variates of 'initial liveweight' and 'growth BV' were deemed to be insignificant and removed from the analysis.

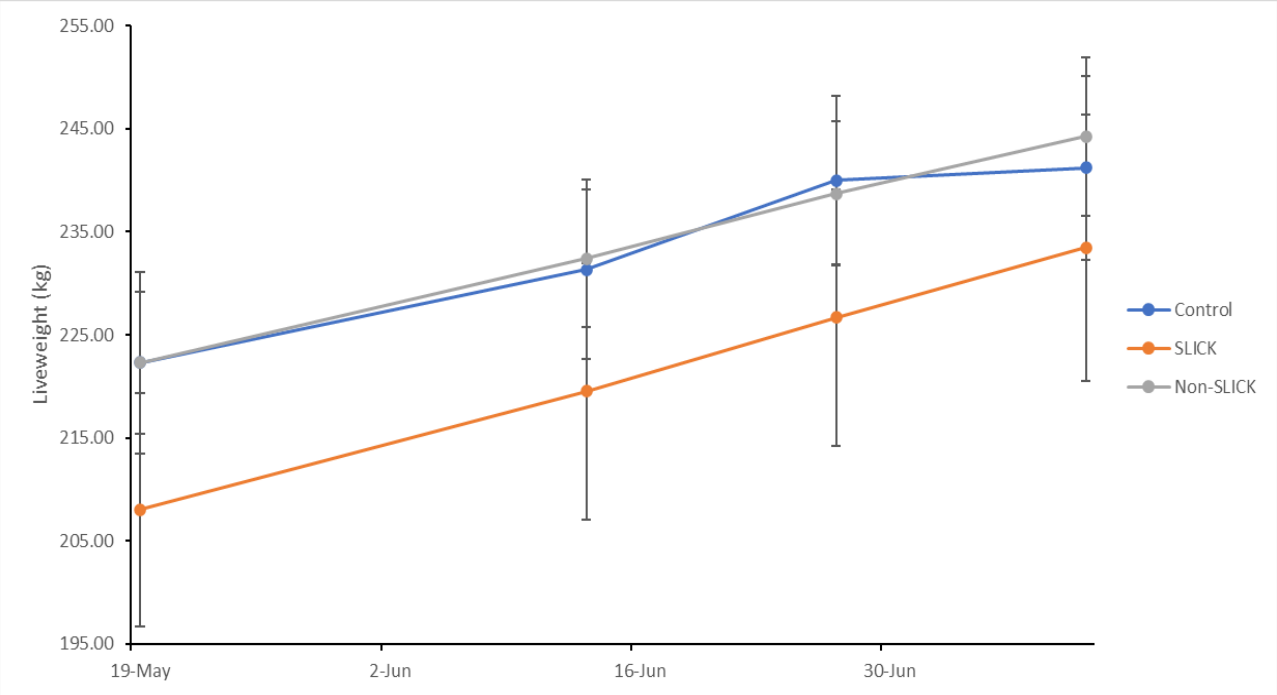

Figure 12. Heifer liveweight on the 19th of May, the 13th of June, the 27th of June, and the 11th of July 2023. Control (blue, n = 9), SLICK (orange, n = 9) and Non-SLICK (grey, n = 9 except July 11th n = 8). Error bars ± SEM.

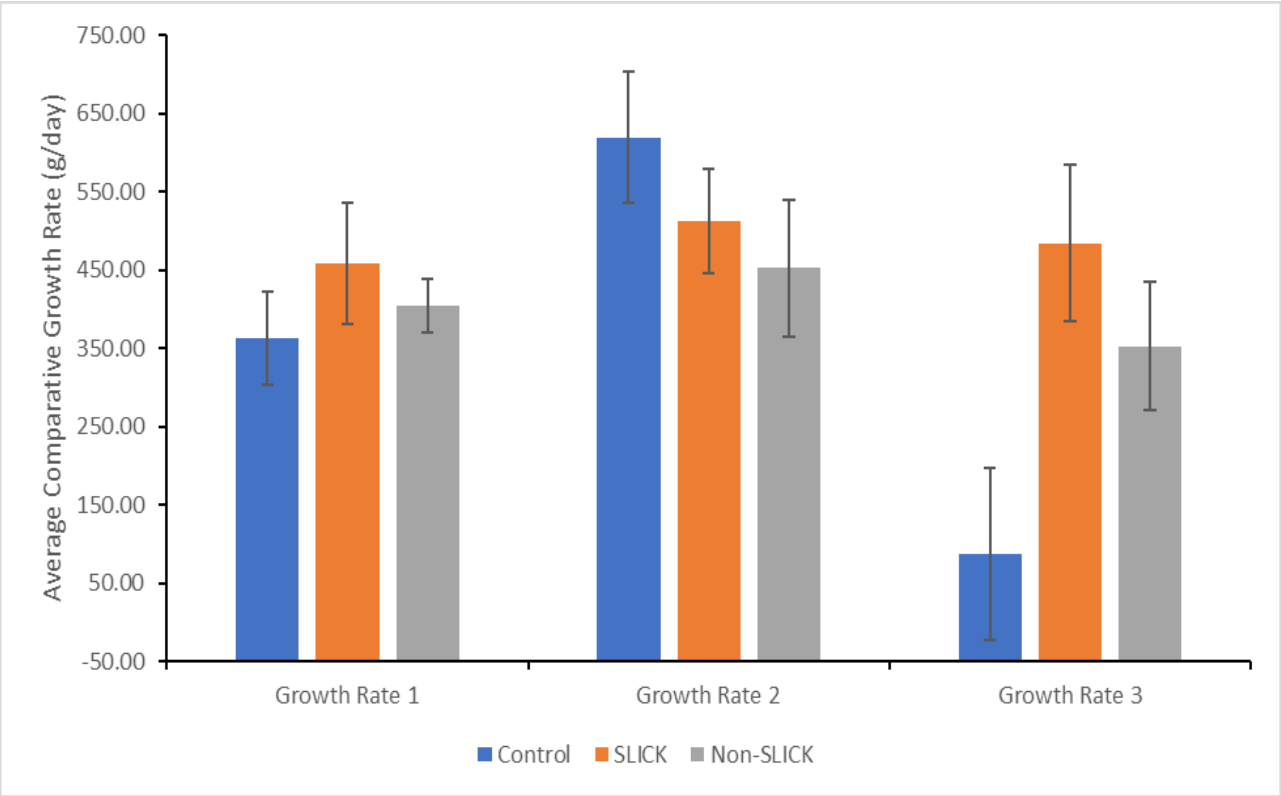

Figure 13. Comparative growth rates of Control (blue), SLICK (orange) and Non-SLICK (grey) heifers over the trial period. Growth Rate 1, 25 days (the 19th of May to the 13th of June), Growth rate 2, 14 days (the 13th of June to the 27th of June), and Growth rate 3, 14 days, (the 27th of June to the 11th of July). Error bars ± SEM

4.4 Behaviour

Daily behaviour categories were similar for all treatments except for inactive time (total average minutes per day) which was greater ($p = 0.008$) for the SLICK heifers compared with the control heifers (Table 6). There was a treatment x time interaction for eating time, which suggests that time spent eating per day changes ($p < 0.001$), and that the change over time was dependent on the heifer group ($p = 0.053$).

Animal behaviour data (Table 6) shows the average total behaviour time (total average minutes per day) shows SLICK heifers spent an average 12% less time eating compared to the control heifers ($p = 0.105$). This time appears to be substituted by an 8% increase time spent not active for SLICK heifer compared with the control heifers ($p = 0.008$).

Table 6. Total minutes per day spent in various behavioural classes over the 14-day Cow Manager observation period for Control, SLICK, and Non-SLICK heifers during the cold stress trial.

Average behaviour (minutes/day)	Control	SLICK	SEM[1]	*p*-value		
				Treatment	***Time***	***Treatment x Time***
Ruminating	253	276	11.60	0.525	< 0.001	0.314
Eating	317	278	8.31	0.105	< 0.001	0.053
Active	195	179	8.53	0.604	< 0.001	0.091
Not Active	426	466	16.46	0.008	< 0.001	0.577
High Active	248	245	19.77	0.829	0.107	0.295
TOTAL	**1439**	**1444**	-	-	-	-

[1]Standard Error of the Mean.

The time (day) x treatment interaction is displayed in Figure 14 which shows the increased eating time per day displayed by the Control treatment, occurs early in the 14 days, with both heifer groups following similar eating time patterns (minutes/hour) but to different degrees. SLICK heifers appear to spend less time eating than the control heifers from the 22nd to the 30th of June, but from then on, the differences become less variable.

