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Biological Responses of Feedlot Cattle to Heat Load

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Abstract

Heat load is a significant animal welfare and cost of production issue worldwide. In the US alone heat load is reported to have an annual economic burden of > $300 million in the beef sector. Furthermore animal growth is often depressed during summer resulting in heat related decreases in weight gain of approximately 10 kg which coincides with a 7 day increase in days on feed. The reduced growth rate increases days on feed, thereby increasing the cost of production. Whilst the effects of heat load on cattle has been researched for a number of years, there is speculation as to the interactions between hot environmental conditions and livestock performance, reproduction, health and overall wellbeing. Therefore there is a need to develop a more comprehensive understanding of the dynamic responses of animals to heat load. The key focus of heat load research is to develop effective management strategies to support animal comfort and performance during hot periods.

While heat load can occur in pasture raised cattle, it is mostly observed within the intensive grain feed feedlot industry. Heat load occurs where a combination of environmental conditions exceed the animals ability to regulate body temperature, thus impacting on homeostasis. However how an animal responds to heat load is also dependent on a number of individual characteristics, including genotype, coat characteristics, health status and days on feed. Therefore no two animals will respond to hot climatic conditions in exactly the same manner. There are numerous responses to heat load that can be measured and/or observed in cattle, including changes in behaviour, respiratory dynamics, blood metabolites and body temperature. The experiments within this thesis were focused on investigating the;

i) *Effectiveness of new technologies in determining body temperature: namely rumen temperature and infrared thermography*
ii) *Influence of genotype and shade availability on the regulation of rumen temperature, behavioural and haematological responses of feedlot cattle*

From the experiments conducted the key findings were;

i) *Rumen temperatures are variable and appear to trend with increasing and decreasing ambient conditions (specifically ambient temperature). Small differences between rectal temperatures and rumen temperatures were observed. Additionally these results indicate that breed, ambient conditions and availability of shade influence rumen temperature, indicating that rumen temperature can be used to assess an animal's thermal status. Overall the data suggest that rumen temperature has the potential to become a functional predictor*

of body temperature, and that it is possible that rumen temperature can be used as a proxy of core body temperature in feedlot cattle.

ii) *Infrared thermography does not appear to be a functional estimate of core body temperature as the results suggest that there was little relationship between the body surface temperature and rumen temperature. However there is the potential that the measurement of body surface temperature can be used to determine the heat flow from the animal, potentially providing an opportunity to further develop knowledge regarding thermal exchange.*

iii) Behavioural observations indicate that;

   a. *Feedlot cattle appear to be consuming small portions of feed at regular intervals;*
   b. *Angus steers had the highest increase (61.3 %) shade utilisation when HLI increased from cool (HLI < 77) to very hot (HLI > 86), followed by Charolais (28.1 %) and Brahman (15.4 %) steers, further highlighting the importance of providing shade structures to feedlot cattle;*
   c. *All breed × treatment groups exhibited a notable increase in panting score as heat load increase, where HLI conditions were very hot (HLI ≥ 86) the mean panting score of all breed × treatment groups differed ($P < 0.05$).*

iv) *Haematological values obtained from feedlot cattle during summer are perplexing.*

   a. *There is large variability in the effect of heat load across studies. However elevated cytokine interleukin 6, glucose and insulin concentrations appear to be indicative of insulin resistance.*
   b. *What appears to be clear is that haematological parameters are closely interrelated and altering concentrations during exposure to stressors ensures animal survival.*

A key aspect in managing heat load is that feedlot personnel are able to recognise the responses of cattle to high heat load. Improvements in animal management have contributed to alleviating some of the negative effects of heat load. However summer conditions are still responsible for significant production losses and welfare concerns worldwide. Furthermore heat load cannot be completely eradicated where there are animal production operations in tropical and sub-tropical regions. Therefore the primary purpose of heat load research becomes focused on the effective use of mitigation strategies prior to and during heat related stress events, thus improving animal survivability and welfare during these events.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis.

The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Refereed Papers

Owen, H., K. Buckle, J. Olm, M. Leitner, S. Pandey, J. B. Gaughan, M. L. Sullivan, **A. M. Lees**, and J. S. Gibson. 2015. Isolation of Nocardia mexicana from focal proliferative tenosynovitis and arthritis in a steer. Australian Veterinary Journal 93 (5): 170-173; DOI:10.1111/avj.12308DOI

Papers in Preparation

Lees A. M., M. L. Sullivan, J. C. Lees, A. T. Lisle and J. B. Gaughan. 2015. Using rumen temperature as a measure of core body temperature in feedlot cattle. Journal of Animal Science (In Preparation).

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Lees A. M., J. C. Lees, A. T. Lisle, M. L. Sullivan and J. B. Gaughan. 2015. Influence of high heat load on rumen temperature. Journal of Animal Science (In Preparation).

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Sullivan. M. L, J. B. Gaughan, N. H Son, J. C. Lees and **A. M. Lees**. 2014. Correlation between milk production, days in milk and temperature humidity index in lactating dairy cows in a sub-

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Lees. A. M. and J. B. Gaughan. 2013. Diurnal Patters in Rumen Temperature of Feedlot Steers During Summer. In: 2013 MLA Postgraduate Conference Proceedings Coffs Harbour, NSW, Australia

Publications included in this thesis

No publications included.

Contributions by others to the thesis

Associate Professor John Gaughan contributed to the concept and design of the project and acquired financial support for this research prior to the commencement of the studies. Associate Professor John Gaughan has maintained the role of primary supervisor throughout the duration of my candidature. Associate Professor John Gaughan, Dr Stephen Anderson and Mr Allan Lisle have contributed to the interpretation and analysis of results. Dr Lisa Elliot (TropBio, James Cook University, Townsville, Queensland) conducted the laboratory analysis of plasma HSP_{70} concentrations. Electrolyte, lipid and glucose analyses were conducted through The University of Queensland's Veterinary Diagnostic Services laboratory (Gatton, Queensland). Critical review of the writing for this thesis was provided by Associate Professor John Gaughan and Dr Stephen Anderson. Review of the writing must also be extended to Mr Jarrod Lees. The adoption, validation and performance of the experimental procedures expressed within this thesis were primarily my responsibility as a PhD candidate. Many of the concluding comments within the chapters and discussion have evolved from discussions with Associate Professor John Gaughan, Dr Stephen Anderson and Mr Jarrod Lees.

Statement of parts of the thesis submitted to qualify for the award of another degree

No sections of this thesis have been submitted for another degree.

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Finally to quote Aristotle *"Excellence is never an accident. It is always the result of high intention, sincere effort and intelligent execution; it represents the wise choice of many alternatives. Choice, not chance determines your destiny."* No truer words can be spoken of the journey through a PhD.

Keywords

Behavioural responses; Biological responses; Body Temperature; *Bos indicus*; *Bos taurus*; Haematological responses; Heat load; Infrared thermography; Rumen temperature; Shade availability

Australian and New Zealand Standard Research Classifications (ANZSRC)

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Fields of Research (FoR) Classification

FoR code: 0702, Animal Production, 80 %

FoR code: 0606, Physiology, 20 %

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List of Abbreviations

Item	Abbreviation	Units
Ambient temperature	T_A	°C
Average daily gain	ADG	kg/day
Black globe temperature	BGT	°C
Bovine serum albumin	BSA	- - - -
Centimetre	cm	- - - -
Day of Experiment	d	- - - -
Dry matter	DM	- - - -
Dry matter intake	DMI	- - - -
Dulbecco's phosphate buffer	D-PBS	- - - -
Enzyme-linked immunosorbent assay	ELISA	- - - -
Heat Load Index	HLI	- - - -
Heat Shock Protein 70	HSP_{70}	- - - -
Heat Shock Protein 82	HSP_{82}	- - - -
Heat Shock Protein 90	HSP_{90}	- - - -
Heat Shock Protein/s	HSP	- - - -
Megahertz	MHz	- - - -
Metabolisable energy	ME	MJ
Metre	m	- - - -
Radio-frequency identification	RFID	MHz
Radioimmunoassay	RIA	- - - -
Relative humidity	RH	%
Solar radiation	SR	W/m^2
Temperature Humidity Index	THI	- - - -
The University of Queensland	UQ	- - - -
Time of Day	h	- - - -
United States of America	US	- - - -
Wind speed	WS	m/s

Chapter 1
General Introduction

Heat stress is a significant welfare and production issue for the feedlot industry worldwide. In sub-tropical and tropical regions feedlot cattle may be exposed to heat stress year round (Buffington et al., 1981), while in temperate regions heat stress is seasonal. Chronic heat stress is present in many regions worldwide during the summer months and is often a major stressor for healthy feedlot cattle (Gaughan et al., 2013). Heat stress in the US beef industry is reported to have an economic burden of > $300 million annually (St-Pierre et al., 2003). Periods of heat stress are associated with reductions in growth, i.e. live weight gains (Mitlöhner et al., 2002), dry matter intake (**DMI**) (Beede and Collier, 1986; Brown-Brandl et al., 2005a), and under severe circumstances death may occur (Bushby and Loy, 1997; Hahn, 1999; Entwistle et al., 2000; Gaughan, 2002; Brown-Brandl et al., 2006a; Brown-Brandl et al., 2006b).

The term 'stress' however, lacks a clear definition in terms of the effects that hot climatic conditions have, on animal performance and welfare. Heat stress was defined by Buffington et al. (1981, pp 711) as "*any combination of environmental conditions that will cause the effective temperature of the environment to be higher than the temperature range of the animal's thermoneutral zone.*" The thermoneutral zone was defined by Ames (1980, pp 457) as "*the optimum thermal environment in which the animal enjoys optimum health and maximum productivity.*" Accumulation and dissipation of heat from the body is constantly adjusting in order to maintain optimum health and productivity; however as ambient conditions increase above a given threshold, which is largely species specific, heat accumulation often becomes greater than dissipation, thereby influencing the overall wellbeing of the animal. Heat accumulation and dissipation is regulated through thermal exchange pathways via conduction, convection, radiation and evaporation. For an animal to maintain thermal balance, the heat accumulated through heat thermal exchange pathways and metabolic functions, must equal that of heat dissipated from the body (Hahn, 1985). As ambient conditions become hotter, thermal exchange between the animal and its surrounding environment may become less effective therefore disrupting homeostasis (Ravagnolo and Misztal, 2002). However environmental conditions are not the only factors that influence thermal exchange in livestock. For feedlot cattle, animal factors which influence thermal balance include: genotype; coat type and coat colour; number of days on feed; body condition, i.e. fat coverage and deposition; performance, i.e. growth rate; health status; and adaptation (to both the feedlot and environment). Therefore the term heat stress tends to be misleading as by definition it refers to the combination of environmental conditions alone without consideration of animal factors

(Buffington et al., 1981; Gaughan, 2002). Throughout this thesis the term heat load will be used rather than heat stress. Heat load accounts for the cumulative effects of animal factors and environmental conditions on the thermal comfort of animals (Gaughan, 2002) and therefore becomes a better descriptor of an animal's thermal balance.

When an animal encounters challenging climatic conditions, i.e. those which are well outside of the animal's thermoneutral zone, the immediate response is self-preservation characteristically at the cost of production, i.e. reduced weight gain (DeShazer et al., 2009). However animal responses to their thermal environment are derived from both acute and chronic exposure to high heat load (Hahn and Mader, 1997). Any imbalance in heat accumulation and dissipation of heat results in a change in core body temperature (Brown-Brandl et al., 2005b), which consequently influences other physiological functions. In response to increasing heat load there are a number of physiological responses exhibited by cattle that may illustrate the extent to which an animal is stressed, including increased sweating rate (g/m^2); respiration rate (breaths per minute); panting score and body temperature (°C); reductions in DMI and variations in haematological parameters. A small increase in respiration rate potentially increases maintenance energy requirements by approximately 7 % (NRC, 1981). Furthermore a significant increase in respiration, i.e. laboured panting, potentially increases energy requirements by 11 to 25 % (NRC, 1981). Therefore as ambient heat load increases, cattle divert energy that is typically partitioned for growth towards maintaining homeostasis (Kadzere et al., 2002; Ravagnolo and Misztal, 2002). The diversion of energy towards homeostasis is associated with depressed growth rates, whereby heat related decreases in weight gain are approximately 10 kg, which coincides with a 7 day increase in days on feed (Baumgard and Rhoads, 2012b). Cattle, however, are able to regulate the impact of adverse conditions by adjusting behaviourally and immunologically to minimise the effects of adverse climatic conditions (Hahn, 1999; Gaughan et al., 2008b) to support survival.

During hot conditions cattle will decrease DMI, decrease the amount of time spent lying, and increase water consumption (Brown-Brandl et al., 2006b). As ambient heat load increases, behavioural observations can therefore provide some insight into how cattle are coping with hot conditions as described by Young and Hall (1993) below;

- Alignment of the body with the sun; reduce exposure to solar radiation (**SR**; W/m^2)
- Shade seeking; from shade structures, fence lines, feed bunks and other animals
- Refusal to lie down; increased proportion of time standing
- Reduction in DMI, also associated with a reduction in rumination
- Crowding at water troughs, may include body splashing
- Increased agitation and restlessness
- Open mouthed breathing/panting, can be combined with excessive salivation

The reduction of DMI influences other biological mechanisms within the body that include an alteration to the function of the endocrine system (Baumgard and Rhoads, 2007). Given the role of the endocrine system in the coordination of metabolism, the alteration of blood hormone concentrations due to the thermal environment are not unexpected (Beede and Collier, 1986). Changes in biological markers, such as haptoglobin, creatine kinase, cytokines, insulin, glucose, heat shock proteins (**HSP**) and electrolyte balance, may be used as an indication of thermal stress in cattle (Mitlöhner et al., 2002). An increase in respiration rate is also associated with an increase in carbon dioxide being exhaled (Baumgard and Rhoads, 2007). In order for the blood to remain as an effective pH buffering system, the body needs to maintain bicarbonate to carbon dioxide ratio of 20:1 (Baumgard and Rhoads, 2007). Therefore an increase in respiration rate results in a decrease in carbon dioxide in blood which leads to a reduction in the bicarbonate:carbon dioxide ratio. Furthermore heat shock induced by hot weather conditions acutely decreases DNA synthesis and negatively affects the ability of the cells to maintain their cytoskeleton, resulting in a collapse of the cell structure (Roy and Collier, 2012). During these periods a HSP response may be elicited where HSP become responsible for the stabilisation of proteins as well as the destruction of damaged protein structures (Pockley, 2003).

Within feedlots the ability to forecast hot climatic conditions on livestock is important to producers in terms of welfare and performance, as it provides an opportunity to implement abatement strategies (Gaughan et al., 2008b). These may include providing access to shade (Mitlöhner et al., 2002), or increasing access to water (Arias and Mader, 2011). By using a combination of local ambient climatic conditions, including ambient temperature (**T_A**, °C), relative humidity (**RH**; %), SR, wind speed (**WS**; m/s) and rainfall (mm), feedlot managers are able to monitor the impact of the thermal environment on the animals.

There have been a number of indices developed that represent the net effect that environmental conditions impose on cattle, including the Black Globe Humidity Index (Buffington et al., 1981); Wet-Bulb Globe Thermometer Index (Lee, 1980); Temperature Humidity Index (**THI**) (Thom, 1959); Heat Load Index (**HLI**) (Gaughan et al., 2008b); and Comprehensive Climatic Index (Mader et al., 2010b). Historically, the development of climatic indices have been for human application (Mader et al., 2010b); however the THI, adapted from Thom (1959), has been used extensively in livestock systems. The THI exists in various forms which accounts for the net impact of T_A, wet bulb temperature or dew point temperature, and RH (Buffington et al., 1981; Bohmanova et al., 2007). Numerous authors have noted the limitations of the THI, primarily as the model does not account for WS or SR (Mader et al., 2006; Gaughan et al., 2008b; Dikmen and Hansen, 2009;

Mader et al., 2010b). Gaughan et al. (2008b) identified the limitations of the THI and developed the HLI which incorporates the combined effects of RH, WS and black globe temperature (**BGT**; °C). The HLI generates a single unit value which represents the thermal load an animal is experiencing (Gaughan et al., 2010b). In conjunction with the HLI, a forecasting system was developed by Katestone Environmental (http://chlt.katestone.com.au/) to assist Australian feedlots in implementing abatement strategies in preparation for hot climatic conditions. The primary purpose of these indices is to provide a tool for the strategic management of livestock during adverse climatic conditions.