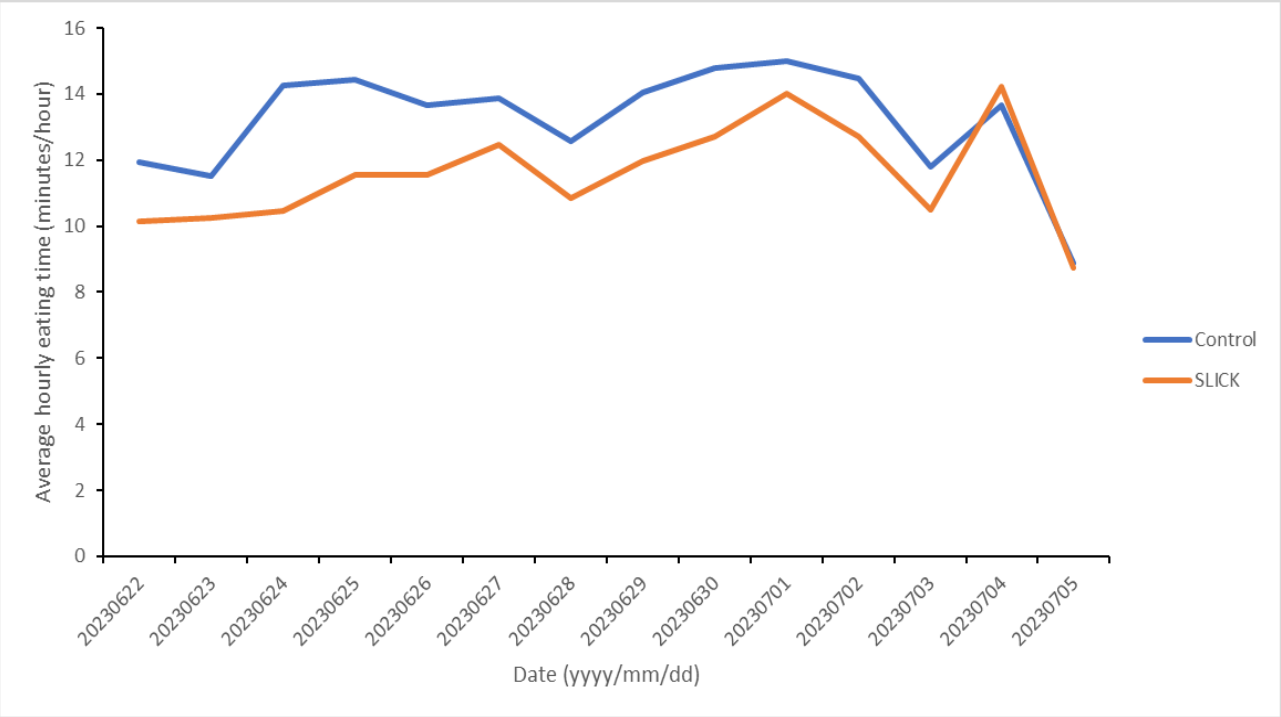

Figure 14. Average daily eating time (minutes per hour) for Control (blue), and SLICK (orange) heifers from the 22nd of June to the 5th of July.

The average diurnal pattern for ‘not active’ time reveals no difference between the heifer treatments throughout the days without cold stress (Figure 15). However, there tends to be an 18% increase (from 30 minutes to 37 minutes) in inactive time between 0700 and 0800 hours (see red circle, Figure 16), on cold days for SLICK heifers compared with the same time on non-cold days which suggests that the SLICK heifers spend more time inactive in these periods of the night (Figure 16). The difference between control and SLICK heifers at 2200 hours suggests the SLICK heifers spend an average 58% more time inactive at this time of night on cold days compared to control heifers. Whilst the overall trend shows no difference, these two examples suggest that there could be periods of inactive behaviour which differs between heifers.

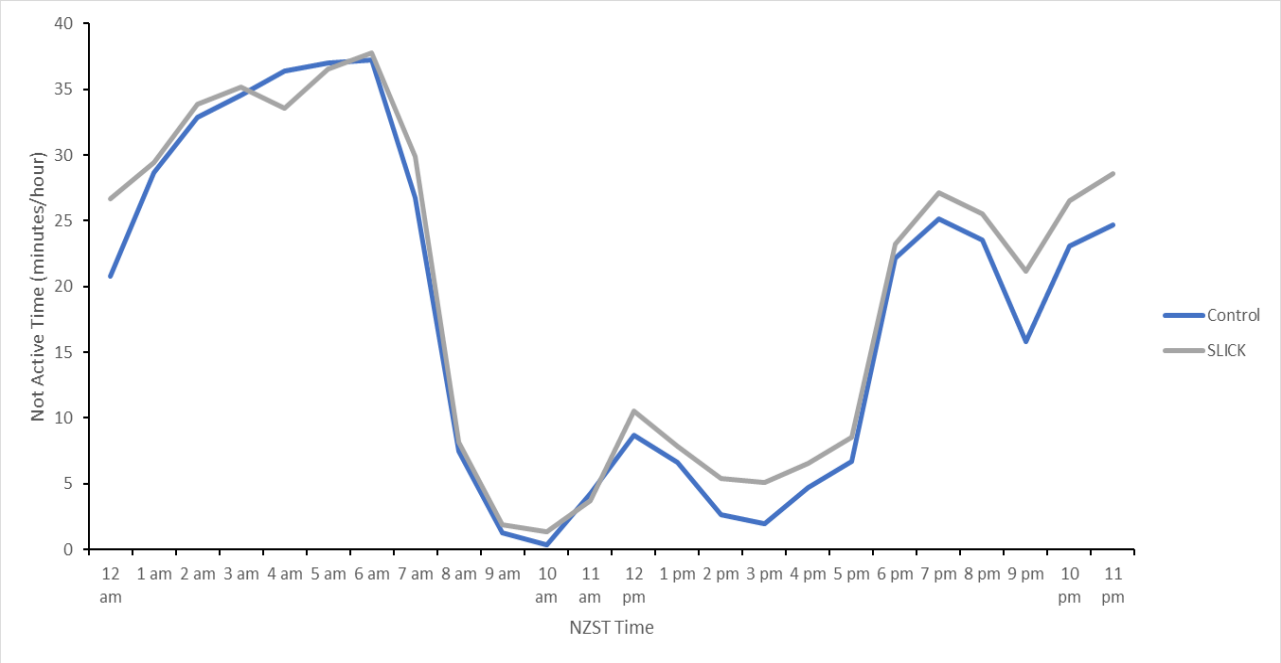

Figure 15. Daily variation in time spent not active (minutes/hour) for Control (blue) and SLICK (grey) heifers for Non-cold stress days.

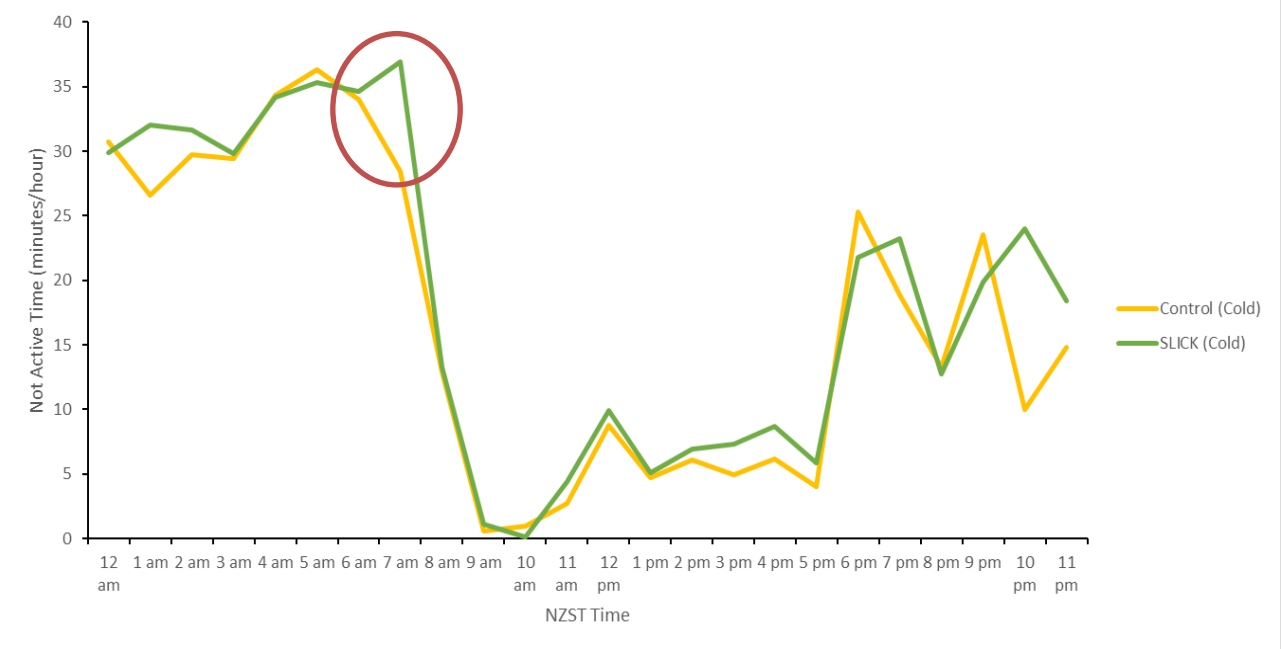

Figure 16. Daily variation in time spent not active (minutes/hour) for cold stress days for Control (yellow), and SLICK (green) heifers. The red circle highlights increased SLICK inactive time compared to control on non-cold days.

The average diurnal eating time during non-cold stress days reveals lower eating times (under 10 minutes per hour) for both heifer groups at night (between the hours 1800 and 1900 hours; Figure 17). Eating time peaks between the hours 0900 and 1000, with the control heifers tending to spend more time eating throughout the day compared to the SLICK heifers, albeit this is not significant.

The average diurnal eating time for cold stress days during this period reveals similar patterns to total diurnal patterns (Figure 18). The lack of difference between cold-stress and non-cold-stress days, suggests that the time x treatment interaction observed has no correlation to cold stress days.

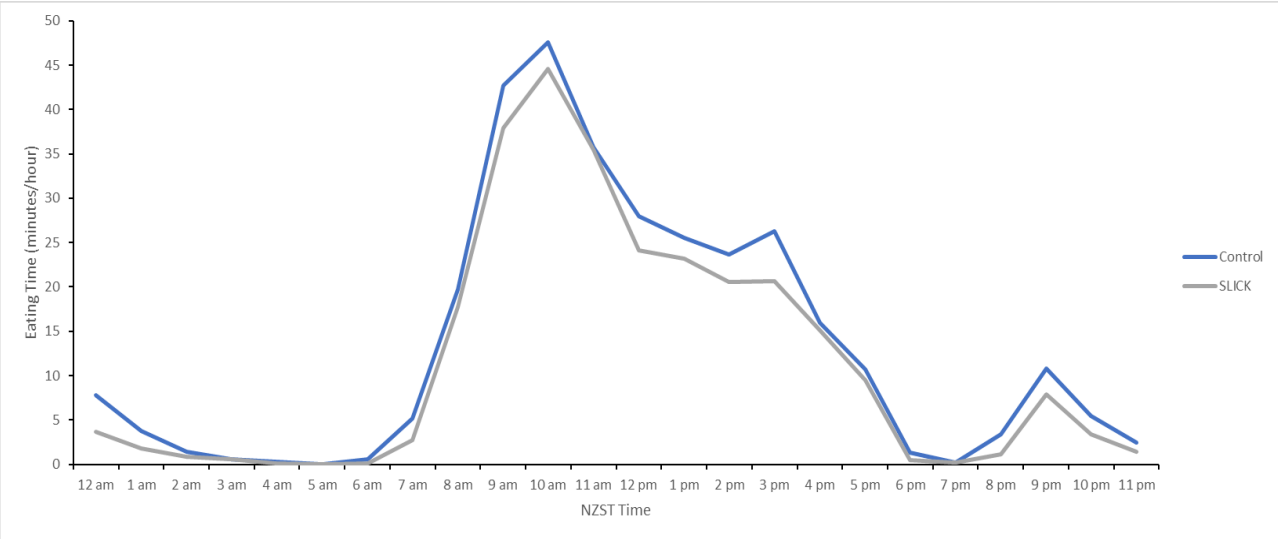

Figure 17. Daily variation in time spent eating (minutes/hour) for Control (blue), and SLICK (grey) heifers for non-cold-stress days.

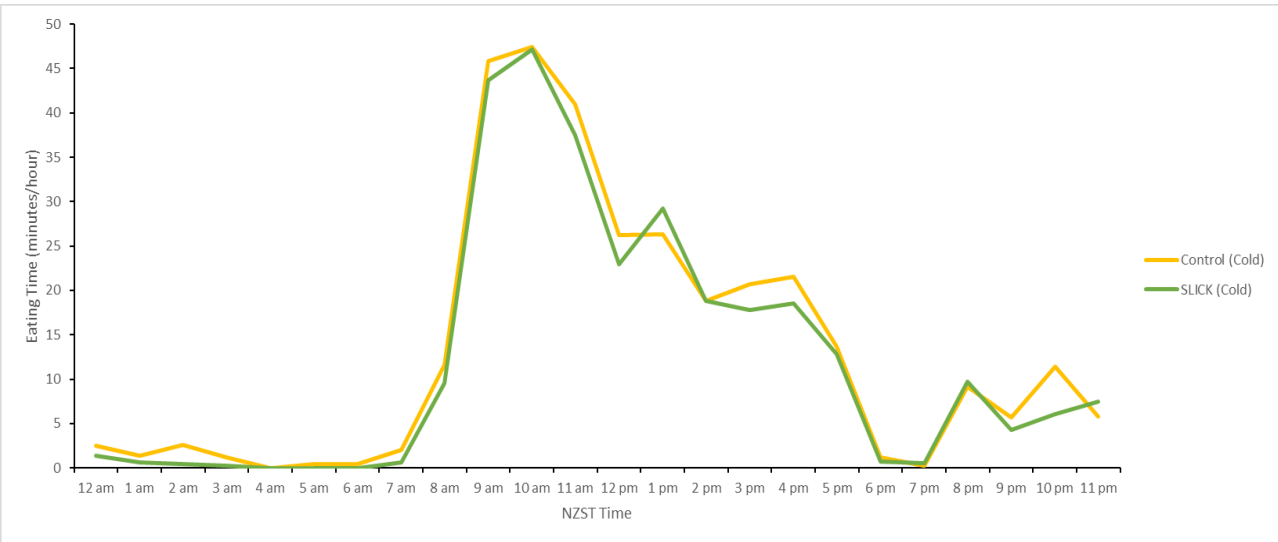

Figure 18. Daily variation in time spent eating (minutes/hour) for cold stress days for Control (yellow), and SLICK (green) heifers.

4.5 Rectal Temperatures

Rectal temperatures (°C) for Control (blue), SLICK (orange) and Non-SLICK (grey) heifers were measured on seven occasions and are presented in Figure 19, with horizontal lines at the lower critical temperature for rectal temperatures (38°C). There was no interaction between time and treatment ($p = 0.509$, nor a treatment effect ($p = 0.120$). There was a time effect ($p < 0.001$). On all cold stress days, there were individuals in all treatments with rectal temperatures below the 38°C indicator of cold stress conditions, with 19th June being the only day where one whole group (the Control heifers) sat below this threshold. This suggests that on all days there were some individuals in each group who were experiencing mild cold stress.

Rectal temperature measurement 2 (on the 19th of June) tended to be lower for the Control heifers (37.4 ± 0.18 °C) compared to the SLICK heifers (37.9 ± 0.12 °C; $p = 0.085$), which was confirmed with a Fisher's unprotected least significant difference test. The median rectal temperature for all heifers sat below the 38°C threshold for all treatments on the 19th of June.

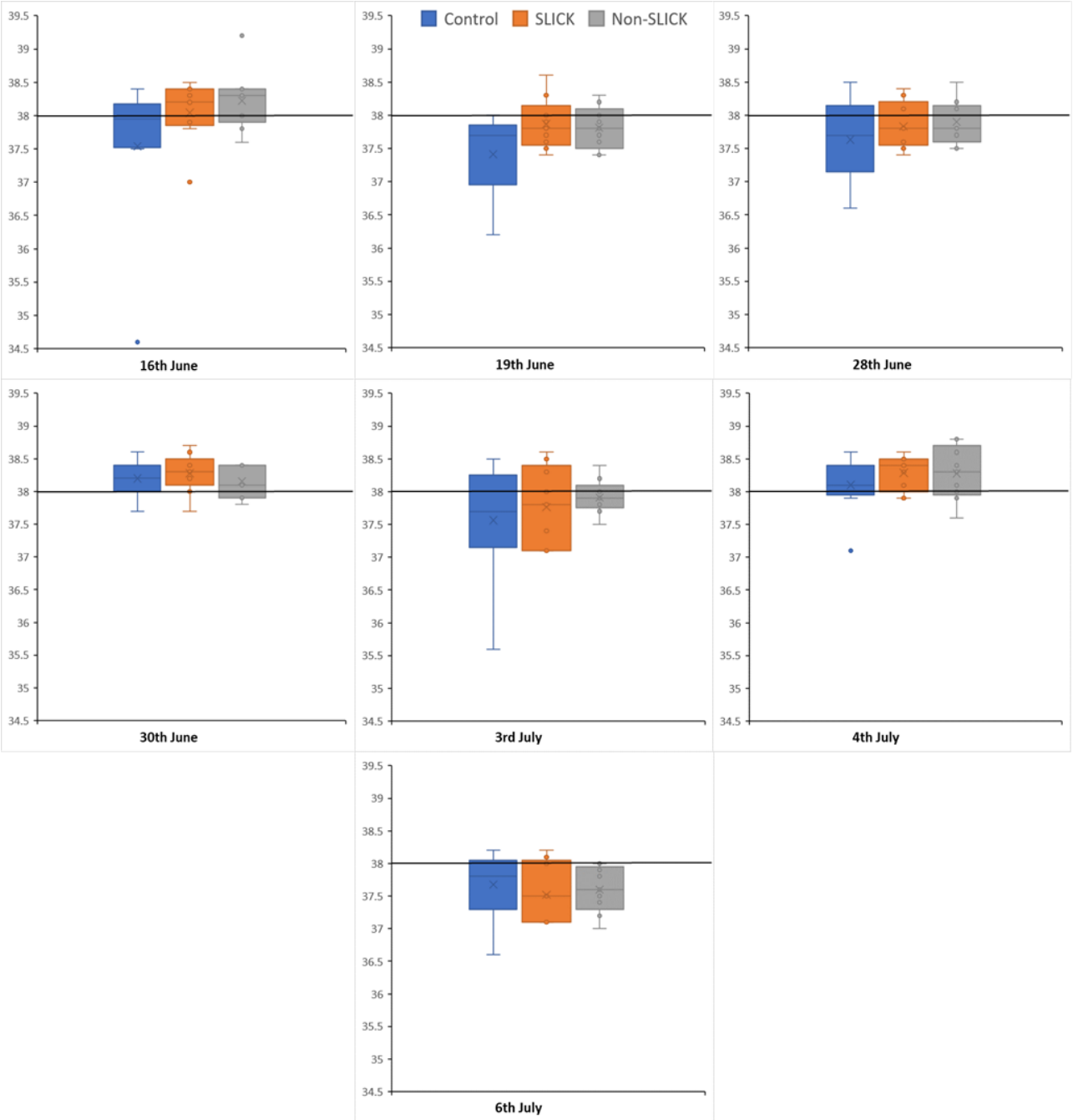

Figure 19. Rectal temperature (°C) of Control (Blue), SLICK (Orange) and Non-SLICK (Grey) heifers on days of potential wind chill. Horizontal black bars at 38°C, lower critical rectal temperature (Andersson & Jonasson 1993).

4.6 Hair samples analysis

The SLICK heifers have a 6-micron larger mean fibre diameter (MFD; micron) on average, when compared to the Non-SLICK heifers ($p = 0.005$, Table 7). Coarse edge, which is the percentage of fibres in a sample that are more than 10 microns above the MFD is greater for SLICK heifers compared to non-SLICK heifers (21.77 ± 1.79% vs 14.00 ± 0.90%; $p = 0.005$). The SLICK heifers also have a higher standard deviation of MFD, (15.63 ± 1.04 micron) compared to the Non-SLICK heifers (11.48 ± 0.39 micron; $p = 0.003$) which suggests there is more variation in SLICK heifer coats across all measurements when compared to the Non-SLICK heifers.

Average medulation (%) was not different between SLICK and Non-SLICK heifers (p = 0.197), but there was a large lower outlier for SLICK heifers, which when removed revealed a 20% higher average percent of medulated fibres in SLICK heifer coats compared with Non-SLICK heifer coats (p = 0.047).

Mean staple length (MSL; mm), and mean fibre curvature (MFC; Â°/mm) were not different when comparing the SLICK and Non-SLICK heifers (p = 0.167 and p = 0.401 respectively).

Table 7. Mean fibre diameter (micron), average medulation (%), mean staple length (mm), mean curve (Â°/mm), coarse edge (%) and standard deviation (± SEM) of SLICK or non-SLICK heifer hair samples.