Hahn and Mader (1997) investigated the progression of a heat wave event in July 1995 in mid-central US where over 4 000 feedlot cattle deaths were reported. During this heat wave event, climatic conditions presented with unusually high minimum and maximum T_A in conjunction with high RH over 3 to 4 consecutive nights (Hahn and Mader, 1997). This heat wave event emphasised the importance of night time recovery on the ability of cattle to cope with hot conditions. Moreover Gaughan et al. (2008b) indicated that when developing forecasting models if night time conditions are not incorporated, the overall heat load status of cattle may be underestimated. When conditions are above specified thresholds, cattle will accrue thermal load throughout the day and dissipate the accumulated heat load at night, contributing to the diurnal variation in core body temperature. However if night time conditions are insufficient to support heat dissipation, cattle will enter the subsequent day with an accumulated heat load and thus are more likely to have an increased core body temperature. During heat wave events cattle may be continually exposed to increasing accumulated heat load and when combined with limited night time heat dissipation, this situation may result in excessive heat load (Mader et al., 2006). At this point cattle are no longer able to regulate core body temperature (Mader et al., 2006), which may result in feedlot mortalities.

It is clear that a dynamic relationship exists between animals and their thermal environment. The research for this thesis was primarily undertaken to determine whether ***'the current HLI model thresholds are adequately describing the effect of hot ambient conditions on physiological, behavioural and biological responses of feedlot cattle.'*** A number of experiments were carried out at The University of Queensland, Gatton Campus to evaluate this hypothesis. The objectives of the experiments within this thesis were to;

- *Assess the effectiveness of new technologies in the measurement of body temperature;*
- *Further understand the dynamic nature of the physiological, behavioural and haematological responses of feedlot cattle to hot climatic conditions, with particular emphasis on differences in response of Bos taurus and Bos indicus breeds*

Chapter 2

Review of the Literature

2.1 Introduction

Animal responses to environmental stressors have been investigated for some time, and although knowledge continues to be developed, managing livestock to reduce the negative impact of hot climatic conditions remains challenging (Hahn, 1999; Mader, 2003). With the forecasted changes to the global thermal environment there is the potential that summer conditions will result in an increase in ambient temperature (**T_A**; °C) and relative humidity (**RH**; %) which could exacerbate the impact of hot climatic conditions on intensively raised animals (Hahn, 1999). Climate change models indicate that an expected outcome of global warming is the increased prevalence and severity of heat wave events (Solomon et al., 2007). Heat wave events are of particular interest as these climatic extremes contribute to substantial economic and physical impact on both people and animal production enterprises (Perkins and Alexander, 2012). Feedlot cattle are particularly susceptible to changes in climatic conditions, often exhibiting reduced performance and wellbeing during periods of hot climatic conditions (Mader, 2003).

For the development of any prediction models knowledge and understanding of the thermal status of livestock and the heat exchange between animals and environment is essential (Parsons et al., 2001). Therefore it is important to gain an understanding of the impact of changing climatic conditions on feedlot cattle and factors influencing thermal exchange between cattle and their environment. Investigating these areas will allow for the development of measures to accurately forecast and predict the impact of heat load conditions and to minimise the negative impact of hot climatic conditions on the feedlot industry. By providing accurate forecasting models, persons responsible for the management and care of feedlot cattle will be able to implement mitigation strategies to reduce the negative effect of hot climatic conditions. There have been a number of indices developed that provide a forecast on the effect of environmental conditions, where the Temperature Humidity Index (**THI**) and Heat Load Index (**HLI**) have been extensively used in both the dairy and feedlot industries (Silanikove, 2000; Bohmanova et al., 2007; Gaughan et al., 2008b). However for the forecasting models to be applicable in commercial situations, animal carers are required to understand and identify animal responses, to thermal challenges in order to make informed decisions on how to reduce the impact on the animals (Hahn, 1999). Quantifiable measures such as physiological, behavioural and biological responses to heat load are all useful indicators of thermal stress. Physiological responses to heat load include increased sweating rate (Mader et al., 2010a); respiration rate (breaths per minute) (Gaughan et al., 2000); panting score (Mader et al., 2006); and body temperature (°C) (Robertshaw, 1985). Biological markers in the blood are also indicators in determining the level of stress an animal is under (Collier et al., 2008). Cattle also use adaptive behaviours to reduce heat load, primarily consisting of shade seeking, under

shade structures or other animals, and the alignment of the body in accordance with solar radiation (**SR**; W/m^2) to reduce whole body exposure to direct sunlight (Nienaber et al., 2003).

Cattle can become exposed to heat load, not only during extreme heat wave events, but also to sudden and rapid changes to local ambient conditions (Mader, 2003). However the way in which cattle respond to hot climatic conditions is also dependent on a number of individual characteristics, including genotype (Brown-Brandl et al., 2006b); coat characteristics (Gebremedhin and Wu, 2002); and days on feed (Brown-Brandl et al., 2006b). Other factors such as dry matter intake (**DMI**) (Hahn et al., 1992); diet composition (Mader et al., 1999b); water intake and temperature (NRC, 1981); and availability of shade (Blackshaw and Blackshaw, 1994) also influence the thermoregulatory capacity of feedlot cattle during hot conditions.

2.2 Climatic Conditions Contributing to Heat Load

When cattle are exposed to conditions outside of their thermoneutral zone they may exhibit signs of heat load however T_A on its own is somewhat arbitrary, as other climatic factors influence how the animal responds (Gaughan et al., 2013). Ambient weather conditions that influence the heat load placed on cattle are i) T_A; ii) RH; iii) thermal radiation, i.e. SR including long and short wave radiation; iv) wind speed (**WS**; m/s) ; and v) rainfall (mm) (Bond et al., 1967; Blackshaw and Blackshaw, 1994; Brown-Brandl et al., 2006b). Combined these parameters determine the heat load placed on the animal. Heat load occurs when an animal gains more heat than it is able to dissipate. The heat gained by the body originates from the environment, metabolism and physical activity, i.e. locomotion, and accounts for diurnal variations in core body temperature. A major driver of heat load in feedlot cattle are overnight conditions, particularly the amount of night time cooling (Hahn, 1999; Mader and Davis, 2004). Hahn and Mader (1997) suggested that feedlot cattle require night time temperatures to fall below 23 °C to allow for effective body temperature regulation. However the number of hours below a specific temperature threshold required to effectively dissipate any, accumulated heat load is yet to be adequately determined for feedlot cattle. This is largely because the relationship between intensity and duration of the heat load exposure, along with numerous animal factors, are difficult to quantify (Gaughan et al., 2013). However previous studies conducted by Hahn and Mader (1997) and Gaughan et al. (2010a) suggest that cattle require between 6 and 8 hours exposure to T_A below 23 °C to adequately recover from high heat load. In the event that ambient conditions are insufficient to allow for heat dissipation during the night, cattle enter the subsequent day with an accumulated heat load (Gaughan et al., 2008b). Climatic conditions that develop into periods where cattle are exposed to prolonged accumulated heat load can be present almost all year long in sub-tropical and tropical climates (Buffington et al., 1981). Chronic exposure

to heat load is present in many regions worldwide during the summer months and is often a major stressor for healthy feedlot cattle (Gaughan et al., 2013).

2.2.1 Ambient Temperature

Ambient temperature refers to the temperature of the air surrounding an animal. Ambient temperature follows a temperature gradient, indicating that heat transfer will occur between an animal and the environment (Hahn, 1985). When T_A surpasses skin temperature the animal is no longer able to dissipate heat to the surrounding air via convection (Silanikove, 2000). Furthermore if the core body temperature of an animal is lower than that of the surrounding air the animal becomes a heat "sink" where it will accumulate heat from the environment (Silanikove, 2000), thus increasing body temperature. Increased body temperature as a result of high T_A affects animal bioenergetics and also has a negative impact on animal performance and welfare (Hahn, 1999). Additionally when T_A is equal to body temperature, heat loss via evaporation becomes the predominant method of heat dissipation (Esmay, 1969), where at 32 °C evaporative heat loss accounts for 85 % of an animals' total heat loss (Avendaño-Reyes et al., 2010). However a reduction in T_A does not necessarily indicate that there will be an automatic increase in heat dissipation (Gaughan et al., 2008a), as there are numerous other factors that influence heat dissipation from the animal.

2.2.2 Relative Humidity

Relative humidity, or water vapour, is a measure of the amount of moisture present within the air (Yousef, 1985). Relative humidity influences heat dissipation, via evaporation, from the skin and respiratory surfaces (Berman, 2009; Dikmen and Hansen, 2009). Once the air becomes saturated with moisture, an animal is no longer able to dissipate heat from the respiratory surfaces and evaporative heat loss ceases (Esmay, 1969). Increased RH lowers the accumulated heat load threshold due to limited evaporative cooling (Berman, 2005) particularly when T_A is high. Therefore the moisture content of the air has a considerable effect on homeostasis in cattle.

2.2.3 Solar Radiation

Heat accumulation by cattle via SR is dependent on a number of factors including body surface temperature; coat colour; hair follicle characteristics; and texture of the coat surface (King et al., 1988; Becerril et al., 1993; Silanikove, 2000; Maia et al., 2005). Solar radiation is delivered in electromagnetic waves from the sun and is reflected by infrastructure surrounding the animal (Bond et al., 1967). Absorbance of radiation though short wave radiation (from the sun) and long wave radiation (from the terrestrial environment) has the potential to exceed the amount of heat produced

by metabolic processes (Brown-Brandl et al., 2005b). Short wave radiation is more easily absorbed by dark coated animals when compared with lighter coated animals, which reflect short wave radiation to the surrounding environment (Robertshaw, 1985). Cattle with white or lighter coloured coat colours have been reported to absorb 40 to 50 % less SR than animals with darker coats (King et al., 1988). However long wave radiation is absorbed equally by all cattle, irrespective of coat colour (Esmay, 1969). Additionally Frazzi et al. (2000) indicated that, given the option, cows would seek out cooled areas, or a barn fitted with water misters and air movement, during hours of the day where SR was at its greatest ($\geq$ 500 W/m^2).

2.2.4 Wind Speed

Wind speed is the velocity at which the air is moving. Wind speed influences the rate of thermal exchange via convection and evaporation (Silanikove, 2000). The intensity of WS influences both evaporative and non-evaporative heat loss mechanisms (Esmay, 1969). Variations in WS affects the rate of convective heat loss from the skin and hair surface of the animal (Silanikove, 2000), where high WS ($\geq$ 4 m/s) increase convective heat loss through the coat (Gebremedhin, 1985). The increase in heat exchange occurs with the movement of air around the coat (Gebremedhin, 1985), where there is a disruption to the layer of air that surrounds the animal. However heat dissipation due to air movement is proportional to the surface area of the animal exposed to air movement and not the entire surface area of the animals body (Mader et al., 2010b).

2.2.5 Heat Wave Events

A heat wave event as defined by American Meteorological Society (1989) is "*a period of abnormally uncomfortable hot and usually humid weather of at least one day duration, but conventionally lasting several days to several weeks*." However for intensively housed livestock Nienaber et al. (2007) and Mader et al. (2010a) defined a heat wave event as a number of successive days, typically 3 to 5, where maximum ambient conditions are above a specific threshold, i.e. HLI above 86 for an un-shaded black Angus steer (Gaughan et al., 2008b).

One predicted consequence of global warming is the increased prevalence and intensity of heat wave events (Solomon et al., 2007). Climatic trends of heat wave events differ from summer to summer and future predictions indicate that there will continue to be large variability in the climatic behaviour of these adverse events (Robinson, 2001; Westcott, 2011). However significant advancement has been made in the last 50 years in predicting and forecasting climatic conditions (Westcott, 2011), enabling producers to prepare for forthcoming adverse climatic events.

Heat waves can result in compromised animal welfare and productivity; however the effect on the individual is influenced by the intensity and duration of the heat wave. During prolonged heat wave events, particularly where there is limited night time relief, the death of vulnerable animals can occur (Hahn, 1999), as a result of excessive heat load. Numerous authors have reported heat wave conditions where feedlot cattle have succumbed to heat load, for example;

- February 1991 – 4 000 deaths were recorded in Queensland (Gaughan, 2002), with one feedlot reporting 2 680 deaths (Entwistle et al., 2000)
- July 1995 – 3 750 deaths were estimated in Western Iowa, (Bushby and Loy, 1997), total deaths for the mid-central US were over 4 000 cattle (Hahn and Mader, 1997). This particular heat wave event was associated with an estimated economic loss of approximately $ 28 million contributed from production losses (Hahn, 1999)
- Hahn (1999) reported the loss of 100 feedlot cattle in central Nebraska over a heat wave event that had three spikes in thermal loads. Deaths occurred during the third spike where it was hypothesised that *ad libitum* feed intake resulted in large metabolic heat load, which in conjunction with environmental heat load, surpassed the animals' ability to regulate thermal balance (Hahn, 1999)
- 1999 – over 5 000 feedlot cattle died during an extreme heat wave event in north-eastern Nebraska (Brown-Brandl et al., 2006a; Brown-Brandl et al., 2006b)
- February 2000 – 1 255 cattle died in south western New South Wales with deaths occurring after a rainfall event where climatic conditions presented high RH and high overnight T_A (Entwistle et al., 2000)
- January 2013 & 2014 – feedlot deaths were experienced in south-east Queensland; however mortality statistics are not readily available (Reinhart *pers. comm.*).

The literature for heat stress related deaths indicates that the US feedlot industry experiences higher economic and animal losses compared to the Australian industry. However there are noticeable differences between grain feeding cattle to meet the market specifications of the US and Australian domestic markets. Cattle being fed for the US domestic market are typically fed for 150 to 180 days whereas the Australian domestic market cattle are typically fed for 70 to 90 days (Gaughan *pers. comm.*). Furthermore cattle for the US market tend to enter feeding programs during October and are finished in July and typically do not have access to shade (Gaughan *pers. comm.*). Therefore it could be concluded that the differences in feeding cattle for their respective domestic markets has an impact on the susceptibility of each country's industry to heat load and ultimately the occurrence of mortalities within the US industry.

2.3 Thermal Exchange

The thermal environment influences animal performance through the net effects of heat energy exchanges between the animal and its surrounding environment, highlighted in Figure 2.1 below (Hahn, 1985). Heat exchange mechanisms for heat accumulation and dissipation are conduction, convection, radiation and evaporation. For an animal to maintain core body temperature, heat accumulated through energy exchange mechanisms and metabolic functions, must equal that of heat energy dissipated from the body (Hahn, 1985). As T_A increases, heat exchange between the animal and its surrounding environment becomes less effective. At this point cattle redistribute energy from growth towards heat dissipation in an attempt to maintain homeostasis (Ravagnolo and Misztal, 2002).

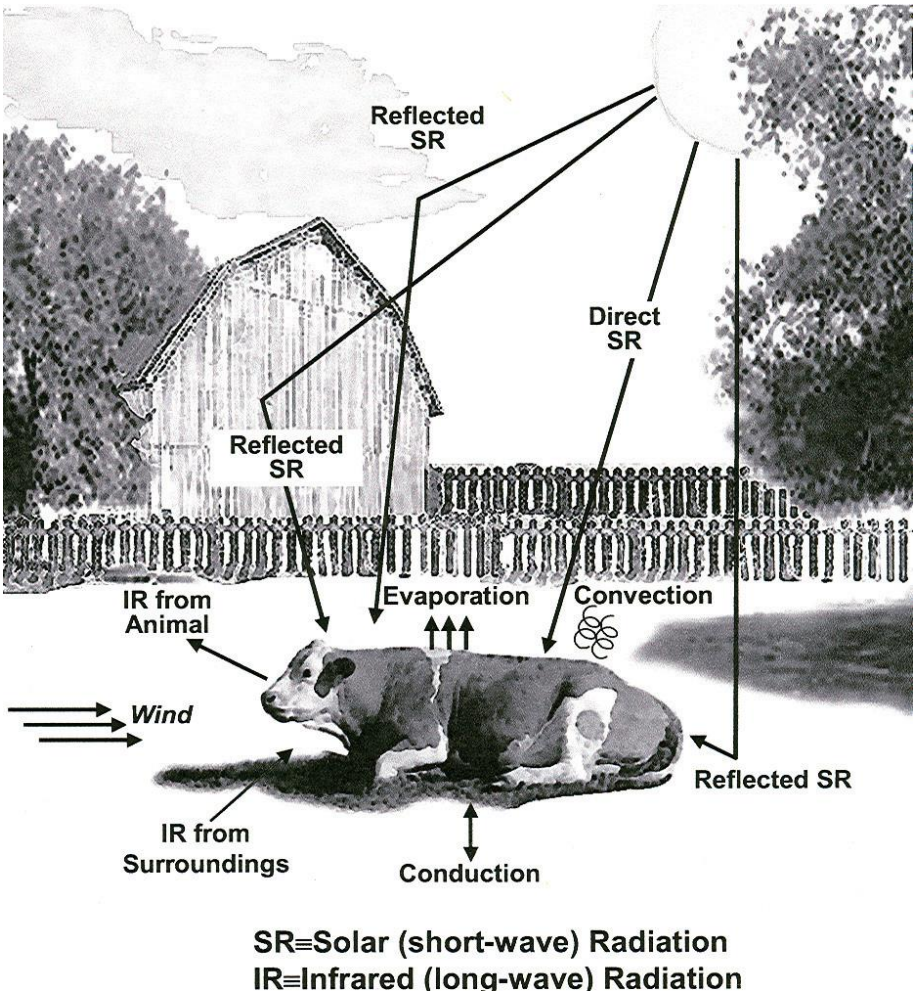


Figure 2.1: Thermal exchanges between an animal, with a body temperature of 39 °C, and its surrounds in a hot environment (adapted from Hahn, 1985; Hahn, 1994; DeShazer et al., 2009)

The net thermal exchange that the animal undergoes is dependent on environmental conditions, animal factors and the animal's surroundings (Hahn, 1985). The interactions between heat transfer and some of the animal and housing factors are presented in Table 2.1. Hahn (1985) indicated that

thermal exchange is influenced by biological factors such as tissue insulation; vasodilation, particularly under the skin; and postural position. However the dissipation of accumulated heat load from the body during hot conditions is often ineffective as heat accumulation is often greater than heat dissipation (Bertipaglia et al., 2007).