	SLICK	Non-SLICK	P-Value
Mean fibre diameter (micron)	36	30	**0.005**
Average medulation (%)	26.5	22.3	0.197
Average medulation (- outlier)	*28.18*	*22.3*	***0.047***
Average mean staple length (MSL; mm)	54.66 ± 5.99	65.22 ± 2.41	0.167
Average mean fibre curvature (MFC; Â°/mm)	48.65 ± 1.74	50.96 ± 0.92	0.401
Average coarse edge	21.77 ± 1.79	14.00 ± 0.90	**0.005**
Standard deviation (micron)	15.63 ± 1.04	11.48 ± 0.39	**0.003**

Standard deviation explains the average standard deviation of all measurements for each group.

4.7 Animal Thermography Images

Thermography images were taken to show the heat loss from different areas of the heifers. After the thermal images were analysed, it was apparent that this approach to assessing heifer temperatures was not a useful measure. A series of the images have been included in Figure 20 but have not been statistically analysed, recognising that the results were not considered to be robust. The images of the rump are shown in Figure 20 of heifer numbers 71 (image a) a SLICK heifer, heifer 45 (image b) a Non-SLICK heifer and heifer 23 (image c) a Control heifer. Marked as R1, the green squares when analysed reveal an average temperature of 11.1^{o}C, 13.3^{o}C and 11.2^{o}C for images a, b, and c, respectively.

Images were also taken of the eye region of the animal (Figure 20) for heifers 71, 45 and 23. Marked as Cr1, the green circles are the analysed area with an average temperature of 29.3^{o}C, 26.2^{o}C and 26.5^{o}C for images a, b, and c, respectively.

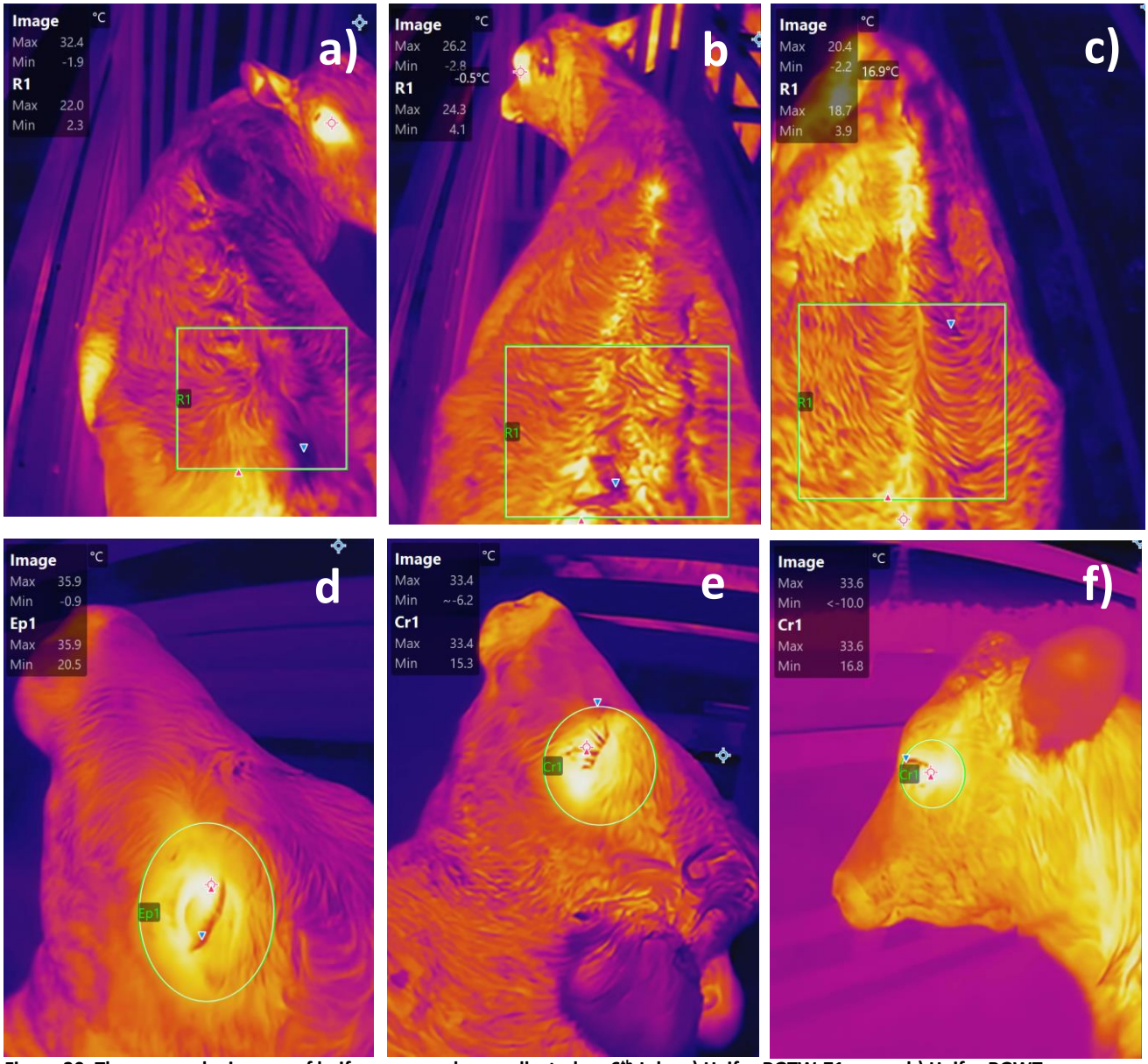

Figure 20. Thermography images of heifer rump and eye collected on 6th July. a) Heifer RGTW-71 rump, b) Heifer RGWT-45 rump, c) Heifer RGWT-23 rump d) Heifer RGTW-71 eye, e) Heifer RGWT-45 eye & f) Heifer RGWT-23 eye.

Chapter 5

Discussion

In this study, we were unable to detect significant physiological, behavioural, or temperature changes between the SLICK heifers and Control heifers during periods of cold stress. However, this does not necessarily mean that heifers carrying the SLICK allele are reacting in the same way to a cold stress.

The heifers likely did not face sufficiently long periods of cold stress that could induce rectal temperature changes. This is evident, as of the seven days predicted to have a windchill temperature within 2°C of the predefined critical temperature for the climatic conditions, only two days, the 19th of June and the 30th of June ended up presenting a significant cold challenge. The 19th of June was however the only day where there tended to be a higher mean rectal temperature for the SLICK, and Non-SLICK heifers compared with the Control ($p = 0.085$). The 19th of June posed a greater cold challenge, with a wind chill temperature of 0.7°C, and 10 mm of rain in the 2 hours prior to measurements, which means that the wind chill temperature was 14.3°C below the critical temperature for wet days of 15°C. The slightly greater rectal temperatures of the SLICK calves were unexpected, due to the expectation these heifers would be more sensitive to cold. However, Bergen *et al.* (2001) revealed that beef heifers exposed to intermittent cold temperatures of -6°C and -15°C had internal vaginal temperatures that were consistently higher than the mean temperatures of control heifers at constant thermoneutral conditions ($p = 0.34$). In this trial, heifers that were exposed to prolonged cold followed by eating did not see a spike in rectal temperatures but maintained their lower temperature as their bodies attempted to maintain body heat through metabolic heat production. This suggests that Control heifers favoured eating to maintain body temperature causing them to have a lower body temperature on average, compared to the SLICK heifers who favoured inactivity to conserve body heat. However, due to the 19th of June being the only day where significant cold stress conditions were presented to the heifers, it perhaps indicates that the heifers were not exposed to cold stress for long enough to induce observable temperature differences on multiple days.

Although there was no difference between treatments, the heifers' rectal temperatures were different over time. A change over time suggests that rectal temperatures changed in response to the climate conditions of the day, but the change did not differ based on their treatment. This is evident with four out of the seven days, seeing mean rectal temperature below the lower normal threshold of 38.0°C, and by up to 0.5°C. On the other three days, mean rectal temperatures were within normal thresholds. Andersson & Jonasson (1993) reported a mean rectal temperature for

dairy cows of 38.6°C, with a normal range from 38.0 – 39.3°C and suggested that rectal temperatures below 38.0°C meant the cold environment is overcoming any heat producing mechanisms. This suggests that on days where mean temperatures were below the 38.0°C threshold, all the heifers in this study were slightly cold stressed. However, without normal baseline temperatures for the heifers in the three treatments, it is difficult to conclude if this is a cold stress response or within the range of natural fluctuations for this group of heifers. In this respect, behavioural differences might be able to help determine the influence of cold stress on the heifers.

On cold mornings, SLICK heifers favoured not moving to preserve body heat. There was a 14% increase in 'not-active time' during the morning on cold days for the SLICK heifers compared to the warmer days (Figure 15 and 16). This behaviour has been shown to be favoured by young animals in cold conditions, as they lose less heat when lying down, compared to when they are standing ($p < 0.05$ (Rawson *et al.*, n.d.). Additionally, the SLICK heifers favoured not moving in the evening, with this illustrated by a 58% increase in not-active time at 2200hrs on cold days compared to the Control heifers on the same days. Evening variation in time when cows stop grazing (become inactive) has been surmised to be caused by cows preferring to lie down and conserve body heat, than to continue standing up and grazing (Linnane *et al.,* 2000). Even though the difference in not active time suggests that this could be a valid explanation, the difference in eating time tends to be more for Control heifers on cold days compared with SLICK heifers, but it is not a significant difference.

Eating time could be further investigated by analysing heifer growth. This trial suggested that SLICK heifer liveweight tended to be lower than both Control and Non-SLICK heifers, despite having similar growth rates. R. Bryant (pers comm.) revealed that the average birth weight for Non-SLICK and SLICK heifers was approximately 6 kg heavier than the Control heifers, but post-weaning liveweight was 5-7 kg lower for the SLICK heifers compared to the Non-SLICK and Control heifers (Table 8).

Table 8: Average birth weight, and post weaning weight (18th November 2022) for Control, SLICK, and Non-SLICK heifers. From R. Bryant (pers. comm. 21st of November 2023)

Values	Control	SLICK	Non-SLICK
Birth weight (mean 8th August) (kg)	30.20	36.80	36.45
Post weaning weight (18th November 2022) (kg)	107.88	99.61	105.70

This trend has continued, with the SLICK heifers being an average 9-14 kg lighter than the Non-SLICK and Control heifers over the winter trial, but with the difference closing during growth rate period 3, during the lower intake period.

Previous research comparing SLICK-haired Puerto Rican Holstein heifers to so-called 'wildtype (WT) heifers', suggested that SLICK heifers have a lower mature weight and reach maturity more quickly than wildtype heifers (Sánchez-Rodríguez, 2019). The difference in mature weight and time to reach maturity was observed through a 'linear' type of growth trend for the WT heifers, compared to a 'quadratic' growth trend in the SLICK heifers. This suggests that the trend for SLICK liveweight to be lower than Control and Non-SLICK heifers, reflects a difference in growth rate due to a different age x coat type interaction, causing these cattle to have a different mature weight and time to reach maturity.

Growth rate can be used to indicate whether energy is being used for heat production or growth. An apparent intake of 6.62 kgDM/heifer is 18% more than the reported feed requirements for a 9-month-old heifer, at 40% mature liveweight (DairyNZ, 2017). Furthermore, the apparent intake was not consistent during the trial period, with apparent pasture intake declining from 6.4 to 4.8 kg DM/heifer/day from the first to the second half of the trial. This caused the average growth rate for the Control heifers to be 82% less during growth rate period 3 (27th of June to the 11th of July) compared to the SLICK heifers. This decrease in apparent intake could account for the lack of difference in eating time, as the heifers had less feed to eat during the second half of the trial, and which may explain the trend of eating time being more similar from the 30th of June onwards (Figure 14).

Restricted feeding has been shown to increase the intensity of a cold challenge by decreasing the metabolic heat production from an animal. Heat production (megajoules per m^2 of surface area per day) has been described to increase 22% for 250 kg store cattle from maintenance feeding to feeding for 0.4 kg gain per day (Monteith & Mount, 1973). Due to the restricted feeding experienced during the trial, all the heifers should have been more disposed to cold stress, as their feed level changed during the second half of the trial. However, the similarity in rectal temperatures during the period of feed restriction would suggest that all heifers were able to maintain body temperature. The Control calves appeared to maintain temperature through indulging increased activity and longer eating times, compared to the lower activity of the SLICK heifers, which may have been an approach to conserving energy. The Control calves also potentially had higher intakes, but the design of this study prevented determination of their treatment intake.

The hair characteristics of the SLICK heifers may have enabled better energy conservation. The fibres in the coats were different between the SLICK and Non-SLICK heifers, with the SLICK heifers having coats that appeared to be better adapted to cold weather conditions. There was a 17% increase in mean fibre diameter (MFD), 6% more medulated fibres, and increased average coarse edge and standard deviation characteristics of the SLICK calf fibre, which is consistent with the characteristics

expected of winter adapted animals. A 1950's analysis of Zebu cattle compared with Australian Illawarra shorthorn (AIS) cattle in the winter, revealed that the Zebu (heat tolerant cattle) had denser and more medulated coats during the winter compared to the AIS cattle, even though appearing to have their 'summer' sleek and thin coats (Dowling, 1958). This suggests that the hair analysis conducted here, successfully revealed that although not visually different from summertime, the SLICK carrier cattle have a coat that might be better adapted to cold weather.

The SLICK allele is characterised as a variant in the prolactin receptor. Recent correlation between slick hair coats, and blood prolactin concentration suggests that beef cattle with lower prolactin levels (21 ± 4 ng/mL PRL) had a higher coat score ($p < 0.05$) (Davis, 2019), which in the context of this study means heavier, more insulative coats. Additionally, Roy *et al.* (2007) demonstrated that prolactin levels in the summer (248.50 – 369.63 ng/mL), were higher than winter levels (3.10 – 9.17 ng/mL) of prolactin ($p < 0.01$) in Murrah buffalo heifers. Seasonal variation in prolactin concentration, and the interaction between prolactin and coat characteristics suggests that the phenotypic differences between the SLICK and Non-SLICK heifers during the summer are not as strongly expressed in winter when prolactin concentrations are low. This suggests that during the winter, the SLICK heifers display normal adaptations to cold climates, just like *Bos taurus* cattle do.

5.1 Limitations

A limitation of this trial was the sample size for each treatment group. Due to the restricted number of SLICK heifers born, the total heifer number per treatment was only nine, which was possibly enough to see trends, but not confidently conclude if the differences were statistically significant or not. This is like the study of Monteith & Mount (1973), who conducted a cold-tolerance trial on 22 mixed Hereford and Charolais/Angus beef cattle, and eight Friesian and Brown Swiss dairy cattle. In their trial, trends and tendencies were able to be reported, but the sample size of each breed was insufficient to enable conclusions to be drawn about their cold-tolerance. This limitation was highlighted further in the current study, with the smaller Cow Manager behaviour data analysis.

Another limitation to this study were errors in the study design. The procedures to ensure that the thermal images could be effectively used as an assessment of heat loss were inadequately developed. Due to poor planning, the images collected were not consistent or taken against the skin and so they did not allow for rigorous analysis. Suggestions for further study, would be to use standard operating procedures produced in past studies to shave areas of hair to expose skin, and to take a series of images over a number of days to provide reliable data. Additional to the errors in thermal image collection, there were issues with the Cow Manager data. As a consequence of not using more Cow Manager tags, the Non-SLICK heifer group was excluded from the analysis. Whilst still being able to use SLICK and Control heifer treatments, having a larger sample size including the

Non-SLICK treatment would have allowed for any potential differences to be more clearly expressed as much as the limitations of the total sample size would allow.

5.2 Further research

The limitations of this trial present a number of areas for further study to better quantify the responses observed. Conditions that enabled greater cold stresses may better reveal the small differences and trends observed between SLICK and Control heifers for the rectal temperature and behavioural data measurements. Changing the experimental design to separate treatments to measure intake by group, rather than overall would also help to reveal more behavioural differences. Additionally, baseline rectal temperatures might better describe what is considered normal for the trial group, rather than relying on textbook definitions that do not account for the specific genotype and environmental conditions of a trial group.

Other measurements that could be investigated could include analysing summer coat characteristics to assess the relative change in coat characteristics inside trial groups, as well as between groups, so as to more confidently draw conclusions around the adaptation to cold conditions. To support this, blood prolactin levels could be assessed during the summer and winter to investigate the interaction between prolactin and coat development, and better understand if the SLICK heifers are able to adapt to cold conditions. Another measurement that might have helped further explain the behavioural differences between heifers is postural measurement. Much of the literature presented around cold stress demonstrates clear postural differences between different genotypes, and ages of animals.

Chapter 6

Conclusions

Welfare concerns around the impact of cold stress on dairy cattle with heat tolerant genes are valid, with the periods of mild cold stress inducing behavioural and physical responses to cold stress. The results presented in this study suggest that in periods of low-mild cold stress, SLICK carrier heifers display heat conservation behaviours through increased inactive time to overcome the cold stress challenge. This differs from Control heifers who appear to favour activity and intake in order to produce body heat, which became more apparent in the growth rate period 3 when feed availability was reduced, and Control heifers' growth rate was checked.

Analysis of the coat characteristics also suggest that SLICK heifers can adapt their coats to the environment to increase their insulative protection, a characteristic that was not expected to be seen given the lack of visual difference between the summer and winter coats. Coat data suggests that under the mild winter environment experienced during this trial that the SLICK heifers are no more pre-disposed to cold stress than Non-SLICK heifers, as expected through visual assessment and the coat adaptations to aid heifer cooling in the summer. The specific interaction between the prolactin receptor variant, characteristic of SLICK heifers, and changes in prolactin leading to heifer coat adaptations would also justify this adaptation. However, the limitations of this study should be considered when applying these results to more harsh conditions from other locations due to the variables explained by literature which reveal more marked differences between heat tolerant cattle and control cattle which show stronger negative influences during times of cold stress. Further investigation into the relationship between prolactin and cold stress responses would help to validate and strengthen the argument for the cold-tolerance adaptability of heat tolerant animals such as SLICK carriers.

References

Aggarwal, A., & Upadhyay, R. (2013). Heat Stress and Animal Productivity (1st ed. 2013.). Springer India. https://doi.org/10.1007/978-81-322-0879-2

Allen, J.D., Hall, L.D., Collier, R.J., & Smith, J.G. (2015). Effect of core body temperature, time of day, and climate conditions on behavioural patterns of lactating dairy cows experiencing mild to moderate heat stress. *Journal of Dairy Science 98*(1), 118–127. https://doi.org/10.3168/jds.2013-7704

Andersson, B.E. and Jonasson, H. (1993) Temperature regulation and environmental physiology. In: Dukes' Physiology of Domestic Animals, 11th edn (eds M.J. Swenson and W.O. Reece). Cornell University Press, Ithaca, NY.