Table 2.1: Physical Factors Influencing Heat Energy Transfer from an Animal

Factor	Mode of Heat Exchange			
	Radiation	**Convection**	**Conduction**	**Evaporation**
Surface area of animal	X[a]	X	X[b]	X[c]
Temp. of animal surface	X	X	X	X[d]
Temp. of surroundings	X		X[e]	
Temp. of air		X		X
Velocity of air		X		X
Vapour pressure of air				X
Shape factor of radiation source or sink	X			
Emissivity of animal surface	X			
Conductivity of surroundings			X[e]	
Emissivity of surroundings	X			

[a] The area of the animal directly exposed to the radiation source or sink
[b] For animals standing, conduction heat transfer is negligible; for animals lying down the area of the animal surface in contact with the supporting structure becomes a factor
[c] The wetted area of the animal, including respiratory passages
[d] The temperature of the animal surface is an indirect factor, since vapour pressure is a function of temperature
[e] Only that portion of the surroundings actually in contact with the animal
(Adapted from Hahn, 1985, pp 160)

2.3.1 Conduction

Heat exchange by conduction occurs when there is direct contact with an object or surface at a different temperature (Gebremedhin, 1985). Temperature gradients influence heat exchange, i.e. if direct contact occurs where the temperature of the object is greater than that of the animal heat will be gained by the animal. For example Mader and Davis (2004) suggested that when ground surface temperature exceeds skin temperature, the animal will accumulate heat. This heat gain must then be later dissipated for the animal to return to a thermal equilibrium. Conductive heat exchange can be represented in the following equation;

$$K = A \times h_c \times (\overline{T_s} - T_A)$$

Where K = heat exchange via conduction; A = surface area of the animal; h_c = thermal conductivity of the object in contact with the skin; $\overline{T}_s$ = average skin temperature; and T_A = ambient temperature (Robertshaw, 1985).

2.3.2 Convection

Convective heat exchange occurs by the loss of heat from the movement of air adjacent to the skin, where there are temperature changes due to conduction of heat from the skin to the air surrounding the animal (Robertshaw, 1985). There are two types of convective heat dissipation i) free and ii)

forced. As air temperature increases, air density decreases resulting in air movement upwards and away from the animal (Robertshaw, 1985). This process is referred to as free convective heat loss. Forced convective heat loss refers to increased air movements and can be due to natural winds or artificial air movement, e.g. fans. Modifying the environment by increasing air movement enhances an animal's ability to dissipate heat via convection (Berman, 2008). Berman (2008) indicated that forced ventilation is principally effective when T_A is in the lower range during heat load conditions. Modifying air movements to produce air flows at a velocity of 1 m/s are capable of producing air streams of between 0.3 and 0.6 m/s over an animals' surface (Berman, 2006). Berman (2005) reported that the effects of RH on thermoregulation are minimised when air velocities are between 1.5 to 2.0 m/s. Providing heat dissipation opportunities through forced ventilation does not directly modify the ambient conditions. It does, however, provide relief from heat load (Berman, 2010). However during periods where T_A is high there is a reduced ability to dissipate heat via convection (Silanikove, 2000). When ambient conditions surpass skin temperature, the animal is no longer able to dissipate heat to the surrounding air via convection (Silanikove, 2000).

2.3.3 Evaporation

Evaporative heat loss occurs by the exchange of heat from the skin and respiratory surfaces. As T_A increases, the proportion of heat exchange via evaporative heat loss also increases (Kadzere et al., 2002). When T_A is equivalent to the body temperature of the animal, evaporative heat loss becomes the predominant means of heat dissipation (Esmay, 1969). Evaporative heat exchange occurs at the skin's surface and throughout the respiratory tract (Kadzere et al., 2002). During periods of high heat load evaporative heat loss is increased via respiration, panting and sweating (McLean, 1963).

2.3.4 Radiation

Radiant heat exchange is the transfer of heat energy through the exchange of electromagnetic waves from the sun and surrounding structures (Robertshaw, 1985). The degree of radiation absorbed by an animal is dependent on a number of factors including body surface temperature, coat colour, hair follicle characteristics and coat surface texture (Silanikove, 2000). Cattle with white or lighter coloured coats absorb approximately 40 to 50 % less radiation, when compared with animals of darker or black coloured coats (King et al., 1988). However, Mader et al. (2002) indicated that as body condition score and days on feed increases, their ability to detect randiant heat from other animals diminishes.

2.4 Assessment of Heat Load Using Climatic Indices

The development of any climatic indices requires knowledge and understanding of the thermal status of livestock and the heat exchange between animal and the environment (Parsons et al., 2001). Over the years numerous indices have been developed; notably the Black Globe Humidity Index (Buffington et al., 1981); Wet-Bulb Globe Thermometer Index (Lee, 1980); THI (Thom, 1959); HLI (Gaughan et al., 2008b); and Comprehensive Climatic Index (Mader et al., 2010b). The THI and HLI are currently the most frequently used in beef production systems. Historically the development of climatic comfort indices has been for human application, particularly the THI, developed by Thom (1959), although the THI has had extensive application in livestock production particularly in the dairy industry. As knowledge about thermal exchange continues to be developed, limitations in earlier climatic index models are identified. This has led to the development of a number of indices that represent the net effect that environmental conditions have on heat load in cattle.

2.4.1 Temperature Humidity Index

The THI incorporates the combined effects of air temperature, either wet bulb or dew point temperature, and RH (Buffington et al., 1981; Bohmanova et al., 2007). The combined effects of T_A and RH are represented by single a value that can be used as a reference for the level of thermal stress an animal is experiencing at a particular time point (Bohmanova et al., 2007). The discomfort index described by Thom (1959) was further developed as a Livestock Weather Safety Index (Figure 2.2) to forecast and prevent heat stress related production losses (Bohmanova et al., 2007). The THI has been successful in adequately describing the impact of hot conditions on livestock performance. The success of the THI is due to the influence of T_A and RH on thermal exchange mechanisms of animals (St-Pierre et al., 2003; Brown-Brandl et al., 2006a; Hahn et al., 2009). Historically the THI has been the gold standard in classifying the intensity of the thermal environment on livestock (Hahn et al., 2009).

Temperature, °C \ Relative Humidity, %	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
21	64	64	64	65	65	65	66	66	66	67	67	67	68	68	68	69	69	69	70	70
22	65	65	65	66	66	67	67	67	68	68	69	69	69	70	70	70	71	71	72	72
23	66	66	67	67	67	68	68	69	69	70	70	70	71	71	72	72	73	73	74	74
24	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75	76	76
26	68	68	69	69	70	70	71	71	72	73	73	74	74	75	75	76	76	77	77	78
27	69	69	70	70	71	72	72	73	73	74	75	75	76	76	77	78	78	79	79	80
28	69	70	71	71	72	73	73	74	75	75	76	77	77	78	79	79	80	81	81	82
29	70	71	72	73	73	74	75	75	76	77	78	78	79	80	80	81	82	83	83	84
30	71	72	73	74	74	75	76	77	78	78	79	80	81	81	82	83	84	84	85	86
31	72	73	74	75	76	76	77	78	79	80	81	81	82	83	84	85	86	86	87	88
32	73	74	75	76	77	78	79	79	80	81	82	83	84	85	86	86	87	88	89	90
33	74	75	76	77	78	79	80	81	82	83	84	85	85	86	87	88	89	90	91	92
34	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94
36	76	77	78	79	80	81	82	83	85	86	87	88	89	90	91	92	93	94	95	96
37	77	78	79	80	82	83	84	85	86	87	88	89	90	91	93	94	95	96	97	98
38	78	79	80	82	83	84	85	86	87	88	90	91	92	93	94	95	97	98	99	100
39	79	80	81	83	84	85	86	87	89	90	91	92	94	95	96	97	98	100	101	102
40	80	81	82	84	85	86	88	89	90	91	93	94	95	96	98	99	100	101	103	104
41	81	82	84	85	86	88	89	90	91	93	94	95	97	98	99	101	102	103	105	106
42	82	83	85	86	87	89	90	92	93	94	96	97	98	100	101	103	104	105	107	108
43	83	84	86	87	89	90	91	93	94	96	97	99	100	101	103	104	106	107	109	110

Categories of Livestock Weather Safety Index associated with THI values:
Normal: ≤ 74 Alert: 75-78 Danger: 79-83 Emergency: ≥84

Figure 2.2: Temperature Humidity Index (THI) values based on temperature humidity index equation by Thom (1959) showing the United States of America's Livestock Weather Safety Index categories (Adapted from LCI (1970) and Hahn et al. (2009))

The THI can be calculated using the following equation as adapted from Thom (1959);

$$THI = 0.8 \times T_A \left[\left(\frac{RH}{100} \times (T_A - 14.4) \right] + 46.4 \right.$$

Where RH = Relative Humidity (%) and T_A = wet bulb or dew point temperature

The THI equation produces a unit value that is associated with stress categories in order to provide an indication of the severity of heat load conditions. The THI thresholds are ≤ 74 no stress, indicating thermoneutral conditions; 75 to 78 alert, mild to moderate stress; 79 to 83 danger moderate to severe stress; and ≥ 84, emergency, extreme stress where deaths due to excessive heat load may occur.

Numerous authors identified the limitations of the THI where it does not account for WS or SR, which are known to influence thermal exchange and ultimately the animal's response to heat load conditions. Gaughan et al. (2008b) identified the limitations of the THI, which lead to the development of the HLI model incorporating RH, WS and BGT. By using BGT within the HLI

model Gaughan et al. (2008b) were able to incorporate the combined effects of T_A, RH, SR and WS in a single unit measure (Hammond et al., 1996).

2.4.2 Heat Load Index

The HLI was developed primarily for feedlot cattle, specifically for *Bos taurus*, where it has been established as a management tool during hot conditions. Development of the HLI improved the ability of indices to predict the impact of hot climatic conditions on livestock performance and welfare (Gaughan et al., 2008b). Incorporating the net effects of RH, WS and BGT, where the index takes the following forms;

i) A nonlinear regression which applies when BGT is greater than 25 °C

$$HLI_{BGT>25} = 8.62 + (0.38 \times RH) + (1.55 \times BGT) - (0.5 \times WS) + [e^{2.4-WS}]$$

ii) A linear model which applies when BGT falls below 25 °C;

$$HLI_{BGT<25} = 10.66 + (0.28 \times RH) + (1.3 \times BGT) - WS$$

Where RH = Relative Humidity (%); BGT = Black Globe Temperature (°C); WS = wind speed (m/s); and e = the base of the natural logarithm (approximate value of e = 2.71828)

In the development of the HLI model, thresholds and adjustments were identified, allowing for numerous animal factors and management strategies to be incorporated within the model. Animal factors that were incorporated into the model included genotype, coat colour and health status (Gaughan et al., 2008b). Management factors influencing the threshold adjustments were shade availability, days on feed, manure management and drinking water temperature (Gaughan et al., 2008b). Threshold adjustments (+ and -; Table 2.2) were modelled from a reference animal (a healthy, un-shaded Angus steer, < 100 days on feed), where a positive value indicates that the threshold has been increased and a negative value indicates that the threshold has decreased. The development of the adjustment factors allows for the HLI model to be adapted to countless locations and also across different areas. From Table 2.2, a healthy un-shaded Angus prior to 100 days on feed, the upper HLI threshold at which the animal accumulated heat load was established at 86 (HLI = 86), and the lower threshold at which the animal dissipates heat was defined at 77 (Gaughan et al., 2008b). Conversely a healthy un-shaded Brahman prior to 100 days on feed, the upper threshold was defined as 96 (HLI = 86 + 10); however the authors acknowledged that the upper threshold of a purebred *Bos indicus* animal may be greater than 96 (Gaughan et al., 2008b). The authors acknowledged that there was not sufficient data where HLI > 95 to provide a definitive HLI threshold for these animals, indicating that further investigation in this area is required.

Although it is worthy to acknowledge that conditions where HLI ≥ 95 are difficult to replicate and assess in natural and controlled environments.

Table 2.2: Animal (genotype, coat colour, health status, acclimatisation) and management (access to shade, days on feed, manure management and drinking water temperature) adjustments (+ and -) to the heat load index (HLI) threshold (86) of the reference steer (a healthy, un-shaded Angus, < 100 days on feed)

Item	Relative effect on upper HLI threshold of the reference steer (HLI = 86)
Genotype[1]	
Bos taurus (British)	0[2]
Bos taurus (European)	+ 3 (i.e. 86 + 3)
Wagyu	+4
Bos indicus (25 %)	+4
Bos indicus (50 %)	+7
Bos indicus (75 %)	+8
Bos indicus (100 %)	+10
Coat Colour[1]	
Black	0
Red	+1
White	+3
Health Status	
Healthy	0
Exhibiting Illness/ Recovering	-5
Acclimatisation	
Acclimated	0
Not Acclimated	-5
Shade[3]	
No Shade	0
Shade (> 1.5 – 2.0 m^2/animal)	+3
Shade (> 2.0 – 3.0 m^2/animal)	+5
Shade (> 3.0 m^2/animal)	+7
Days on Feed[4]	
0 – 80 days	+2
80 – 130 days	0
130 + days	-3
Manure Management (maximum depth)[5]	
Manure pack – 50 mm	0
Manure pack – 100 mm	-4
Manure pack – 200 mm	-8
Drinking Water Temperature[6]	
15 – 20 °C	+1
21 – 30 °C	0
31 – 35 °C	-1
> 35 °C	-2

[1]Not all cattle were assessed within each threshold trait. For example coat colour was assessed only in *Bos taurus* cattle, manure management was assessed at 5 feedlots and drinking water temperature was assessed on 3 feedlots.
[2]The values for the reference steer are presented as 0 (i.e. no change from the threshold of 86).
[3]For shade that provides 70% block out (includes shade cloth and also steel structure with gaps in the roof). Un-shaded *Bos indicus* cattle > 25 % not included.
[4]Not all cattle were assessed for this trait. Wagyu cattle excluded from 130 + days.
[5]Mean manure pack depth over 54 days.
[6]Only un-shaded Angus cattle were assessed for this trait.
(Adapted from Gaughan et al. 2008b, page 230)

In addition to the HLI, Meat and Livestock Australia and Katestone Environmental developed the Cattle Heat Load Toolbox (http://chlt.katestone.com.au/) to assist Australian feedlots by forecasting hot weather conditions. This forecasting system provides a five day forecast showing hourly changes in localised climatic conditions. The website also allows feedlots to predict the impact of climatic conditions on their cattle, providing an opportunity to make management changes in preparation for heat load conditions.

2.4.3 Accumulated Heat Load

Cattle have the ability to accrue heat energy throughout the day, influencing the variability of core body temperature, and dissipate the accumulated heat load at night if conditions permit (Hahn, 1999; Mader and Davis, 2004; Gaughan et al., 2008b). If the conditions are insufficient to allow for night time heat dissipation, cattle will enter the subsequent day with an accumulated heat load, consequently increasing an individual's susceptibility to heat load (Gaughan et al., 2008b). During periods of sustained hot climatic conditions, particularly high T_A and RH, cattle can continually gain accumulated heat load with limited night time relief resulting in excessive heat load (Mader et al., 2006). In conditions where excessive heat load is prolonged, cattle are no longer able to regulate core body temperature, which may result in mortalities (Mader et al., 2006).

Following the development and validation of the HLI model Gaughan et al. (2008b) established the accumulated heat load model. The accumulated heat load model incorporates the amount of time the animal is exposed to the HLI upper threshold and animal heat balance (Gaughan et al., 2008b). When an animal is exposed to conditions above the upper threshold, i.e. HLI = 86, for a healthy un-shaded Angus < 100 days on feed, the individual is unable to dissipate sufficient accumulated heat load back to the environment therefore increasing core body temperature (Gaughan et al., 2008b). Furthermore if the animal is exposed to its HLI lower threshold, i.e. HLI = 77, for the reference animal, the individual is able to dissipate the accumulated heat load to the environment, returning core body temperature to a 'normal' body temperature (Gaughan et al., 2008b). Gaughan et al. (2008b) established the following equations to calculate accumulated heat load;

i) If $[HLI_{ACC} < HLI_{Lower\ Threshold}, (HLI_{ACC} - HLI_{Lower\ Threshold})/M]$; and

ii) If $[HLI_{ACC} > HLI_{Upper\ Threshold}, (HLI_{ACC} - HLI_{Upper\ Threshold})/M, 0]$

Where HLI_{ACC} = the actual HLI value at a point in time; $HLI_{Lower\ Threshold}$ = the HLI lower threshold where cattle will dissipate heat (e.g. 77); $HLI_{Upper\ Threshold}$ = the HLI upper threshold where cattle will gain heat (e.g. 86); and M = number of measures per hour, i.e. number of times HLI data are collected per hour; If every 10 minutes, then M = 6 (Gaughan et al., 2008b).

2.4.4 Limitations of Indices

Over the years there has been the development of a number of climatic indices. Each consecutive index has established the limitations of the previous index. The purpose of climatic indices is to provide a tool for the strategic management of livestock during adverse climatic conditions. Climatic indices need to be dynamic in order to provide an index that is broadly applicable across a range of climatic conditions and different life stages and species, to maximise animal welfare (Mader et al., 2010b). Therefore the development of indices that are comprehensive in nature and allow for greater application across a broad range of environmental conditions are required (Mader et al., 2010b). Whilst each consecutive index has provided a better understanding of i) the impact of the thermal environment on animals and ii) animals' responses to the thermal environment, each index cannot completely account for biological and physiological responses of each individual animal; therefore highlighting the importance of recognising animal responses to heat load.

2.5 Animal Factors Influencing Heat Load

How an animal responds to hot climatic conditions is dependent on a number of individual characteristics. Therefore no two animals will respond to hot climatic conditions in the same manner. There are a number of factors that influence how an animal will respond to heat load conditions including genotype, coat characteristics, health and days on feed. Vulnerable animals have been described as those with black or dark coats (and skin); compromised immune systems, i.e. a history of pneumonia; greater fat cover (greater body condition scores) and animals that are excitable in temperament (Brown-Brandl et al., 2006a).

2.5.1 Genotype

An animal's genotype is a major factor contributing to its susceptibility or tolerance to heat load. It is widely accepted that *Bos indicus* breeds have a greater heat tolerance compared to *Bos taurus*. Gaughan et al. (2010b) indicated that the identification of heat tolerant cattle is not a new concept, as many breeds are already known for their thermal tolerance, i.e. Brahman and other *Bos indicus* breeds (Brown-Brandl et al., 2006b). However in many cases heat tolerance comes at the cost of growth and reproduction when compared to non-heat tolerant counterparts (Gaughan et al., 2010b). Moreover there are *Bos taurus* genotypes that are tropically adapted and able to cope with hot climatic conditions. However the heat tolerance of *Bos taurus* genotypes does not compare those animals of *Bos indicus* heritage (Carvalho et al., 1995). Heat tolerance also varies within genotype, therefore the identification of individual heat tolerant animals within a breed may be useful if these animals are able to maintain productivity throughout summer conditions (Gaughan et al., 2010b). Brown-Brandl et al. (2006b) found that Angus cattle had higher respiration rates, panting scores and

skin temperatures when compared to other *Bos taurus* genotypes. Gaughan et al. (2010b) concluded that Angus × Hereford cattle had a lower heat tolerance compared to purebred Angus under heat stress conditions.

2.5.2 Coat Characteristics

Coat characteristics are crucial in an animal's ability to dissipate excess heat from the body (Gebremedhin and Wu, 2002), as the characteristics of the coat determine the absorption of SR by the animal. The absorbance properties, more so the reflection and absorption characteristics of the coat, has an effect on the balance of radiant heat exchange mechanisms (Blackshaw and Blackshaw, 1994). This is of particular importance as the absorption of SR can potentially outweigh heat produced by metabolic processes (Brown-Brandl et al., 2005a; Gebremedhin et al., 2011). The coat characteristics of different breeds of cattle, i.e. thick and woolly versus fine and glossy, highlight potential differences in the insulative properties of the hair coat (Blackshaw and Blackshaw, 1994). Cattle with black coats have higher body surface temperatures, respiration rates and panting scores when compared with animals that have white or lighter coloured coats (Brown-Brandl et al., 2006b). Brown-Brandl et al. (2006a) concluded that Black Angus and dark coated MARC III heifers had similar respiration rate responses associated with increasing T_A where mean respiration rates were 94.0 ± 1.2 bpm and 93.4 ± 1.2 bpm respectively. Furthermore similar trends of increasing respiration rates in dark/black coated animals, the Angus and MARC III heifers had higher ($P < 0.05$) respiration rates compared with heifers of lighter coat colouration of Gelbvieh (84.6 ± 1.0 bpm) and Charolais (78.1 ± 1.0 bpm) heifers (Brown-Brandl et al., 2006a).

A study by Gebremedhin et al. (2011) determined that coat colouration influences the regulation of body temperature. King et al. (1988) reported that cattle with white or light coloured coats absorbed 40 to 50 % less SR compared to cattle with black or darker coloured coats. Finch et al. (1984) indicated that dark red coat coloured *Bos taurus* had rectal temperature that were on average 0.3 °C higher compared to white coat coloured *Bos taurus* cattle. Mader et al. (2002) indicated that dark coat coloured animals generally reached peak body temperature 1 to 2 hours earlier, compared to light coat coloured animals. Furthermore Gebremedhin et al. (2011) concluded that black and dark coloured coats absorb significantly higher rates of SR compared to their lighter coloured counterparts (Figure 2.3). Gaughan et al. (1998) reported that predominantly white coloured dairy cattle did not actively seek shade during hot conditions. However Mader et al. (2002) reported that as body condition score and days on feed increases, cattle with light coloured coats tend to behave similarly to dark coloured animals during hot conditions.

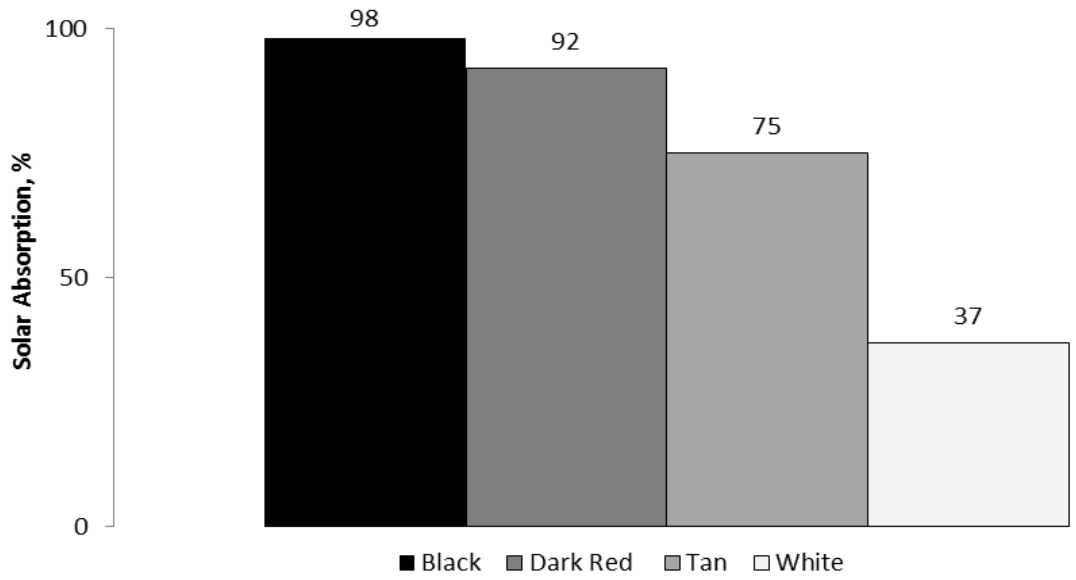


Figure 2.3: Difference in solar radiation absorption of different coat colours (Adapted from Gebremedhin et al., 2011)

Coat characteristics, particularly colouration, need to be taken into consideration when assessing heat tolerance in cattle. The practical implication of coat colouration within a commercial setting continues to amplify, as the population of Angus within Australian feedlots continues to increase. The coat characteristics of individual cattle not only impacts on accumulated heat load, through absorbed SR, but also the behavioural responses of cattle to ambient conditions. Therefore it is important to assess the coat characteristics of cattle and make adjustments to predictive models, i.e. HLI, to accurately forecast the impact of hot conditions.

2.5.3 Body Condition and Days on Feed

It is widely accepted that the more time cattle spend in a feedlot the heavier, and greater body fat composition, the cattle will become. Visual body condition scores are often used to determine the physical condition of feedlot cattle typically using a 1 (emaciated) to 5 (obese) scale (Table 2.3) (Houghton et al., 1990; Department of Agriculture Fisheries and Forestry, 2009). During a heat wave event in February 2000, 1 255 cattle died due to heat load, where a majority of the deaths occurred in un-shaded pens, where the cattle were > 70 days on feed (Entwistle et al., 2000). Brown-Brandl et al. (2006a) indicated that finished feedlot heifers with higher body condition scores had respiration rates that were 6.8 % higher compared to heifers with a lower body condition score. Yeates (1956) reported that heat tolerance of "fat" well-fed cattle was lower compared with "thin" poorly fed cattle. Comparing heavy body condition score Angus and Charolais heifers, Angus cattle had a 23.6 % higher respiration rates than that of their Charolais counterparts (Brown-Brandl et al., 2006a). A survey of heat load mortalities after a heat wave event in Iowa during July

1995, reported that light weight cattle (362 to 476 kg) had lower mortalities (3.4 %) compared to moderate (487 to 535 kg; 5.0 %) and heavy (544 to 567 kg; 5.9 %) cattle (Bushby and Loy, 1997). The literature supports that heavier and greater fat density, i.e. fatter, cattle are more vulnerable to the negative effects of heat load and heavier cattle are more likely to succumb to excessive heat load.

Table 2.3: Visual assessment of body condition

Body condition score	Condition	Description
1	Emaciated	Severe muscle wasting; no fat reserves; extremely prominent skeletal body outlie i.e. backbone, shoulder, pins and ribs
2	Thin	Little to no muscle wasting; little to no fat coverage; body outline bony; prominent skeletal body with normal appearing muscle structure
3	Ideal	Normal muscle structure; visible fat deposits on body; body outline almost smooth i.e. hip bones visible faintly, ribs generally not visible, some smoothness around shoulder
4	Fat	Normal muscle structure; considerable fat deposits on body, body outline becomes rounded; very smooth over backbone with no skeleton visible; ribs well covered
5	Obese	Normal muscle structure; extreme fat deposits over body, body curvature becomes square with 'bulging' appearance, prominent brisket; broad flat top line; ribs very well covered

Adapted from Houghton et al. (1990) and Department of Agriculture Fisheries and Forestry (2009)

2.5.4 Health Status

Hot ambient conditions have a negative influence on animal bioenergetics, and as such have negative flow on effects on animal performance, health and well-being (Brown-Brandl et al., 2005a). However the health status of an animal is also likely to have a significant influence on the animal's ability to cope with heat load conditions. Animals that are immunocompromised are more vulnerable during heat load conditions (Brown-Brandl et al., 2006a). Animals that are suffering from illness typically have an elevated body temperature. The net effect of illness related fever and exposure to heat load conditions could potentially result in mortalities (Silanikove, 2000). A study by Brown-Brandl et al. (2006a) reported that animals with previous treatment history for pneumonia, anytime from birth to slaughter, had respiration rates that were on average 10.5 % higher compared to those never diagnosed or treated. A study by Gardner et al. (1999) reported that there were differences in the average daily gain (**ADG**) of steers with no (1.58 kg/d), in-active (1.43 kg/d) and active (1.17 kg/d) respiratory tract lesions. Brown-Brandl et al. (2006a) also reported that animals previously treated for pneumonia had significantly ($P < 0.05$) lower ADG (1.46 ± 0.04 kg/d) compared to non-treated (1.54 ± 0.02 kg/d) cattle. Sowell et al. (1997) also reported that cattle that had been clinically diagnosed and treated for illnesses spent 23 % less time at the feed bunk

compared to clinically healthy cattle. These studies suggest that previous diagnosis and treatment of illnesses have a lasting influence on the health status and overall performance and welfare of individual animals within a feedlot.

2.6 Adaptation and Acclimation

All animals possess the capacity to adapt to their thermal environment. Animals are capable of modifying their behavioural, physiological and morphological, or a combination of these, characteristics in response to the thermal environment (Angilletta Jr., 2009). Thus all animals have developed survival techniques that minimise the effect that the thermal environment has on the body as a whole. The coping mechanisms developed by animals can be summarised into adaptation and acclimation. Gaughan (2002) indicated that adaptation and acclimation have different meanings, which are often interchanged.

Acclimation is a homeostatic process that is driven by the endocrine system, resulting in cellular, metabolic and systemic changes, enabling animals to respond and cope with thermal stressors. Acclimation can be separated into i) developmental and ii) reversible (Angilletta Jr., 2009). Developmental acclimation refers to irreversible changes, and reversible acclimation refers to regulated animal responses, i.e. changes in response to the changing seasons (Angilletta Jr., 2009) such as changing coat characteristics. Therefore acclimation can be considered as a within a lifetime process whereby continuous exposure to a particular stressor, i.e. hot climatic conditions, results in biological adjustments thereby increasing the fitness of that individual animal to survive in those conditions (Horowitz, 2001). Horowitz (2001) also indicated that a part of the acclimation response is a widening in the dynamic range of body temperature, resulting in greater shifts in upper and lower critical temperature. Hahn and Mader (1997) reported that cattle appear to be acclimating where post heat wave event body temperature transitioned and stabilised around a new elevated temperature. Changing the dynamic range in body temperature will have a positive influence on the regulation of body temperature through adjustments to heat accumulation and dissipation from the body.

Adaptation, however, refers to the biological change in successive generations by favouring genetic selection within a population due to continuous stressor exposure that supports species survival (Roy and Collier, 2012). *Bos indicus* cattle evolved in tropical regions, with high T_A and RH, and as a result these breeds of cattle have a number of genetic differences that support thermotolerance (Hansen, 2004; Roy and Collier, 2012). Therefore the survivability of *Bos indicus* breeds in tropical environments arises from the adaptations developed throughout successive generations.

2.7 Nutritional Influences and Response to Heat Load

Feedlot cattle are particularly susceptible to heat load due partially to the nature of the diets they are fed (Blackshaw and Blackshaw, 1994), i.e. high energy concentrate feeds. High energy dense feeds have the potential to increase core body temperature (Cho et al., 2014). The heat increment for feedlot cattle is high, 35 to 70 % of metabolisable energy (**ME**), depending on the balance of nutrients within the diet (Blackshaw and Blackshaw, 1994). As heat load conditions increase, the energy required for maintenance increases. An increase in energy requirements are associated with the behavioural and physiological responses, i.e. panting, initiated by cattle for maintaining core body temperature in response to thermal loads (Beede and Collier, 1986). One response to increasing thermal loads is to reduce DMI (Ray, 1989; Hahn et al., 1992). The reduction in DMI subsequently results in a reduction of available ME and essential nutrients to support bodily functions (Beede and Collier, 1986). However the reduction in DMI is also associated with a decrease in heat production, via ruminal fermentation and metabolism, thus aiding in maintaining the overall heat balance of the animal (Beede and Collier, 1986; Hahn, 1999). Beede and Collier (1986) indicated that a confounding factor in voluntary DMI reductions was reduced gut motility and rumination. Furthermore, the less digestible the diet fed during heat load conditions, the greater the rate and extent of reduction in DMI (Beede and Collier, 1986).