Angrecka, S. & Herbut, P. (2015). Conditions for Cold Stress Development in Dairy Cattle Kept in Free Stall Barn During Severe Frosts. *Czech journal of Animal Science 60* (2), 81-87. doi: 10.17221/7978-CJAS

Bergen, R. D., Kennedy, A. D., & Christopherson, R. J. (2001). Effects of intermittent cold exposure varying in intensity on core body temperature and resting heat production of beef cattle. *Canadian Journal of Animal Science*, *81*(4), 459–465. https://doi.org/10.4141/a00-118

Berman, A. (2003). Effects of Body Surface Area Estimates on Predicted Energy Requirements and Heat Stress. *Journal of Dairy Science*, *86*(11), 3605–3610. https://doi.org/10.3168/jds.s0022-0302(03)73966-6

Brody, S. (1956). Climatic Physiology of Cattle. *Journal of Dairy Science*, *39*(6), 715–725. https://doi.org/10.3168/jds.s0022-0302(56)91194-8

Bryant, J.R., Huddart, F., and Schutz, K.E. (2022). Development of a heat load index for grazing dairy cattle. *New Zealand Journal of Agricultural Research, 66* (6), 665-679. https://doi.org/10.1080/00288233.2022.2114504

Bryant, J., López-Villalobos, N., Pryce, J. E., Holmes, C. W., & Johnson, D. L. (2007). Quantifying the effect of thermal environment on production traits in three breeds of dairy cattle in New Zealand. *New Zealand Journal of Agricultural Research*, *50*(3), 327–338. https://doi.org/10.1080/00288230709510301

Carvalho, C., de, G., Nivaldo Karvatte, Junqueira, D., Bungenstab, D. J., & Alves, F. V. (2021). Daytime ingestive behaviour of grazing heifers under tropical silvopastoral systems: Responses to shade and grazing management. *Applied Animal Behaviour Science*, *240*, 105360–105360. https://doi.org/10.1016/j.applanim.2021.105360

Carvajal, M. A., Alaniz, A. J., Constanza Gutiérrez-Gómez, Vergara, P. M., Veerasamy Sejian, & Bozinovic, F. (2021). Increasing importance of heat stress for cattle farming under future global climate scenarios. *Science of the Total Environment*, *801*, 149661–149661. https://doi.org/10.1016/j.scitotenv.2021.149661

Chanda, T., Debnath, G.K., Khan, K.I., Rahman, M.M., & Chanda, G.C. (2017). Impact of heat stress on milk yield and composition in early lactation of Holstein Friesian crossbred cattle. *Bangladesh Journal of Animal Science 46 (3): 192-197.*

Council, R. (2023). *Animal-Environment Interactions*. Nih.gov; National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK232338/

Cow Manager (2023). "Cow Manager Ear Sensors" retrieved on 23/11/2023 from https://www.cowmanager.com/cow-management/system/

Cox, B., Gasparrini, A., Boudewijn Catry, Delcloo, A., Esmée Bijnens, Jaco Vangronsveld, & Nawrot, T. S. (2016). Mortality related to cold and heat. What do we learn from dairy cattle? *Environmental Research*, *149*, 231–238. https://doi.org/10.1016/j.envres.2016.05.018

DairyNZ, (2017). Facts and Figures for New Zealand Dairy Farmers (2nd Edition). Page 57 (Dairy heifer requirements).

Davis, E. (2019). *Determining the Relationship Among Cattle Genotype, Hair Coat Score, and Productivity Through the Investigation of Single Nucleotide Polymorphisms within Prolactin, Dopamine Receptor D2, and Melatonin Receptor 1A*. ScholarWorks@UARK. https://scholarworks.uark.edu/anscuht/30/

Delfino, J. G., & Mathison, G. W. (1991). Effects of cold environment and intake level on the energetic efficiency of feedlot steers. *Journal of Animal Science*, *69* (11), 4577–4587. https://doi.org/10.2527/1991.69114577x

De Rensis, F., & Scaramuzzi, R. J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow—a review. *Theriogenology 60* (6), 1139–1151. https://doi.org/10.1016/s0093-691x(03)00126-2

Donkersloot, E.G., Worth, G.M., Yeates, A.F., Littlejohn, M.D., McNaughton, L.R., Spelman, R.J., & Davis, S.R. (2014) The benefit of a SLICK Hair Coat for Heat Tolerance in New Zealand Dairy Cattle. *Proceedings of the Association of Animal Breeding and Genetics 24*, 94-97

Dowling, D. (1958). Seasonal Changes in Coat Characters in Cattle. In *Animal Genetics Section*. Retrieved November 7, 2023, from https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=4fdd16653520a6b02bb7cbc66f9249e8e1804159

Farooq, U., Samad, H.A., Shehzad, F., & Qayyum, A. (2010). Physiological Responses of Cattle to Heat Stress. *World Applied Sciences Journal 8 (Special Issue of Biotechnology & Genetic Engineering),* 38-43. ISSN 1818-4952

Gatti, F., Cris, H., Rodrigues, G., Guimarães, E. C., & Regina, M. (2019). What is the best temperature-humidity index equation to indicate heat stress in crossbred dairy calves in a tropical environment? *Ciencia Rural*, *49*(1). https://doi.org/10.1590/0103-8478cr20180132

Gaughan, J. B., Mader, T. L., Holt, S. M., Sullivan, M. J., & Hahn, G. L. (2009). Assessing the heat tolerance of 17 beef cattle genotypes. *International Journal of Biometeorology*, *54*(6), 617–627. https://doi.org/10.1007/s00484-009-0233-4

Gonyou, H. W., Christopherson, R. J., & Young, B. A. (1979). Effects of cold temperature and winter conditions on some aspects of behaviour of feedlot cattle. *Applied Animal Ethology*, *5*(2), 113–124. https://doi.org/10.1016/0304-3762(79)90083-x

Hahn G.L. (1999) Dynamic Responses of Cattle to Thermal Heat Loads. *Journal of Animal Science 77* (2). 10-20 https://doi.org/10.2527/1997.77suppl_210x.

Hayman, R., & Nay, T. (1961). Observations on hair growth and shedding in cattle. *Australian Journal of Agricultural Research*. https://doi.org/10.1071/ar9610513

Huson, H. J., Kim, E.-S., Godfrey, R. W., Olson, T., McClure, M., Chase, C. C., Rizzi, R., Pérez, A. M., Tassell, van, José Fernando Garcia, & Sonstegard, T. S. (2014). Genome-wide association study and ancestral origins of the slick-hair coat in tropically adapted cattle. *Frontiers in Genetics*, *5*. https://doi.org/10.3389/fgene.2014.00101

Idris, M., Uddin, J., Sullivan, M. J., McNeill, D., & Clive. (2021). Non-Invasive Physiological Indicators of Heat Stress in Cattle. *Animals*, *11*(1), 71–71. https://doi.org/10.3390/ani11010071

Kava, R., Peripolli, E., Mariana Piatto Berton, Lemos, F., Raysildo Barbosa Lôbo, Nedenia Bonvino Stafuzza, Cravo, S., & Baldi, F. (2021). Genome-wide structural variations in Brazilian Senepol cattle, a tropically adapted taurine breed. *Livestock Science*, *253*, 104708–104708. https://doi.org/10.1016/j.livsci.2021.104708

Lavínia, F., Miqueo, E., Donizete, M., Thaís Manzoni Torrezan, Nathália Brito Rocha, Vieira, S., & Maris, C. (2021). Thermoregulatory Responses and Performance of Dairy Calves Fed Different Amounts of Colostrum. *Animals*, *11*(3), 703–703. https://doi.org/10.3390/ani11030703

Lees, A. M., Veerasamy Sejian, Wallage, A. L., Steel, C. C., Mader, T. L., Lees, J. C., & Gaughan, J. B. (2019). The Impact of Heat Load on Cattle. *Animals*, *9*(6), 322–322. https://doi.org/10.3390/ani9060322

Linnane, M. L, A. J. Brereton, and P. S. Giller. 2001. Seasonal changes in circadian grazing patterns of Kerry cows (Bos taunts) in semi-feral conditions in Killarney National Park, Co. Kerry, Ireland. Appl. Anim. Behav. Sci. 71:277.

Mateescu, R. G., Sarlo, K. M., Hernandez, A., Nunez, A. N., Zayas, G. A., Rodriguez, E. E., Serdal Dikmen, & P.A. Oltenacu. (2023). Impact of Brahman genetics on skin histology characteristics with implications for heat tolerance in cattle. *Frontiers in Genetics*, *14*. https://doi.org/10.3389/fgene.2023.1107468

Maxy-Mariasegaram, Robinson, N., & Goddard, M. E. (2007). Empirical evaluation of selective DNA pooling to map QTL in dairy cattle using a half-sib design by comparison to individual genotyping and interval mapping. *Genetics Selection Evolution*, *39*(3), 267–283. https://doi.org/10.1051/gse:2007003

Meyerhoeffer, D., Wettemann, R.P., Coleman, S.W., & Wells, M.E. (1985). Reproductive Criteria of Beef Bulls during and after Exposure to Increased Ambient Temperature. *Journal of Animal Science 60*(2), 352–357. https://doi.org/10.2527/jas1985.602352x

Ministry for the Environment (2016). *Climate Change Projections for New Zealand Atmospheric projections based on simulations undertaken for the IPCC 5th Assessment 2nd edition.* https://environment.govt.nz/assets/Publications/Files/Climate-change-projections-2nd-edition-final.pdf

Mishra, S.R. (2021). Behavioural, physiological, neuro-endocrine and molecular responses of cattle against heat stress: an updated review. *Tropical Animal Health and Production, 53(3).* https://doi.org/10.1007/s11250-021-02790-4

Monteith, J.L., and Mount, L.E. (1973). Heat loss from animal and man. The Butterworths Group. ISBN 0 40870652 X.