2.7.1 Feed Intake and Eating Behaviour

Feed intake and feeding behaviours are not a suitable measure of thermal status as these behaviours are intermittent (Brown-Brandl et al., 2005a). However the pattern in feeding behaviour may be highly repeatable (Hicks et al., 1989). Alterations to DMI also have a lag effect where animals are adjusting their feed intake based on numerous factors, including ambient conditions and previous feed intake (Brown-Brandl et al., 2005a). Voluntary feed intake appears to decline when T_A is approximately 25 °C to 27 °C (Beede and Collier, 1986). However the T_A at which DMI begins to decline is influenced by diet type and composition (Beede and Collier, 1986). Mader et al. (1999b) indicated that high roughage diets, those containing greater than 25 % of diet dry matter (**DM**), and lower in ME density, appear to contribute less to metabolic heat load. Brosh et al. (1998) concluded that heifers on a high ME (10.6 MJ/kg) diet had significantly ($P < 0.001$) higher DMI (1.76 times) compared with heifers offered low ME (7.2 MJ/kg) rations (Brosh et al., 1998). Variations in DMI are also influenced by breed (genotype); production status; health status; body condition; and days on feed. Brosh et al. (1998) indicated that time of feeding also impacts on heat production and heat balance. However the authors concluded the effects of time of feeding were confounded by ambient conditions (Brosh et al., 1998). Differences in feed intake indicate that cattle compensate for hotter conditions by consuming smaller meals, more frequently (Brown-Brandl et al., 2005a). Ray and

Roubicek (1971) indicated that there was a peak in feeding activity where a majority of feed consumption occurred in the late afternoon and early evening. Brown-Brandl et al. (2005a) also reported that un-shaded cattle appear to be adapting feed consumption times by shifting feed intake to the cooler hours of the day, typically between 0200 h and 0600 h.

Brown-Brandl et al. (2003) conducted a study investigating the difference in DMI in three groups of beef steers housed in environmental chambers at different temperature treatments (18 ± 7 °C, 30 ± 7 °C, and 34 ± 7 °C). The authors concluded that the highest DMI intake was recorded in the lowest temperature range (18 ± 7 °C); whilst the steers exposed to high temperatures (30 ± 7 °C and 34 ± 7 °C) recorded lower DMI. The authors found that the two heat stress groups reduced DMI during the first several days and DMI then stabilised for the remainder of the heat stress period. Hahn (1985) reported similar findings with mean DMI over 5 days at thermoneutral conditions as 15.93 ± 1.03 kg with a reduction of 69 % on day 3 of a heat wave event. Reducing DMI decreases the quantity of heat produced by rumen fermentation and other associated metabolic functions (Sanchez et al., 1994). Therefore by reducing DMI, cattle are able to decrease heat production within the body, thus decreasing the amount of heat that must be dissipated from the body to maintain homeostasis.

2.7.2 Water Requirement

Water accounts for approximately 60 % of body weight and accounts for 98 % of all molecules within the body (NRC, 1981). There are numerous factors that influence daily water intake including ambient conditions; diet type; breed (genotype); weight; and physiological functions, all of which confound the determination of water requirement in cattle (Arias and Mader, 2011). Daily water intake and consumption is influenced by a number of body functions, including but not limited to (NRC, 2000);

i. the regulation of core body temperature;
ii. growth,
iii. reproduction and lactation; and
iv. digestion, metabolism; and
v. hydrolysis of proteins, fats and carbohydrates;

There are three primary sources of water available to cattle (NRC, 1981);

i. free drinking water;
ii. water available within feed; and
iii. water produced in the oxidation of organic compounds (metabolic water)

However daily water intake appears to be primarily driven by DMI, where the level of intake (kg/d) and type of ration offered, i.e. concentrates versus roughage, influence the amount of water consumed by cattle (McDowell and Weldy, 1967). Brosh et al. (1998) indicated that there was an

interaction between ME and SR that overall resulted in an increase in daily water intake. Arias and Mader (2011) reported that feedlot cattle finished in the summer consumed 87.3 % more ($P < 0.01$) water compared to cattle finished during winter (32.4 L/d versus 17.3 L/d). Additionally Arias and Mader (2011) indicated that thermal exchange is often impeded during heat wave events where adequate water supplies are unavailable. Parker et al. (2000) concluded that increased water consumption during summer can be attributed to the thermoregulatory mechanisms evoked to regulate core body temperature. Therefore ambient conditions do cause variations in daily water intake; the effect of increasing T_A can be viewed in Table 2.4 below.

Table 2.4: Water Requirements of Beef Cattle under Different Ambient Conditions

Ambient Conditions	Water Requirements (kg/ kg DMI)
> 35 °C	8 to 15
25 to 35 °C	4 to 10
15 to 25 °C	3 to 5
-5 to 15 °C	2 to 4

(Adapted from NRC, 1981)

Arias and Mader (2011) concluded that the THI and SR were the primary factors influencing daily water intake during summer. However Beede and Collier (1986) attributed an increase in daily water intake during summer to the animal attempting to regulate core body temperature during hot conditions. The study by Arias and Mader (2011) identified daily water intake increased at a THI threshold of 67.2, where the authors concluded that the THI threshold of 67.2 may have represented the THI value at which cattle were activating thermoregulatory mechanisms to cope with heat load. However the Livestock Weather Safety Index indicates that a THI value between 70 and 74 as the lower threshold where cattle are experiencing no stress. This highlights that response to heat load is exceptionally varied between not only groups of animals but also individual animals.

2.8 Animal Responses to Heat Load

Cattle are often exposed to numerous stressors in natural and built environments (Gaughan et al., 2013). Stressors affecting cattle may be chronic, lasting from a few weeks to months, i.e. heat load through summer, or acute, lasting a few minutes to a few days, i.e. heat wave events (Gaughan et al., 2013). It is not uncommon for feedlot cattle to be exposed to a number of low level stressors including non-heat stressors such as variations in nutrition and housing management, which over a period of time potentially lead to chronic stress (Gaughan et al., 2013). Cattle responses to the ever changing thermal environment are resultant of both acute and chronic exposure (Hahn and Mader, 1997).

Under most climatic conditions, T_A represents a significant portion of the driving force that allows/ prohibits heat exchange between an animal and the environment (Hahn 1999). The total heat exchange of the animal is dependent on T_A, RH, SR and WS (Mader and Davis, 2004; Mader et al., 2006). Therefore the apparent temperature at which an animal responds to the thermal environment is the animal's response to a combination of all climatic variables (Mader et al., 2010b). Figure 2.4 provides a schematic representation of the effect of environmental stressors and the response mechanisms utilized by an animal to cope with thermal stress (Hahn, 1999; DeShazer et al., 2009). The responses to thermal stress can be divided into three sections i) physiological; ii) behavioural; and iii) haematological (DeShazer et al., 2009).

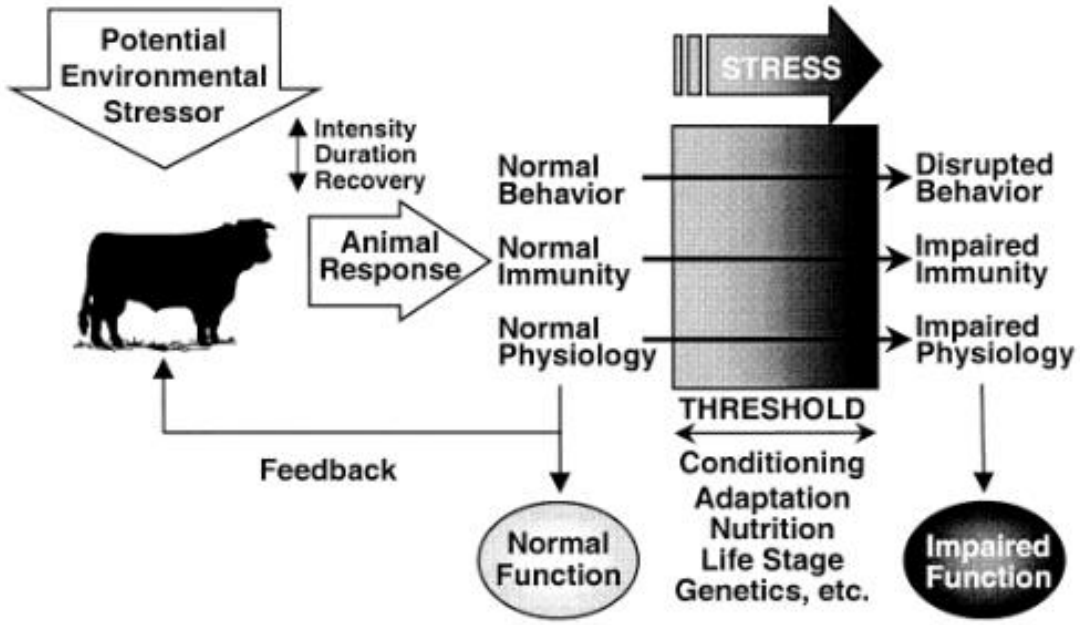


Figure 2.4: A schematic representation the effect of environmental stressors and animal responses that can disrupt normal function and impair animal performance and welfare (adapted from Hahn, 1999)

As ambient heat load conditions increase, animal observations can provide some insight into the severity of heat load the animal is experiencing. Diagnosis of the severity of heat load can be summarised, in order of progression, by the following responses (Young and Hall, 1993, pp 145);

1. Alignment of the body with the sun; reduce exposure to SR
2. Shade seeking
3. Refusal to lie down
4. Reduction in DMI
5. Crowding at water troughs
6. Body splashing
7. Agitation and restlessness
8. Reduced or stopped rumination
9. Grouping together, i.e. seeking shade from pen mates

10. Open mouthed breathing/panting
11. Excessive salivation
12. Ataxia, i.e. an inability to move
13. Collapse; convulsions; coma;
14. Death

Young and Hall (1993) indicated that up until number 10, open mouth breathing/panting, feedlot cattle are able to cope with heat load. However the onset of laboured open mouthed breathing/panting is suggestive of an animal's inability to cope with heat load (Young et al., 1997). When climatic conditions do not ease and these observations continue to persist, abatement strategies need to be implemented to circumvent death as a result of excessive heat load (Young and Hall, 1993; Young et al., 1997).

2.8.1 Behavioural Responses to Heat Load

In response to increasing thermal loads, cattle will initiate purposeful behavioural changes. However cattle have an ability to recognise and learn these behaviours to support thermoregulation. Mader et al. (2002) indicated that previous learned behaviours may influence an animal's ability to cope with thermal stress. These learnt behaviours are continually developed through exposure to hot environmental conditions where the experience provides the animal with strategies to cope with thermal loads (Castaneda et al., 2004). Mitlöhner et al. (2001b) reported difficulties in describing the differences in feeding and drinking responses to heat load as animals were exhibiting learned behaviours, i.e. seeking shade from feed bunks and body splashing behaviour at water troughs. Dikmen et al. (2012) reported that light body weight (353.8 ± 15.5 kg) cattle ate more frequently during daylight hours (1300 h and 1600 h; $P < 0.05$) and early evening (2000 h; $P = 0.09$) compared with heavy body weight (737.1 ± 15.8 kg) cattle that were observed eating during night time hours (2300 h; $P < 0.05$). This appears to indicate that cattle may adjust feeding behaviours, in accordance with changes to body composition, to regulate metabolic heat production during hot conditions. Other behavioural changes exhibited by cattle may include alterations in posture, including increasing the proportion of time standing; increased duration in shaded areas or increased shade seeking, including shade provided from other animals; and body splashing at water troughs (Young and Hall, 1993). Behavioural changes are the animal's first response to increasing thermal loads. If behavioural responses are insufficient to support the regulation of core body temperature, cattle will adjust physiologically and haematologically to negate the adverse effects of hot conditions (Hahn, 1999; Gaughan et al., 2008b).

2.8.1.1 Posture

Numerous authors have indicated that cattle will alter their posture during periods of hot weather. Young and Hall (1993) indicated that cattle will alter their position in terms of alignment of the

body with the sun in order to reduce the degree of exposure to SR. Dairy cows tend to spend considerable periods of time standing when conditions are hot (Igono et al., 1987; Frazzi et al., 2000). Brown-Brandl et al. (2006b) indicated that in feedlot heifers standing behaviour increased from 42.0 % during thermoneutral conditions to 48.1 % during periods of heat load. The increase in proportion of time standing should be considered as a sign of heat load in both dairy and feedlot cattle (Dikmen et al., 2012). Shultz (1984) indicated that un-shaded dairy cows spend a greater proportion of time ($P < 0.05$) standing compared to those animals with access to shade during summer. Furthermore Gaughan et al. (2008a) also indicated that during excessive heat load conditions dairy cows without access to cooling, i.e. water misting and air movement, spent more time standing compared to cows that were cooled. An increase in the proportion of time spent standing during heat load conditions may be representative of the animals' inability to dissipate body heat via evaporation. When pen surface temperature exceeds that of skin temperature, the animal will accumulate heat from the ground, thus increasing the overall accumulated heat load that the animal must dissipate (Mader et al., 2002). By increasing the amount of time standing the animal is exposing greater body surface area potentially attempting to increase the proportion of heat dissipation via evaporative, from the coat surface, and convective, air movement around the body, exchange mechanisms.

2.8.1.2 Rumination

Young and Hall (1993) refer to stage 8 in the diagnosis of the degree of heat load an animal is exposed to as a reduction of, or a complete termination of rumination. Shultz (1984) indicated that daytime rumination in un-shaded dairy cows decreased during periods of hot weather. However rumination is a necessary component of digestion and it is well reported that there is a voluntary reduction of DMI during hot weather, further emphasised during heat wave events. Therefore the reduction in ruminating behaviour is potentially directly related to the amount of DM consumed. Hicks et al. (1989) reported that when cattle spent more time eating, the time spent ruminating also increased ($P < 0.01$). Furthermore in their study animals that had a higher number of observations ruminating also had heavier slaughter weights ($P < 0.19$; Hicks et al., 1989). Dikmen et al. (2012) indicated that light cattle (353.8 ± 15.5 kg) had higher observed eating (38.7 %) and rumination (1.18 %) events compared to heavy cattle (737.1 ± 15.8 kg). However results from both studies may indicate that increased rumination is determined by a higher proportion of feed intake due to increased time spent eating. Therefore the reduction or termination of rumination cannot be solely dependent on the degree of heat load an animal is experiencing; as the volume of DM consumed will also impact the time spent ruminating.

2.8.1.3 Shade Seeking

During hot conditions cattle will seek shade where available (Blackshaw and Blackshaw, 1994). Shade seeking behaviour can be considered as a thermoregulatory mechanism whereby the shaded areas provide a change in microclimate and as such assist in the regulation of core body temperature (Bennett et al., 1985). Ray and Roubicek (1971) concluded that there were seasonal differences in shade usage, where feedlot cattle fed during the summer months utilise shaded areas at greater proportions compared to winter fed cattle. Shade seeking behaviours are not limited to feedlot cattle. McIlvain and Shoop (1971) reported observing grazing cattle seeking shade during hot summer days. *Bos taurus* breeds of cattle exhibit shade seeking behaviours earlier and more frequently compared with *Bos indicus* breeds (Blackshaw et al., 1987). Bennett et al. (1985) indicated that *Bos indicus* breeds will continue to graze in hot conditions, with high solar load, whereas *Bos taurus* will seek shade.

The provision of shade structures for feedlot cattle is beneficial as the animals are able to utilise shaded regions voluntarily. However feedlot cattle in un-shaded pens will also express shade seeking behaviours where they will exploit the shade footprint of other animals and from structures around the pen. Mitlöhner et al. (2001b) and Castaneda et al. (2004) indicated that un-shaded cattle seek shade by placing their heads in the shade footprint of feed bunks during the hot hours of the day. Additionally Gaughan and Mader (2014) recorded observations of un-shaded cattle utilising the shade footprint of other animals, water troughs and fence posts. These findings suggest that it is impossible to completely remove access from shade footprints in feedlot pens. Mitlöhner et al. (2002) indicated that shaded heifers utilised shaded areas during most daylight hours; however the authors acknowledged that this was likely to be a result of lower radiant heat and ground surface temperature. A study by Tucker et al. (2008) reported that dairy cows were more likely to utilise shade structures where SR were highest throughout the day. Ray and Roubicek (1971) concluded that the use of shade in the early morning hours during summer may be a reflection on the degree of SR rather than increasing T_A. Therefore the overall voluntary use of shaded areas throughout the day is likely to correspond with increasing SR.

2.8.2 Physiological Responses to Heat Load

As heat load increases, cattle need to use energy to dissipate excess heat in order to maintain homeostasis (Kadzere et al., 2002; Ravagnolo and Misztal, 2002). However the utilisation of energy to maintain homeostasis increases the overall energy requirements of the animal. A slight increase in respiration rate, increases maintenance energy expenditure by approximately 7 %; furthermore a significant increase in respiration, i.e. laboured panting, potentially increases energy requirements

by 11 to 25 % (NRC, 1981). Additionally the dissipation of body heat content during hot summer conditions can be ineffective as heat accumulation is often greater than heat loss (Bertipaglia et al., 2007). During these circumstances body temperature increases which consequently influences other physiological functions, ultimately resulting in increased respiration rate and core body temperature, as well as reductions in DMI, all of which have a negative influence on the performance and welfare of the animal (Ravagnolo and Misztal, 2002).

2.8.2.1 Core Body Temperature

Cattle are homoeothermic indicating that they are able to maintain a constant core body temperature despite a wide range in climatic conditions (Robertshaw, 1985). However, body temperature in homeothermic animals is not absolute with all animals exhibiting a diurnal pattern (Robertshaw, 1985). Variations observed within an animals' body temperature can be attributed to the equilibrium between the amount of heat energy produced/accumulated and heat energy dissipated from the body (Legates et al., 1991). Even when animals are housed within environmental conditions where ambient conditions remain constant, animals will still exhibit diurnal variations in body temperature, typically within a ± 1 °C gradient (Robertshaw, 1985). The amount of heat accumulated, from the environment and metabolic functions, must equal the amount of heat dissipated to the surrounding environment (Robertshaw, 1985), for an animal to maintain a state of equilibrium with its external environment. Homeothermy can be expressed as the following equation;

$$M = \pm K \pm C \pm R + E$$

Where M = metabolic heat production; K = heat exchange via conduction; C = heat exchange via convection; R = heat exchange via radiation; and E = heat exchange via evaporation (Robertshaw, 1985).

Under moderate conditions the diurnal rhythm of body temperature is thought to lag ambient conditions by 8 to 10 hours (Hahn and Mader, 1997). However during heat wave events it is thought that body temperature lags ambient conditions by 3 to 5 hours (Hahn and Mader, 1997). This may be suggestive of reduced ability to cope with heat load and may indicate that the animal is more susceptible to accumulated heat load at lower HLI thresholds during heat wave events. Furthermore during heat wave events it has been reported that daily means and ranges in body temperature are markedly increased (Hahn and Mader, 1997), thus increasing the dynamic range in body temperature. Under thermoneutral conditions the core body temperature of cattle is between 38 °C to 38.5 °C (Sjaastad et al., 2003), where a rectal temperature greater than 42 °C is considered to be lethal (Findlay, 1958). Mehla et al. (2014) indicated that as body temperature increases towards 42 °C there are numerous effects on bodily functions. Notably there is i) direct damage to

cells where there is an increase in membrane fluidity and permeability; ii) an increase in the animal's metabolic rate; and iii) a reduction in blood flow around the body (Mehla et al., 2014). Above 42 °C homeostatic systems within the body reach their upper critical limits for normal function (Mehla et al., 2014), resulting in death.

During periods of high heat load an increase in core body temperature is a function of the amount of heat gained by the animal from the thermal environment. However an animal's normal metabolic processes, i.e. digestion and locomotion, also result in an increase in core body temperature and therefore contribute to the diurnal pattern. Robertshaw (1985) indicated that controlling and maintaining the temperature of the central nervous system, particularly the brain, is the most important function of the body, as the brain is highly susceptible to changes in temperature. In a study conducted by Verwoerd et al. (2006), the authors showed that cattle were able to isolate their body temperature from the thermal environment during moderate temperatures, however when conditions become hot cattle are no longer able to cope with increasing ambient conditions. Furthermore Spiers et al. (2004) indicated that rectal temperature of beef cattle increased within 24 hours after the introduction of thermal stress. Therefore body temperature is considered a reliable indicator of thermal balance.

2.8.2.2 Respiration Rate

When exposed to heat load conditions many mammalian species rely on respiratory dynamics to assist in heat dissipation, thus the regulation of body temperature (Hales and Findlay, 1968). Respiration rate has been identified as a reliable early indicator of increasing heat load (Gaughan et al., 2008b). Brown-Brandl et al. (2005a) also suggested that respiration rate was a good indicator of total thermal load and concluded that respiration rate was a suitable measure to assess an animal's thermal status. As ambient heat load increases, the thermal status of the animal changes thus increasing respiration rates, where no stress would be considered as $\leq$ 60 breaths per minute; significant stress 120 to 150 breaths per minute; excessive stress $\geq$ 150 breaths per minute (Brown-Brandl et al., 2006a; Mader et al., 2006). An increase in respiration rate is indicative of an imbalance between heat accumulation and dissipation (Brown-Brandl et al., 2006a). Respiration rate is thought to precede T_A by approximately 1 hour and lag SR by approximately 1 hour (Brown-Brandl et al., 2005a).

Respiration rate can be visually assessed, however this can be difficult in field conditions where observations can occur 30 to 40 m away from the cattle (Gaughan et al., 2010b). Respiration rate is also subject to rapid changes, where the animal may be required to lower respiration rate and take

deep breaths in order to stabilise blood pH (Baumgard and Rhoads, 2007). Young and Hall (1993) described open mouth breathing/panting, where the onset of laboured open mouthed breathing is suggestive of an inability to cope with thermal loads (Young et al., 1997). Respiration rate does not provide a descriptive indication of the respiratory dynamic of the animal, where cattle may be panting and showing signs of significant or extreme heat load, i.e. open mouth panting, excessive salivation and tongue extended (Gaughan et al., 2010b). Therefore respiration rate alone does not provide a conclusive indication of the thermal status of feedlot cattle.

2.8.2.3 Panting

Panting score is not a measure of respiration rate, although it is a good indicator of heat load in cattle (Mader et al., 2006; Gaughan and Mader, 2014). Panting score provides a visual assessment of respiratory dynamics in cattle and assesses the breathing/panting condition that the animal is displaying (Young and Hall, 1993). Under field conditions the assessment of panting score (Table 2.5) is a viable alternative to using body temperature to assess the heat load status of cattle (Brown-Brandl et al., 2006b; Mader et al., 2006; Gaughan et al., 2008b; Gaughan and Mader, 2014). As ambient conditions change, changes to panting score provide a good indication of the changing thermal status of the animal (Mader et al., 2006). Panting score can be used to determine an individual's heat load status (Gaughan et al., 2010b), or a group of animals by calculating a mean panting score for the group of animals (Brown-Brandl et al., 2006b; Gaughan et al., 2008b). Mean panting scores are classified as: no stress mean panting score = 0 to 0.4; low stress mean panting score = 0.4 to 0.8; high stress mean panting score 0.8 to 1.2; and severe stress mean panting score > 1.2 (Gaughan et al., 2008b). Mean panting score of a group of animals can be used to determine the severity of heat load (Gaughan et al., 2010b), and can be calculated using the following equation (Gaughan et al., 2008b);

$$Mean\ Panting\ Score = \frac{\sum_{i=0}^{4.5} N_i \times i}{\sum_{i=0}^{4.5} N_i}$$

Where N_i = the number of cattle observed at PS i

Table 2.5: Assessment of panting score (PS), description of breathing/panting condition and associated respiration rate (RR; breaths per minute)

PS	Breathing Condition	RR
0	No panting	≤ 60
1	Slight panting, mouth closed, no drool, easy to see chest movement	60 – 90
2	Fast panting, drool present, no open mouth	90 – 120
2.5	As for 2, but occasional open mouth panting, tongue not extended	90 – 120
3	Open mouth and excessive drooling, neck extended, head	120 – 150
3.5	As for 3, but with tongue out slightly and occasionally fully extended for short periods	120 – 150
4	Open mouth with tongue fully extended for prolonged periods with excessive drooling. Neck extended and head up	≥ 160
4.5	As for 4, but head held down. Cattle "breath" from flank. Drooling may cease.	Variable RR may decrease

Adapted from Brown-Brandl et al. (2006a), Mader et al. (2006) and Gaughan et al. (2008b)

Classification of animals using a panting score system provides an indication of the extent at which the individual is suffering from heat load (Brown-Brandl et al., 2006a; Mader et al., 2006). Cattle exhibiting severe heat load as described by excessive heat load responses, i.e. panting score ≥ 4, display opened mouth breathing with the tongue fully extended for long periods where the neck is fully extended and head held up (Brown-Brandl et al., 2006a). Death may occur at this point if conditions persist and there are insufficient cooling opportunities to allow the animal to dissipate body heat.

2.8.2.4 Sweating Rate

Sweating, or cutaneous evaporation, is an important method of evaporative heat loss in cattle. Mader et al. (2010a) indicated that as T_A approaches body temperature, sweating becomes a key physiological mechanism for heat dissipation. In cattle, each individual hair follicle is associated with an apocrine sweat gland; therefore hair follicle density has a direct association to the number of sweat glands an animal has and its ability to dissipate excess body heat via evaporation (Collier et al., 2008). Carvalho et al. (1995) reported that sweating rates of *Bos indicus* cattle are greater and increase at a faster rate compared with temperate, *Bos taurus*, cattle. Berman (2005) indicated that the peak sweating rate of cattle is between 200 and 300 g/m^2, approximately 10 % of the sweating rate of the horse, which is estimated at 2000 g/m^2. Collier et al. (2008) added that the difference in maximal sweating rate of the horse and bovine is not due to differences in sweat gland type, as both have apocrine sweat glands. Therefore there may be an opportunity to improve the sweating rate and subsequently evaporative heat loss in cattle through selective breeding.

2.8.2.5 Digestion, Blood Flow and Nutrient Partitioning

Digestion and absorption processes carried out by the animal are affected by the thermal environment. Primarily during heat load, absorbable nutrients are diverted from growth and development and directed to maintaining body temperature (Baumgard and Rhoads, 2012b), within a physiologically acceptable range. Heat load conditions are associated with a reduction in gut motility and rumination (Beede and Collier, 1986). When cattle begin to enter a state of accumulated heat load there is a redistribution of blood flow from the internal organs to the extremities (Baumgard and Rhoads, 2007), thus away from the gastrointestinal tract. Engelhardt and Hales (1977) indicated that exposure to heat load (40 °C, dry bulb temperature; and 27 °C, wet bulb temperature) reduced blood flow to the mucosa of the dorsal rumen (32 %) and reticulum (31%) compared with animals considered within thermoneutral conditions (18 °C, dry bulb temperature; and 14 °C, wet bulb temperature). Given that there is a reduction in DMI and blood flow to the gastrointestinal tract during heat load, the concentration of absorbable nutrients per unit of blood volume must increase if the animal is to satisfy daily requirements (Beede and Collier, 1986) and maintain normal bodily functions.

During heat load there is an increase in maintenance energy requirements of approximately 7 to 25 % (NRC, 2001), which is associated with energy costs for dissipating accumulated heat load (Baumgard and Rhoads, 2007), i.e. increased respiration rate. However this increase in maintenance energy requirements does not adequately describe the total increase in energy requirements as it does not include the energy costs associated with protein synthesis or haematological responses that occur outside normal homeostasis (Baumgard and Rhoads, 2013; Carroll and Burdick Sanchez, 2014). Therefore a voluntary reduction in DMI is not beneficial to animal performance and wellbeing; however the reduction in DMI is an important contributing factor to the maintenance of core body temperature. Additionally the effect of heat load on digestion and nutrient partitioning cannot be completely explained by the reduction in DMI. Therefore these metabolic changes can potentially become classified as a part of the acclimation and adaptation to hot environments, where many of the changes in metabolic pathways are not yet defined and/or understood. However it is clear that these changes are potentially imperative to animal survival.

2.8.3 Haematological Responses to Heat Load

The relationship between the body's stress response and immune function is exceptionally complex and dynamic (Carroll and Burdick Sanchez, 2014). Hyperthermia as a result of heat load can compromise cellular function and result in physiological changes (Hansen, 2004), thus influencing animal welfare and performance. The compromise of cellular functions is somewhat the result of

electrolyte imbalances as well as inhibited protein synthesis that may be experienced during heat load (Mehla et al., 2014). However the negative impact of heat load on animal performance can partly be explained by the reduction in DMI. The reduction of DMI influences other biological mechanisms within the body that include an alteration to the function of the endocrine system (Baumgard and Rhoads, 2007). Given the role of the endocrine system in the co-ordination of metabolism, the alteration of blood hormone concentrations due to heat load is not unexpected (Beede and Collier, 1986). Therefore it becomes important to consider the net impact that DMI reductions and heat load have on changes in circulating haematological parameters. Currently there are relatively large gaps in the knowledge regarding the metabolic and biochemical changes that occur during heat exposure (Rhoads et al., 2013b). Nevertheless, haematological parameters can be sensitive indicators of thermal stress in cattle (Mitlöhner et al., 2002).

2.8.3.1 Acute Phase Proteins

When tissue damage or inflammation occurs the body responds by activating a systemic response, i.e. the acute phase response (Ceciliani et al., 2012). The acute phase response is activated as an innate response to inflammation, infection, disease and/or trauma (Carroll et al., 2009). The systemic response is a highly co-ordinated response involving a diverse range of cell types and proteins to correct tissue damage or the cause of inflammation (Ceciliani et al., 2012). Thus acute phase proteins are also actively involved in the repair and remodelling of damaged tissues (Carroll and Burdick Sanchez, 2014). A characteristic reaction to hyperthermia involves a shift in liver synthesis to promote the production of acute phase proteins (Carroll et al., 2009). Additionally acute phase proteins are good indicators of immunocompetence, whereby a decrease in circulating concentrations would indicate that the animal has become immunocompromised (Mehla et al., 2014).

2.8.3.1.1 Haptoglobin

In the plasma of cattle the acute phase protein, haptoglobin exists as a polymer in association with albumin (Eckersall and Conner, 1990). Haptoglobin is a haemoglobin binding protein that prevents oxidative damage by utilising free haemoglobin (Carroll and Burdick Sanchez, 2014), and is integral in the formation of a haptoglobin-haemoglobin complex (Ceciliani et al., 2012). Lomborg et al. (2008) reported that serum haptoglobin concentration increased, ranging between 264 to 2577 mg/L, in response to complex physical and psychological (a combination of transport and solitary housing in novel surroundings) stress, whereby the reference range of haptoglobin reported by Horadagoda et al. (1999) is < 350 mg/L in bovines. However Alsemgeest et al. (1995) were not able to detect haptoglobin concentration in calves exposed to the physical stress of standing on different

floorings, concluding that there was no effect on haptoglobin. However Alsemgeest et al. (1994) reported that the plasma haptoglobin concentration of clinically healthy dairy cows diagnosed with pathologically acute inflammatory diseases had plasma haptoglobin concentrations that were lower ($P < 0.01$; 21.6 ± 14.5 haemoglobin binding capacity /100 mL) compared with cows diagnosed with chronic inflammatory diseases (100.3 ± 11.9 haemoglobin binding capacity /100 mL). Whilst Lomborg et al. (2008) reported an increase in serum haptoglobin to a combination of stressors, these stressors are unlikely to be representative of the complex multifactorial systemic stress of heat load. Furthermore the effect of physical stress on haptoglobin concentrations in cattle remains unclear.

2.8.3.2 Creatine Kinase

Creatine kinase is a muscle specific enzyme (Sattler and Fürll, 2004), where circulating concentrations can be used as a marker of muscle degradation (Spears et al., 1986; Kanelov et al., 2008; De la Fuente et al., 2010). Creatine kinase occurs in cattle as three isoenzymes specific to i) skeletal muscle; ii) cardiac muscle; and iii) brain derived (Sattler and Fürll, 2004). Increases in plasma creatine kinase are the result of creatine kinase isozymes leaking from the muscle, particularly due to bruising and/or exercise (Broom et al., 1996). In bovines, creatine kinase has a half-life of 2 to 4 hours and has a normal plasma concentration between 21 and 280 U/L (Radostits and Done, 2007). Plasma creatine kinase typically declines rapidly unless there is continued muscular degeneration (Radostits and Done, 2007). However creatine kinase remains a good biological marker of muscular damage for three days post degeneration (Radostits and Done, 2007). Scharf et al. (2010) reported that there were no breed ($P = 0.80$) or heat exposure ($P = 0.53$) or breed × temperature ($P = 0.98$) effects on serum creatine kinase (U/L) concentrations in Angus (thermoneutral, 126.44 ± 20.90 U/L; heat 114.44 ± 20.90 U/L) or Romosinuano (thermoneutral, 131.00 ± 20.90 U/L; heat 118.00 ± 20.90 U/L) steers. However Nazifi et al. (2003) reported that serum creatine kinase concentration of Iranian fat-tailed sheep increases under heat load (40 °C; 95.90 ± 9.80 U/L) compared with animals housed in thermoneutral (21 °C; 23.39 ± 2.41 U/L) conditions. During heat load there is an increase in respiration rate, i.e. muscular movement; therefore it is likely that as respiration rate increases with heat load, plasma creatine kinase will also increase. However there are limited studies on the effect of heat load on plasma creatine kinase concentrations.

2.8.3.3 Cytokine

Pro-inflammatory cytokines, specifically tumour necrosis factor-alpha, interleukin 1β and interleukin 6, are indicators of acute inflammation in cattle (Carroll and Burdick Sanchez, 2014).