Mostafa, A., Gad, A. E., & Mostafa Abas Atta. (2018). Temperature-Humidity Indices as Indicators to Heat Stress of Climatic Conditions with Relation to Production and Reproduction of Farm Animals. *International Journal of Biotechnology and Recent Advances*, *1*(1), 35–50. https://doi.org/10.18689/ijbr-1000107

Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M., & U. Bernabucci. (2010). Effects of climate changes on animal production and sustainability of livestock systems. *Livestock Science*, *130*(1-3), 57–69. https://doi.org/10.1016/j.livsci.2010.02.011

Nicol, A. M., & Young, B. A. (1990). Effect of feed temperature on cold susceptibility of cattle and sheep. *Canadian Journal of Animal Science*, *70*(1), 191–197. https://doi.org/10.4141/cjas90-021

Nissim Silanikove, & Nazan Koluman (Darcan). (2015). Impact of climate change on the dairy industry in temperate zones: Predications on the overall negative impact and on the positive role of dairy goats in adaptation to earth warming. *Small Ruminant Research*, *123*(1), 27–34. https://doi.org/10.1016/j.smallrumres.2014.11.005

Rahmstorf, S., Foster, G., & Cahill, N. (2017). Global temperature evolution: recent trends and some pitfalls. *Environmental Research Letters*, *12*(5), 054001–054001. https://doi.org/10.1088/1748-9326/aa6825

Rawson, R., Dziuk, H., Good, A., Anderson, J., Bates, D., Ruth, G., & Serfass, R. (n.d.). *Health and Metabolic Responses of Young Calves Housed at -30 C to -8°C*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1255709/pdf/cjvetres00051-0016.pdf

Roman-Ponce, H. Thatcher, W.W., & Wilcox, C.M. (1981). Hormonal interrelationships and physiological responses of lactating dairy cows to a shade management system in a subtropical environment. *Theriogenology 16*(2), 139–154. https://doi.org/10.1016/0093-691x(81)90097-2

Roy, K. S., & Prakash, B. S. (2007). Seasonal variation and circadian rhythmicity of the prolactin profile during the summer months in repeat-breeding Murrah buffalo heifers. *Reproduction, Fertility and Development*, *19*(4), 569–569. https://doi.org/10.1071/rd06093

Sánchez-Rodríguez, H. L. (2019). Growth comparison between wild type and slick-haired Puerto Rican Holstein heifers. *The Journal of Agriculture of the University of Puerto Rico*, *103*(1), 49–58. https://doi.org/10.46429/jaupr.v103i1.17900

SDSU Extension; Beef (2020) *Chapter 8: Cold Stress Impacts on Cattle*. Warren Rusche and Julie Walker. https://extension.sdstate.edu/sites/default/files/2021-05/S-0013-08.pdf retrieved on 24/05/2023.

Sepehr Foroushani, & Amon, T. (2022). Thermodynamic assessment of heat stress in dairy cattle: lessons from human biometeorology. *International Journal of Biometeorology*, *66*(9), 1811–1827. https://doi.org/10.1007/s00484-022-02321-2

Shalit, U., Maltz, E., Nissim Silanikove, & Berman, A. (1991). Water, Sodium, Potassium, and Chlorine Metabolism of Dairy Cows at the Onset of Lactation in Hot Weather. *Journal of Dairy Science*, *74*(6), 1874–1883. https://doi.org/10.3168/jds.s0022-0302(91)78353-7

Shephard, R., & Maloney, S. K. (2023). A review of thermal stress in cattle. *Australian Veterinary Journal*, *101*(11), 417–429. https://doi.org/10.1111/avj.13275

Silanikove, N. & Darcan, N.K. (2015). Impact of climate change on the dairy industry in temperate zones: Predications on the overall negative impact and on the positive role of dairy goats in adaptation to earth warming. *Small Ruminant Research*, *123*(1), 27–34. https://doi.org/10.1016/j.smallrumres.2014.11.005

Smith, D. L. O., Smith, T., Rude, B. J., & Ward, S. (2013). Short communication: Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows. *Journal of Dairy Science*, *96*(5), 3028–3033. https://doi.org/10.3168/jds.2012-5737

Sosa, F., Carmickle, A. T., Esbal Jiménez-Cabán, M. Sofía Ortega, Serdal Dikmen, Negrón-Pérez, V. M., Jannaman, E. A., Baktula, A. M., Rincón, G., Larson, C. C., Pagán-Morales, M., Denicol, A. C., Sonstegard, T. S., & Hansen, P. J. (2021). Inheritance of the SLICK1 allele of *PRLR* in cattle. *Animal Genetics*, *52*(6), 887–890. https://doi.org/10.1111/age.13145

Sparke, E., Young, B., Gaughan, J., Holt, M., & Goodwin, P. (2001). *Heat Load in Feedlot Cattle Project number FLOT. 307, 308, 309. Report prepared for MLA by*. https://www.mla.com.au/contentassets/61e63928432144adbf11c6a17c53ec56/flot.307_final_report.pdf

Statista (2023). Total Export Revenue of the dairy industry in New Zealand from the financial year 2015 to 2024. Published by the Statista Research Department, January 3rd, 2023. Taken from: https://www.statista.com/statistics/1100950/new-zealand-export-revenue-of-dairy-industry/

The Treasury New Zealand (2022) *FEU Special Topic: Medium-term outlook for dairy exports*. (2022, June 30). https://www.treasury.govt.nz/publications/research-and-commentary/rangitaki-blog/feu-special-topic-medium-term-outlook-dairy-exports

Thornton, P., Nelson, G., Mayberry, D., Herrero, M., (2022) Impacts of heat stress on global cattle production during the 21st century: a modelling study. *Lancet Planet Health 6* (3) e192-201. https://doi.org/10.1016/S2542-5196(22)00002-X

Tucker, C.B., Rogers, A.R., & Schütz, K.E. (2008). Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. *Applied Animal Behavioural Science 109*(2-4), 141–154. https://doi.org/10.1016/j.applanim.2007.03.015

Tucker, C.B., Rogers, A.R., Verkerk, G.A., Kendall, P.E., Webster, J.R. & Matthews, L.R. (2006). Effects of Shelter and Body Condition on the Behaviour and Physiology of Dairy Cattle in Winter. *Applied Animal Behaviour Science 105* (1-13).

Webster, A.J.F. (1973). Heat loss from Cattle with particular emphasis on cold. In J.L. Monteith & L.E. Mount, (Eds.), *Heat Loss in Animals and Man (PP 206-214).* Elsevier Science.

Webster, J.R., Stewart, M., Rogers, A.R., & Verkerk, G.A. (2008). Assessment of welfare from physiological and behavioural responses of New Zealand dairy cows exposed to cold and wet conditions. *Animal Welfare 17,* 19-26. DOI: 10.1017/S0962728600031948.

Yadav, B., Singh, G. P., Ashok Kumar Verma, Dutta, N., & Veerasamy Sejian. (2013). Impact of heat stress on rumen functions. *Veterinary World*, *6*(12), 992–996. https://doi.org/10.14202/vetworld.2013.992-996

Appendix A

Heifer Statistic Output

A.1 Liveweight Repeated Measures ANOVA.

Analysis of variance

Variate: LW1_kg_19_May,LW2_kg_13_June,LW3_kg_27_June,LW4_kg_11_July

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Subject stratum						
TMNT	2		3523.11	1761.56	0.48	0.624
Residual	24		87849.85	3660.41	342.85	
Subject.Time stratum						
d.f. correction factor 0.7522						
Time	3		7497.42	2499.14	234.08	<.001
Time.TMNT	6		120.84	20.14	1.89	0.119
Residual	71	(1)	758.03	10.68		
Total	106	(1)	99615.92			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Tables of means

Variate: LW1_kg_19_May,LW2_kg_13_June,LW3_kg_27_June,LW4_kg_11_July

Grand mean 229.99

Time	LW1_kg_19_May	LW2_kg_13_June	LW3_kg_27_June	LW4_kg_11_July
	217.54	227.74	235.13	239.54

TMNT	Control	Non-SLICK	SLICK
	233.71	234.33	221.92

Time	TMNT	Control	Non-SLICK	SLICK
LW1_kg_19_May		222.28	222.28	208.06
LW2_kg_13_June		231.33	232.39	219.50
LW3_kg_27_June		240.00	238.72	226.67
LW4_kg_11_July		241.22	243.94	233.44

Standard errors of differences of means

Table	Time	TMNT	Time TMNT
rep.	27	36	9
s.e.d.	0.889	14.260	14.323
d.f.	53.40	24	24.42
Except when comparing means with the same level(s) of			
TMNT			1.540
d.f.			53.40

(Not adjusted for missing values)
Correction factors have been applied to residual d.f.(see analysis-of-variance table for details)

A.2 Growth rate one (GR1) and growth rate two (GR2) ANOVA

Analysis of variance

Variate: GR1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMNT	2	41274.	20637.	0.57	0.571
Residual	24	863733.	35989.		
Total	26	905007.			

Message: the following units have large residuals.

units 3	462.	s.e. 179.

Tables of means

Variate: GR1

Grand mean 408.

TMNT	Control	Non-SLICK	SLICK
	362.	404.	458.

Standard errors of differences of means

Table	TMNT
rep.	9
d.f.	24
s.e.d.	89.4

Analysis of variance

Variate: GR2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMNT	2	128401.	64201.	1.00	0.383
Residual	24	1540816.	64201.		
Total	26	1669218.			

Tables of means

Variate: GR2

Grand mean 528.

TMNT	Control	Non-SLICK	SLICK
	619.	452.	512.

Standard errors of differences of means

Table	TMNT
rep.	9
d.f.	24
s.e.d.	119.4

A.3 Growth rate three ANOVA and Fisher's unprotected LSD test

Analysis of variance

Variate: GR3

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TMNT	2		735522.	367761.	3.85	0.036
Residual	23	(1)	2195561.	95459.		
Total	25	(1)	2929013.			

Message: the following units have large residuals.

units 27	-587.	s.e. 285.

Tables of means

Variate: GR3

Grand mean 308.

TMNT	Control	Non-SLICK	SLICK
	87.	353.	484.