Pro-inflammatory cytokines act as regulators of the acute phase protein response (Leon et al., 2006). The acute phase protein response is activated by pro-inflammatory cytokines by the release from macrophages and monocytes from the source of inflammation or trauma (Carroll et al., 2009). In human patients, pro-inflammatory cytokines are produced within adipose and skeletal muscle tissues (Suagee et al., 2012). Additionally there tends to be an association between obesity and increased concentration of pro-inflammatory mediators (Suagee et al., 2012). Given that it is widely accepted that the more time cattle spend in a feedlot the heavier and 'fatter', i.e. greater body fat composition, they become it is likely that there will be an increase in pro-inflammatory cytokine concentrations. However there appears to be no studies that investigate the change in cytokine concentration with increasing days on feed in feedlot cattle. Additionally, there does not appear to be any studies investigating the effect of heat load on circulating cytokine concentrations, in feedlot cattle. However pro-inflammatory cytokines, tumour necrosis factor-alpha and interleukin 1, have been reported to increase systemically and in the central nervous system of rats and rabbits suffering heatstroke (Bouchama et al., 2005).

There have been numerous studies supporting an increase in cytokine interleukin 6 in response to hyperthermia. Liu et al. (2011) reported there was a tendency of intestinal cytokine interleukin 6 expression to increase with increasing core body temperature. Parikh et al. (1998) reported in human intestinal epithelial cells (Caco-2) one hour exposure to heat (43 °C) resulted in an increase in cytokine interleukin 6 expression (≈ 30 pg/mL), whilst prior to heat exposure cytokine interleukin 6 was undetectable (< 1 pg/mL). Leon et al. (2006) indicated that plasma cytokine interleukin 6 concentrations showed a tendency of increased circulating concentration at maximum core body temperature in heat stressed mice. Furthermore plasma cytokine interleukin 6 remained elevated (45.0 ± 17.4 pg/mL) 24 hours after heat exposure (Leon et al., 2006). In human patients serum cytokine interleukin 6 concentration increased (1059 ± 757 pg/mL; P = 0.0001) when suffering from hyperthermia (rectal temperature ≥ 40 °C) with neurological symptoms, i.e. decreased mental consciousness, as a result of physical exertion, compared with clinically healthy unaffected patients (0 ± 0 pg/mL) (Chang, 1993). Heat load resulted in an increase (P < 0.0001) in circulating cytokine interleukin 6 concentration in hyperthermic (rectal temperature ≥ 42.5 °C) baboons (Bouchama et al., 2005). Bouchama et al. (2005) concluded that peak plasma cytokine interleukin 6 concentrations coincided with the degree of tissue injury, or death, associated with the severity of hyperthermia experienced. Given the increase in cytokine interleukin 6 in response to hyperthermia in humans, baboons and mice it is likely that heat load will result in an increase in circulating cytokine interleukin 6 concentrations in feedlot cattle, and may be indicative of gut integrity.

2.8.3.4 Electrolytes

Electrolyte losses, particularly sodium and potassium, occur through drooling, salivation, sweating and urination (Mader et al., 2010a). Sodium and potassium play key roles in maintaining osmotic pressure and controlling the passage of nutrients into cells as well as water metabolism (Mader et al., 2010a). Mader et al. (2010a) indicated that as T_A increases and approaches body temperature heat dissipation is increased via sweating and increased urination. This increase ultimately disrupts the water and electrolyte balance within the body. Furthermore Sparke et al. (2001) indicated that the loss of water and electrolytes during hot conditions increases plasma pH and bicarbonate concentration. Changes in plasma pH and electrolyte balance reduce the buffering capacity of the blood impairing normal cellular function (Sparke et al., 2001), therefore influencing the ability of an animal to cope with heat load conditions. In bovines under physiologically normal conditions the basal electrolyte concentrations of bicarbonate, chloride, potassium and sodium are 17 to 29 mmol/L, 97 to 111 mmol/L, 3.9 to 5.8 mmol/L and 132 to 152 mmol/L respectively (Kaneko et al., 1997).

2.8.3.4.1 Bicarbonate

During heat load conditions bicarbonate may be influenced from an increase in respiration rate. However Khelil-Arfa et al. (2014) reported that T_A did not affect concentration of bicarbonate, although blood pH was significantly higher in heat exposed (28 °C; pH ≥ 7.35; $P \leq 0.001$) lactating cows, compared with cows at thermoneutral (15 °C) conditions. Conversely Beatty et al. (2006) showed that bicarbonate concentration of *Bos taurus* and *Bos indicus* heifers was significantly reduced during and after heat exposure (≥ 32 °C, wet bulb temperature). In order for the blood to remain an effective pH buffering system, the body needs to maintain a bicarbonate to carbon dioxide balance of 20:1 (Schneider et al., 1988; Baumgard and Rhoads, 2007). As an increase in respiration rate is also associated with an increase in carbon dioxide being exhaled (Baumgard and Rhoads, 2007), the increase in respiration rate results in a decrease of carbon dioxide in blood. The imbalance of carbon dioxide in the blood initiates bicarbonate secretion from the kidneys that can be utilised, through saliva, to buffer and maintain rumen pH (Baumgard and Rhoads, 2007). However as heat load conditions persist or increase in intensity there is typically an increase in the panting score of animals which can be associated with an increase in drooling, i.e. ≥ panting score 2 as described in Table 2.5. Thus decreasing the amount of saliva that would typically enter the rumen (Baumgard and Rhoads, 2007), would further impede pH stability within the rumen resulting in an increased risk of developing ruminal acidosis (West, 2003).

2.8.3.4.2 Chloride

Sanchez et al. (1994) reported heat load (40 °C), plasma chloride concentration tended to be higher ($P < 0.07$) in cows with *ad libitum* access to feed, compared with cows at thermoneutral (25 °C). Beatty et al. (2006) reported that plasma chloride concentrations increased ($P < 0.05$) in *Bos taurus*, there were no significant changes in *Bos indicus* during heat load. Srikandakumar et al. (2003) concluded that breed did not affect blood chloride concentration, however there was a significant effect of heat load on blood chloride concentration in Merino (thermoneutral, 109.33 ± 1.03 mmol/L; heat 116.33 ± 1.02 mmol/L) and Omani (thermoneutral, 109.50 ± 1.05 mmol/L; heat 116.50 ± 0.55 mmol/L) sheep. Additionally Scharf et al. (2010) reported that there were no breed ($P = 0.13$) or heat exposure ($P = 0.22$) influences on serum chloride (mEq/L) concentrations in Angus (thermoneutral, 101.78 ± 1.57 mEq/L; heat 101.56 ± 1.57 mEq/L) or Romosinuano (thermoneutral, 101.28 ± 1.57 mEq/L; heat 97.78 ± 1.57 mEq/L) steers. These studies suggest that genotype differences potentially affect the maintenance of circulating chloride concentrations.

2.8.3.4.3 Potassium

Srikandakumar and Johnson (2004) concluded that heat load increased ($P < 0.01$) blood potassium concentration in Holstein (thermoneutral, 4.41 ± 0.01; heat 5.21 ± 0.01) and Jersey (thermoneutral, 4.24 ± 0.01; heat 4.34 ± 0.01) cows. However heat load decreased ($P < 0.01$) blood potassium concentration in Australian Milking Zebu (thermoneutral, 4.09 ± 0.01; heat 4.06 ± 0.01) (Srikandakumar and Johnson, 2004). Scharf et al. (2010) reported that there were no breed ($P = 0.28$) or heat exposure ($P = 0.48$) effects on serum potassium (mEq/L) concentrations in Angus (thermoneutral, 4.31 ± 0.12 mEq/L; heat 4.50 ± 0.12 mEq/L) or Romosinuano (thermoneutral, 4.27 ± 0.12 mEq/L; heat 4.22 ± 0.12 mEq/L) steers. However the potassium concentrations reported by Srikandakumar and Johnson (2004) and Scharf et al. (2010) were within the normal reference range (3.9 – 5.8 mmol/L) for bovines (Kaneko et al., 1997). It is unclear the effect of breed or heat load has on circulating potassium concentrations, however electrolyte balance is essential for maintaining a normal acid-base balance.

2.8.3.4.4 Sodium

Heat load is associated with an increase in drooling, salivation, sweating and urination (Mader et al., 2010a). Scharf et al. (2010) reported that there were no breed ($P = 0.55$) or heat exposure ($P = 0.95$) effects on serum sodium (mg/dL) concentrations; however there was an effect of breed × temperature ($P = 0.05$) where serum sodium concentration heat exposure of Angus increased (thermoneutral, 141.11 ± 1.31 mg/dL; heat 144.44 ± 1.31 mg/dL) and decreased in Romosinuano (thermoneutral, 143.63 ± 1.31 mg/dL; heat 140.44 ± 1.31 mg/dL) steers. The authors concluded that

the decrease in serum sodium of Romosinuano steers may be associated with an increased water intake whereby there was an increase in urinary output (Scharf et al., 2010). Beatty et al. (2006) reported that significant ($P < 0.05$) decreases in plasma sodium concentrations were observed in *Bos taurus* and *Bos indicus* heifers under heat load. Given that sodium loss occurs through drooling, salivation, sweating and urination, losses during exposure to heat load are not unexpected.

2.8.3.5 Heat Shock Proteins

The cellular thermal stress response was first observed by Ritossa (1962) in temperature shocked *Drosophila spp* salivary glands. Ritossa (1962) described the response as following a 'new puffing pattern'. The gene expression pattern described by Ritossa (1962) would later be referred to as heat shock proteins (**HSP**). Heat shock proteins are a diverse family of highly conserved molecular chaperones (Pockley, 2003) which exist within all organisms, ranging from archaebacteria to eubacteria, including plants and animals (Lindquist and Craig, 1988). The HSP are molecular chaperones and are categorised into numerous families that are named in accordance with their approximate molecular weight (Pockley, 2003), i.e. a HSP group within 70 kilodaltons (**kDa**) are classified as the **HSP_{70}** family. Heat shock protein families are not only activated during periods of exposure to hot ambient conditions, as their name suggests, as other stressors including oxidative stress, nutritional deficiencies, viral infection and ischaemia can also incite the expression of these molecular chaperones (Pockley, 2003). However proteins classified within the HSP_{70} and **HSP_{90}** families are activated in response to elevated T_A (Lindquist and Craig, 1988). Moreover it is important to note that HSP are present at normal and elevated T_A indicating HSP are involved in fundamental roles of normal cellular function (Lindquist and Craig, 1988).

During periods where the heat shock response is elicited, HSP function as intracellular chaperones or proteases where they become responsible for the assembly, stabilisation, folding and translocation of oligomeric proteins, as well as the degradation of damaged protein structures (Pockley, 2003). Heat shock induced by hot weather conditions acutely decreases DNA synthesis and negatively affects the ability of the cells to maintain their cytoskeleton, resulting in a collapse to the cells structure (Roy and Collier, 2012). Induction, regulation and transcription of HSP are facilitated by the interaction of heat shock factor transcription factors (Pockley, 2003). In vertebrate species, four heat shock factors have been identified with heat shock factor 1 having a predominant role in the response of HSP to physiological and environmental stressors (Pockley, 2003). Under homeostatic conditions heat shock factor 1 is present within the cell cytoplasm as a dormant monomeric molecule, unable to bind to the DNA structure (Pockley, 2003). However when the cell is exposed to a physical or chemical stressor the fluctuation of newly synthesised non-native

proteins activates heat shock factor 1, which is then converted to a phosphorylated trimer that binds to DNA, allowing the heat shock factor 1 to translocate from the cytoplasm to the nucleus (Pockley, 2003). Binding of the heat shock factor 1 to newly synthesised non-native proteins activates the transcription of HSP genes (Pockley, 2003). The primary purposes of HSP are to provide protection from the toxic effects associated with stressors (Lindquist and Craig, 1988), from the accumulation of by-products produced from denatured proteins.

2.8.3.5.1 Heat Shock Protein 70

The HSP_{70} family, ranging between 68 to 73 kDa (Adamowicz et al., 2005), are responsible for a wide range of protein folding processes that include the folding and assembly of newly synthesised proteins; refolding of misfolded and aggregated proteins; membrane translocation of organellar and secretory proteins; and controlling the activity of regulatory proteins (Mayer and Bukau, 2005). During periods of exposure to a stressor, i.e. elevated body temperature, the rapid expression of HSP_{70} is critical for cytoprotection (Silver and Noble, 2012), highlighting HSP_{70} role in supporting homeostasis by maintaining cellular structures (Manjari et al., 2015) in response to climatic stressors.

Fader et al. (1994) indicated that the induction of HSP_{70} in four fish species was incited by seasonal changes. Manjari et al. (2015) also reported that mRNA expression of HSP_{70} in Tarai buffalo was twofold higher ($P < 0.05$) during the summer season. This indicates that the induction of the HSP response occurs at temperature thresholds to provide protection to protein structures during periods of changing climatic conditions. However the temperature threshold and magnitude of the HSP response is likely to be species specific. Lindquist (1980) identified that the induction of **HSP_{82}** occurred in *Drosophila spp.* when T_A increased from 23 °C to 26 °C. Additionally Lindquist (1980) found that HSP_{70} was produced in higher concentrations than other measured HSP. Moreover HSP_{70} was detectable at most T_A, maximum concentrations were detected within a narrow T_A range based around 37 °C (Lindquist, 1980). Mehla et al. (2014) investigated the cellular response of Sahiwal cattle exposed to conditions where T_A and RH were 42 °C and 90 % respectively, representing a THI > 104, compared with animals housed at 37 °C and 45 % (THI = 86). The authors reported that there were 5.76 and 1.74 fold increases in HSP_{70} gene expression associated with protein folding and immunity in the heat exposed cattle (Mehla et al., 2014).

Supporting the influence of seasonal changes as identified by Fader et al. (1994) and Manjari et al. (2015), Gaughan et al. (2013) identified a photoperiod effect on HSP_{70} concentrations ($r^2 = 0.94$; $P < 0.0001$) in Angus feedlot steers. Indicating that the changes in day length, associated with the

changing seasons, potentially influences the induction of the HSP response. Manjari et al. (2015) indicated that mRNA expression of HSP_{70} was highly correlated with respiration rate (r = 0.958; *P* = 0.01) and rectal temperature (r = 0.920; *P* = 0.01). Gaughan et al. (2013) also observed a positive linear relationship between body temperature and HSP_{70}, while acknowledging that the coefficient of determination was small (r^2 = 0.06; *P* < 0.001). Furthermore Gaughan et al. (2013) also identified a strong relationship (r^2 = 0.86; *P* = 0.0001) between T_A and HSP_{70} concentration. However the authors concluded that that the relationship between changes in body temperature and circulating HSP_{70} could be confounded by an influence of T_A on body temperature, indicating that the initiation of the HSP_{70} response is likely to be associated with ambient conditions rather than body temperature.

2.8.3.6 Glucose

Itoh et al. (1998) indicated that in ruminants glucose availability is predominantly supplied by hepatic gluconeogenesis. Heat load is thought to increase glucose usage by skeletal muscle (Rhoads et al., 2013a). Circulating plasma glucose levels are the equilibrium between glucose production and utilisation within the body (Itoh et al., 1998), where normal basal plasma concentration in bovines is between 2.50 and 4.16 mmol/L (Kaneko et al., 1997). Itoh et al. (1998) reported that in lactating dairy cows basal glucose concentration had a tendency to be lower during exposure to heat load (69.5 mg/dL; T_A, 28 ± 0.5 °C; RH 60 ± 5 %) although not significantly different (*P* < 0.1) from thermoneutral conditions (71.9 mg/dL). Achmadi et al. (1993) reported that heat load reduced (*P* < 0.01) circulating glucose levels in sheep. Additionally O'Brien et al. (2010) showed that circulating glucose decreased by 7 % (*P* < 0.04) during exposure to cyclic heat load where T_A varied between 29.4 °C, at 0600 h, and 40.0 °C, at 1600 h, and RH was 20 %. Itoh et al. (1998) reported that exposure to hot conditions did not influence the insulin response to exogenous glucose (0.625 mmol/kg; with constant glucose infusion of 0.022 mmol/kg/minute) in lactating dairy cows. However the authors reported that 60 and 90 minutes after the commencement of constant glucose infusion lactating dairy cows exhibited a higher (*P* < 0.05) insulin response during heat exposure, compared with thermoneutral conditions. Furthermore in growing calves O'Brien et al. (2010) reported that environmental conditions did not influence glucose response (area under curve or slope of glucose disposal) during a glucose tolerance test. However there was a tendency (*P* < 0.07) for a greater insulin response (59 %) during a glucose tolerance test in the bull calves exposed to hot conditions (O'Brien et al., 2010).