Standard errors of differences of means

Table	TMNT
rep.	9
d.f.	23
s.e.d.	145.6

Fisher's unprotected least significant difference test

TMNT

	Mean	
Control	87.3	a
Non-SLICK	352.7	ab
SLICK	484.1	b

A.4 Rumination time Multiple Comparison ANOVA Output

Analysis of variance

Variate: %22_Jun,%23_Jun,%24_Jun,%25_Jun,%26_Jun,%27_Jun,%28_Jun,%29_Jun,%30_Jun,%1_Jul,%2_Jul,%3_Jul,%4_Jul,%5_Jul

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
TMNT	1	19965.	19965.	0.44	0.525
Residual	9	411247.	45694.	30.89	
Subject.Time stratum					
d.f. correction factor 0.4068					
Time	13	637261.	49020.	33.14	<.001
Time.TMNT	13	23460.	1805.	1.22	0.314
Residual	117	173089.	1479.		
Total	153	1265022.			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Subject 5 -136.7 approx. s.e. 51.7

Standard errors of means

Table	Time	TMNT	Time TMNT	
rep.	11	unequal	unequal	
e.s.e.	11.60	25.55	30.46	min.rep
d.f.	47.59	9	17.58	
e.s.e.		23.32	27.80	max.rep
d.f.		9	17.58	
Except when comparing means with the same level(s) of				
TMNT			17.20	min.rep
d.f.			47.59	
			15.70	max.rep
d.f.			47.59	

Correction factors have been applied to residual d.f.(see analysis-of-variance table for details)

A.5 Eating time Multiple comparison ANOVA output

Greenhouse-Geisser epsilon

epsilon 0.3682

Analysis of variance

Variate: %22_Jun,%23_Jun,%24_Jun,%25_Jun,%26_Jun,%27_Jun,%28_Jun,%29_Jun,%30_Jun,%1_Jul,%2_Jul,%3_Jul,%4_Jul,%5_Jul

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
TMNT	1	57262.0	57262.0	3.25	0.105
Residual	9	158472.1	17608.0	23.19	
Subject.Time stratum					
d.f. correction factor 0.3682					
Time	13	196433.8	15110.3	19.90	<.001
Time.TMNT	13	23954.9	1842.7	2.43	0.053
Residual	117	88824.6	759.2		
Total	153	524947.2			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Subject 3 Time %5_Jul -71.4 approx. s.e. 24.0
Subject 5 Time %4_Jul -82.1 approx. s.e. 24.0
Subject 7 Time %30_Jun -87.4 approx. s.e. 24.0

Standard errors of means

Table	Time	TMNT	Time TMNT	
rep.	11	unequal	unequal	
e.s.e.	8.31	15.86	19.81	min.rep
d.f.	43.08	9	20.57	
e.s.e.		14.48	18.09	max.rep
d.f.		9	20.57	
Except when comparing means with the same level(s) of				
TMNT			12.32	min.rep
d.f.			43.08	
			11.25	max.rep
d.f.			43.08	

Correction factors have been applied to residual d.f.(see analysis-of-variance table for details)

A.7 Active time Multiple comparisons ANOVA

Greenhouse-Geisser epsilon

epsilon 0.4226

Analysis of variance

Variate: %22_Jun,%23_Jun,%24_Jun,%25_Jun,%26_Jun,%27_Jun,%28_Jun,%29_Jun,%30_Jun,%1_Jul,%2_Jul,%3_Jul,%4_Jul,%5_Jul

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
TMNT	1	9266.4	9266.4	0.29	0.604
Residual	9	289248.1	32138.7	40.14	
Subject.Time stratum					
d.f. correction factor 0.4226					
Time	13	127734.7	9825.7	12.27	<.001
Time.TMNT	13	20665.7	1589.7	1.99	0.091
Residual	117	93679.2	800.7		
Total	153	540594.1			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Subject 5	103.7	approx. s.e. 43.3
Subject 1 Time %5_Jul	73.9	approx. s.e. 24.7

Standard errors of means

Table	Time	TMNT	Time TMNT	
rep.	11	unequal	unequal	
e.s.e.	8.53	21.43	24.65	min.rep
d.f.	49.44	9	15.48	
e.s.e.		19.56	22.51	max.rep
d.f.		9	15.48	
Except when comparing means with the same level(s) of				
TMNT			12.65	min.rep
d.f.			49.44	
			11.55	max.rep
d.f.			49.44	

Correction factors have been applied to residual d.f.(see analysis-of-variance table for details)

A.8 Not Active time Multiple comparisons ANOVA output

Greenhouse-Geisser epsilon

epsilon 0.3635

Analysis of variance

Variate: %22_Jun,%23_Jun,%24_Jun,%25_Jun,%26_Jun,%27_Jun,%28_Jun,%29_Jun,%30_Jun,%1_Jul,%2_Jul,%3_Jul,%4_Jul,%5_Jul

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
TMNT	1	63203.	63203.	11.42	0.008
Residual	9	49804.	5534.	1.86	
Subject.Time stratum					
d.f. correction factor 0.3635					
Time	13	769783.	59214.	19.87	<.001
Time.TMNT	13	29441.	2265.	0.76	0.577
Residual	117	348742.	2981.		
Total	153	1260973.			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Subject 4 Time %23_Jun	-186.1	approx. s.e. 47.6
Subject 5 Time %4_Jul	-183.7	approx. s.e. 47.6
Subject 7 Time %30_Jun	-204.7	approx. s.e. 47.6
Subject 9 Time %25_Jun	-202.2	approx. s.e. 47.6

Standard errors of means

Table	Time	TMNT	Time TMNT	
rep.	11	unequal	unequal	
e.s.e.	16.46	8.89	25.15	min.rep
d.f.	42.53	9	50.67	
e.s.e.		8.12	22.96	max.rep
d.f.		9	50.67	
Except when comparing means with the same level(s) of				
TMNT			24.42	min.rep
d.f.			42.53	
			22.29	max.rep
d.f.			42.53	

Correction factors have been applied to residual d.f.(see analysis-of-variance table for details)

A.9 High Active Multiple comparisons output

Greenhouse-Geisser epsilon

epsilon 0.3061

Analysis of variance

Variate: %22_Jun,%23_Jun,%24_Jun,%25_Jun,%26_Jun,%27_Jun,%28_Jun,%29_Jun,%30_Jun,%1_Jul,%2_Jul,%3_Jul,%4_Jul,%5_Jul

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
TMNT	1	352.	352.	0.05	0.829
Residual	9	64095.	7122.	1.66	
Subject.Time stratum					
d.f. correction factor 0.3061					
Time	13	115055.	8850.	2.06	0.107
Time.TMNT	13	71667.	5513.	1.28	0.295
Residual	117	502992.	4299.		
Total	153	754161.			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Subject 7	42.1	approx. s.e. 20.4
Subject 4 Time %23_Jun	233.4	approx. s.e. 57.2
Subject 5 Time %4_Jul	326.7	approx. s.e. 57.2
Subject 7 Time %30_Jun	334.7	approx. s.e. 57.2
Subject 8 Time %24_Jun	181.3	approx. s.e. 57.2
Subject 9 Time %25_Jun	184.0	approx. s.e. 57.2

Standard errors of means

Table	Time	TMNT	Time TMNT	
rep.	11	unequal	unequal	
e.s.e.	19.77	10.09	30.00	min.rep
d.f.	35.81	9	42.75	
e.s.e.		9.21	27.39	max.rep
d.f.		9	42.75	
Except when comparing means with the same level(s) of				
TMNT			29.32	min.rep
d.f.			35.81	
			26.77	max.rep
d.f.			35.81	

Correction factors have been applied to residual d.f.(see analysis-of-variance table for details)

A.10 Rectal Temperature Multiple Comparison ANOVA Output

Analysis of variance

Variate: RT1,RT2,RT_3,RT_4,RT5,RT6,RT7

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Subject stratum						
Treatment_Group	2		2.3124	1.1562	2.37	0.115
Residual	24		11.7209	0.4884	2.16	
Subject.Time stratum						
d.f. correction factor 0.5333						
Time	6		9.9076	1.6513	7.30	<.001
Time.Treatment_Group	12		2.4256	0.2021	0.89	0.509
Residual	143	(1)	32.3264	0.2261		
Total	187	(1)	58.4819			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Analysis of variance

Variate: RT2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment_Group	2	1.1119	0.5559	2.74	0.085
Residual	24	4.8778	0.2032		
Total	26	5.9896			

Fisher's unprotected least significant difference test

Treatment_Group

	Mean	
Control	37.41	a
Non-SLICK	37.81	ab
SLICK	37.87	b

A.11 ANOVA for Micron Mean

Analysis of variance

Variate: Micron_Mean

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMNT	1	168.06	168.06	10.46	0.005
Residual	16	256.98	16.06		
Total	17	425.04			

Tables of means

Variate: Micron_Mean

Grand mean 33.10

TMNT	Control	SLICK
	30.04	36.16

Standard errors of differences of means

Table	TMNT
rep.	9
d.f.	16
s.e.d.	1.889

A.12 ANOVA for Medullation

Analysis of variance

Variate: Medulation_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMNT	1	76.47	76.47	1.81	0.197
Residual	16	675.84	42.24		
Total	17	752.31			

Message: the following units have large residuals.

units 2	-15.6	s.e. 6.1
units 16	-13.8	s.e. 6.1

Tables of means

Variate: Medulation_%

Grand mean 24.4

TMNT	Control	SLICK
	22.3	26.5

Standard errors of differences of means

Table	TMNT
rep.	9
d.f.	16
s.e.d.	3.06

A.13 Unbalanced ANOVA for Medulation (post outlier removal)

Analysis of an unbalanced design using Genstat regression

Variate: Medulation_%

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ TMNT	1	144.53	144.53	4.68	0.047
Residual	15	462.98	30.87		
Total	16	607.50	37.97		

Grand mean

25.08

Predictions from regression model

Response variate: Medulation_%

TMNT	Prediction
Control	22.33
SLICK	28.18

Standard error of differences between predicted means 2.700

A.14 ANOVA for coarse edge and standard deviation

Analysis of variance

Variate: Coarse_Edge

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMNT	1	204.54	204.54	11.03	0.005
Residual	15	278.28	18.55		
Total	16	482.83			

Message: the following units have large residuals.

units 13 8.1 approx. s.e. 4.0

Tables of means

Variate: Coarse_Edge

Grand mean 17.3

TMNT	Control	SLICK
	14.0	21.0
rep.	9	8

Standard errors of differences of means

Table	TMNT
rep.	unequal
d.f.	15
s.e.d.	2.09

Analysis of variance

Variate: SD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TMNT	1	48.203	48.203	12.60	0.003
Residual	15	57.388	3.826		
Total	16	105.591			

Message: the following units have large residuals.

units 12 -3.76 approx. s.e. 1.84

Tables of means

Variate: SD

Grand mean 13.08

TMNT	Control	SLICK
	11.49	14.86
rep.	9	8

Standard errors of differences of means

Table	TMNT
rep.	unequal
d.f.	15
s.e.d.	0.950