2.8.3.7 Insulin

Insulin, a peptide hormone produced in the pancreas by β-cells, circulates freely within plasma and has a half-life of 5 to 8 minutes (Sjaastad et al., 2003), with normal circulating concentration of 0 to 5 µU/mL (Kaneko et al., 1997) in bovines. Circulating insulin levels are dependent on two variables; i) the rate of release from the pancreas and ii) the rate of utilisation by the body (Itoh et al., 1998). According to Sjaastad et al. (2003) the principal actions of insulin are to;

- Increase glucose and amino acid uptake, glycogen stores and protein synthesis;
- Decrease gluconeogenesis, reduce circulating glucose and amino acids; and
- Increase triglyceride synthesis

Itoh et al. (1998) indicated that insulin is the most important hormone controlling glucose metabolism. Insulin has an antilipolytic action, i.e. blocks fat breakdown and reduces adipocyte (non-esterified fatty acid) export, and is the primary driver of cellular glucose uptake (Baumgard and Rhoads, 2007; Rhoads et al., 2013a). Rhoads et al. (2013a) stated that under normal physiological conditions, i.e. thermoneutral and non-stressed, an increase in circulating insulin concentrations results in a shift from fat oxidation to glucose usage. Moreover significant reductions in DMI are typically associated with hypoinsulinemia (Baumgard and Rhoads, 2012b). However studies have reported increased circulating insulin levels in lactating dairy cows (Itoh et al., 1998; Wheelock et al., 2010); growing cattle (O'Brien et al., 2010) and pigs (Pearce et al., 2013) during heat load. Conversely Achmadi et al. (1993) reported that heat exposure (T_A, 30 °C; RH, 70 %) did not have an effect on basal insulin levels in sheep, although the authors did indicate that sheep fed a concentrate ration had higher ($P < 0.01$) circulating insulin levels than those sheep fed roughage diets, regardless of ambient conditions (Hot = T_A, 30 °C and RH, 70 %; Thermoneutral = T_A, 20 °C and RH, 70 %; Achmadi et al., 1993). In lactating dairy cows Itoh et al. (1998) reported that basal insulin concentrations were higher ($P < 0.01$) during heat load (T_A, 28 ± 0.5 °C; RH, 60 ± 5 %) compared with thermoneutral conditions (T_A, 18 ± 0.5 °C; RH 60 ± 5 %). It should be noted that in the study by Itoh et al. (1998) each cow acted as their own experimental control, thus removing individual variability. Wheelock et al. (2010) also concluded elevated circulating insulin levels during heat load, in lactating dairy cows. Additionally, in growing bull calves, O'Brien et al. (2010) indicated that exposure to heat load tended ($P < 0.06$) to increase basal circulating insulin by 33 %.

2.8.3.7.1 Insulin and Glucose Relationship

Although insulin secretion is influenced by numerous factors the most important regulator of insulin is blood glucose (Sjaastad et al., 2003). Baumgard and Rhoads (2007) suggest that during heat load, cows have a much greater insulin response to a glucose challenge when compared to under-fed cows. The greater insulin response indicates that the cow becomes metabolically inflexible during

heat load (Baumgard and Rhoads, 2007). With the cow becoming metabolically inflexible, the animal is no longer able to utilise fatty acids and ketones indicating that the cow becomes increasingly dependent on glucose for her energy maintenance requirements, thus decreasing available glucose for milk production (Baumgard and Rhoads, 2007). A tool for estimating glucose as a preferred energy source can be determined through a glucose tolerance test, which provides an estimation of glucose utilisation and insulin action within the body (Wheelock et al., 2010). An increased glucose supply combined with a decrease in circulating glucose, potentially indicates that there is an increase in glucose utilisation representing glucose as the favoured energy source, particularly during heat load (Wheelock et al., 2010).

Whilst the literature is primarily focused on lactating dairy cattle it is important to consider the physiological differences between beef cattle and lactating dairy cows, i.e. energy demands for growth versus lactation. The literature discussed here may not be representative of growing beef cattle due to the different physiological demands for glucose, which overall influences insulin production and glucose utilisation within the body. This indicates that further studies investigating the effect of heat load on circulating insulin and glucose levels, as well as the relationship between glucose and insulin, are warranted particularly in feedlot cattle.

2.8.3.8 Lipids

2.8.3.8.1 Cholesterol

In grazing cattle, the plasma cholesterol concentration of *Bos indicus* bulls, heifers, calves, lactating cows and dry cows were greater ($P < 0.001$) compared with *Bos taurus* (O'Kelly, 1968a). The study by O'Kelly (1968a) highlights that there is potentially a genotype effect on cholesterol concentration. Additionally Scharf et al. (2010) reported that there were breed ($P = 0.01$) and breed × temperature ($P = 0.001$) effects on serum cholesterol concentrations of Angus steers. Heat exposure (26 °C – 36 °C) resulted in an increase in serum cholesterol concentration of Angus (thermoneutral, 64.11 ± 6.17 mg/dL; heat 91.55 ± 6.17 mg/dL) steers (Scharf et al., 2010). However there were no differences in serum cholesterol concentration Romosinuano steers at thermoneutral (58.01 ± 6.17 mg/dL) or heat exposed (64.88 ± 6.17 mg/dL) (Scharf et al., 2010). It is well known that *Bos indicus* cattle are able to survive in harsh conditions, both climatically and nutritionally. Therefore the difference in plasma cholesterol concentration may be a reflection of the metabolic differences between *Bos indicus* and *Bos taurus* genotypes.

2.8.3.8.2 Triglycerides

O'Kelly (1968a) concluded that there were no differences in serum triglyceride concentration of grazing Brahman (23.2 mg/ 100 mL), Brahman × *Bos taurus* (17.9 ± 2.2 mg/ 100 mL), Africander × *Bos taurus* (19.2 ± 1.0 mg/ 100 mL) and *Bos taurus* (17.7 ± 1.9 mg/ 100 mL) bulls (> 22 months of age). However in grazing heifers (24 months of age) the authors found that Africander × *Bos taurus* (31.2 ± 5.2 mg/ 100 mL) and *Bos taurus* (32.1 ± 0.1 mg/ 100 mL) genotypes had serum triglyceride concentrations that were greater ($P < 0.01$) than Brahman (16.4 ± 0.6 mg/ 100 mL), Brahman × Hereford (15.8 ± 3.6 mg/ 100 mL) and Africander (21.6 ± 1.8 mg/ 100 mL) (O'Kelly, 1968a). Similarly, Scharf et al. (2010) reported that breed did not have a significant effect ($P = 0.94$) on serum triglyceride concentration. Additionally Scharf et al. (2010) found that heat exposure (26 °C to 36 °C) increased ($P = 0.05$) serum triglyceride concentration of Angus (heat, 19.22 ± 1.88 mg/dL; thermoneutral, 15.22 ± 1.88 mg/dL) and Romosinuano (heat, 19.88 ± 1.88 mg/dL; thermoneutral, 15.64 ± 1.88 mg/dL) steers. Additionally O'Kelly (1968b) concluded that the nutritional status influences plasma triglyceride concentrations in cattle.

2.9 Methods for Alleviating Heat Load

There are numerous considerations that need to be accounted for whilst managing feedlot cattle during summer, particularly within the confines of a feedlot. Pen location within the feedlot can influence pen microclimate. However Entwistle et al. (2000) reported that there were no differences ($P > 0.05$) in pen location, rows or pens within rows, where cattle deaths were experienced during a heat wave event. However the authors indicated that a greater proportion of deaths occurred in pens and rows towards the centre of the feedlot (Entwistle et al., 2000), indicating that a within feedlot microclimate existed. Furthermore these microclimates have the ability to influence thermal exchange and thus the heat load status of feedlot cattle.

Traditionally strategies for mitigation management of heat load have involved environmental modification where the focus has been on i) reducing SR and ii) increasing WS (Eigenberg et al., 2005), however there have also been studies investigating wetting feedlot cattle (Gaughan et al., 2004a). A study by Gaughan et al. (2008a) investigated the influence of day and night cooling, through the use of water application and air movement, on managing heat load as determined by changes in rectal temperature, respiration rate and DMI. The authors concluded that cooling cattle after peak T_A is reached, was more beneficial compared to animals that were cooled during peak T_A (Gaughan et al., 2008a). At the cessation of the daytime cooling treatment cattle may have been suddenly exposed to hot conditions, resulting in a rapid accumulation of heat load as they had not been required to initiate normal physiological responses to cope whilst being cooled (Gaughan et

al., 2004a). Morrison et al. (1973) reported that cattle sprinkled with water had higher mean DMI as well as greater ADG. The authors also indicated that the water application did not prevent increases in respiration rate and rectal temperature, however it did reduce ($P < 0.05$) the magnitude of increases. The application of water within the Australian industry is illogical due to i) restricted water availability and ii) the potential of water application to increase within pen RH impacting thermal exchange.

2.9.1 Provision of Shade

It has been well established that the provision of shade is advantageous for feedlot cattle. Providing shade to feedlot cattle alters the microclimate within the pen, potentially providing an area for cooling (Mitlöhner et al., 2002), supporting the regulation of core body temperature. The benefits associated with the use of shade structures during hot ambient conditions has been of interest for many years (Brown-Brandl et al., 2005a). The advantage of shade structures is that the application is passive, where animals are able to utilise shaded areas voluntarily (Eigenberg et al., 2005). The provision of shade reduces the animals exposure to direct SR, however shade structures do not alter T_A or RH (Gaughan et al., 2004b). Gaughan et al. (2004b) showed that the beneficial aspects of shade structures, i.e. reduced exposure to SR, may be offset by a lack of air movement under the structure itself.

Entwistle et al. (2000) reported that during a heat wave event shade reduced the impact of severe conditions on excessive heat load related deaths, whereas un-shaded pens had a significantly higher, 5.8 %, mortality rate compared with shaded pens, 0.2 %. Furthermore Gaughan et al. (2004b) showed that un-shaded Angus heifers generally had higher respiration rates compared with their shaded counterparts (Gaughan et al., 2004b). The authors also indicated that these animals showed a preference for shade usage when HLI $\geq$ 83 (Gaughan et al., 2004b). Brown-Brandl et al. (2005a) concluded that shaded cattle had lower mean respiration rates compared to their un-shaded counterparts, however the respiration rate of shaded and un-shaded cattle followed a similar trend until approximately 1100 h where the shaded cattle response stabilised and un-shaded cattle respiration rates continued to increase. Gaughan et al. (2004b) reported that during the afternoon un-shaded Angus heifers had lower rectal temperature but higher respiration rates compared with shaded heifers. The authors concluded that the decreased rectal temperature of un-shaded heifers was a result of the functional relationship between respiration rate and body temperature. In exposure to varying climatic extremes Brown-Brandl et al. (2005a) reported that the core body temperature of shaded cattle was lower compared to un-shaded during daylight hours. However the

results within the literature for cattle performance for shaded and un-shaded feedlot pens are inconsistent.

There is also some conjecture regarding the amount of shade, m^2/animal, required to offset the impact of heat load. Clarke and Kelly (1996) provided shaded areas of 10 m^2/animal and concluded that the shade allocation did not result in the improvement ($P > 0.05$) of animal performance or meat quality. This was supported by the findings of Sullivan et al. (2011) where the authors concluded that the provision of shade at applications of 2.0 m^2/animal or greater were not associated with any additional production benefits for short fed (119 days on feed) cattle. However Mitlöhner et al. (2002) concluded that a shade provision of 2.12 m^2/animal improved ADG, carcass quality, decreased respiration rate and improved overall animal wellbeing. Clarke and Kelly (1996) identified that the provision of shade did reduce rectal temperature and respiration rate compared with un-shaded cattle.

2.10 Conclusion

Heat load, often classified as heat stress, has been a focal point of productivity research for numerous years. Previous research clearly describes the negative impact hot climatic conditions have on the health, performance and welfare of feedlot cattle. Furthermore the literature highlights the importance of developing a comprehensive understanding of the responses of cattle to climate related stressors and factors that further confound the negative impact of heat load particularly on feedlot cattle.

It is well acknowledged that cattle respond to heat load with numerous adjustments to their behaviour as well as physiological, biological and immunological parameters. Numerous studies have investigated the effects of heat load on thermoregulation in livestock. However few studies have utilized techniques that have allowed for continuous recording of body temperature over periods of time ≥ 10 days (Nienaber et al., 1999). Remote sensing technology is a potential method of obtaining body temperature over long periods of time, i.e. months, potentially years, without compromising animal welfare (Gaughan et al., 2010a). Additionally, as identified throughout the literature and further highlighted by Rhoads et al. (2013a), there are inconsistencies in knowledge regarding the haematological changes that occur during heat load. Furthermore where there are bovine haematological studies they are primarily focused on lactating dairy cows, highlighting the need to investigate the haematological responses of feedlot cattle.

Responses initiated by the body have a common purpose to ensure survival. Therefore it is important to gain a comprehensive understanding of the dynamic impact that hot conditions have on feedlot cattle. Investigating these areas will allow for the development of measures to minimise the negative influence that hot conditions have on the feedlot industry.

Based on the literature review the following generalised hypothesis has been developed ***'Genotype, i.e. Bos taurus versus Bos indicus, has an influence on the dynamic nature of the physiological, behavioural and haematological responses of feedlot cattle to heat load.'*** The experiments incorporated within this thesis were primarily designed to investigate ***'the influence of genotype and shade availability on the physiological, behavioural and haematological responses of feedlot cattle to heat load.'***

The impact of hot conditions cannot be completely abated where there are animal production operations particularly intensive systems, i.e. feedlots, occurring in tropical and sub-tropical regions. However with implementation of management strategies producers are able to reduce the impact of heat load conditions. Understanding factors that influence heat load, both environmental and animal, allows producers to establish and implement mitigation strategies prior to and during heat related stress events, thus improving animal survivability and welfare during these events.

Chapter 3

Using Rumen Temperature as a measure of Body Temperature in Feedlot Steers

Experimental Hypothesis: Rumen temperature will have a relationship with rectal temperature and rumen temperature will show a diurnal pattern.

3.1 Introduction

Core body temperature is considered to be a reliable indicator of thermal balance. However, defining core body temperature is somewhat problematic as no clear definition of core body temperature is available. Estimates of core body temperature have been measured from several locations in cattle, i.e. tympanic (Davis et al., 2003; Mader et al., 2010a), abdominal (Lefcourt and Adams, 1996; Gaughan et al., 2010a), rectal (Gaughan et al., 1999; Gaughan et al., 2008a) and more recently rumen (Ipema et al., 2008; Rose-Dye et al., 2011; Mohammed et al., 2014) in bovines. Traditional methods of obtaining body temperature often require relocating the animal to appropriately designed handling facilities (Rose-Dye et al., 2011). The relocation of the animal to handling facilities may subsequently result in an increase in body temperature, due to handling and locomotion, potentially confounding body temperature responses due to ambient conditions. Further to this, data loggers may be used that collect and store body temperature data that is downloaded at the completion of the data collection phase typically $\leq$ 10 days. Few studies have utilised methodologies that have allowed for continuous recording of body temperature over prolonged periods of time (Nienaber et al., 1999), i.e. $\geq$ 10 days, with access to real time data.

Advancement in technology has seen the development of equipment that has allowed the collation of $\geq$ 100 day body temperature data, via surgical implantation of data loggers (Lefcourt and Adams, 1996; Gaughan et al., 2010a). More recently Rose-Dye et al. (2011) and Mohammed et al. (2014) used rumen temperature, via rumen boluses, to monitor body temperature. Remote sensing, particularly rumen boluses, has the potential to obtain body temperature data over prolonged periods of time, months potentially years, without compromising animal welfare. Furthermore access to real time body temperature data has the potential to provide useful information to quantify an animal's true thermal status, therefore allowing for effective management of feedlot cattle during periods of heat load. Whilst respiratory dynamics, i.e. panting scores, are able to provide a reliable visual appraisal of an animal's thermal status (Mader et al., 2006; Gaughan et al., 2008b), cattle are prone to rapid and sudden changes that reflect other physiological functions within the body, i.e. stabilising blood pH (Baumgard and Rhoads, 2007). This indicates that the most reliable method to quantify an animal's thermal status is through measuring body temperature. The objective of this experiment was to *investigate the suitability of rumen temperature as an assessment of body temperature in Bos taurus cattle housed in outside feedlot pens through summer*.

3.2 Materials and Methods

The experiment was undertaken in Southeast Queensland, Australia, at The University of Queensland (**UQ**; 27.54 °S, 152.34 °E; 100 m above mean sea level) research feedlot during a

Southern Hemisphere summer (October to March), over 128 days. The location is characterised by a hot, humid sub-tropical climate. This experiment was conducted with the approval of UQ animal ethics committee (SAFS/210/13/MLA). During this experiment 80 purebred Angus steers, with an initial non-fasted live weight of 388.8 ± 2.1 kg were used.

3.2.1 Animal Management

Steers were vaccinated against clostridial diseases (enterotoxaemia, pulpy kidney disease; tetanus; blacks disease; malignant oedema; and blackleg; Pfizer Animal Health, Australia) on d -19; Bovine Respiratory Disease (Bovillis MH, Inactivated *Mannheimia haemolytica*; Coopers Animal Health, Australia) on d 7 and d 28; and trivalent tick fever (chilled, 3 germ; *Babesia. bovis, Babesia bigemina and Anaplasma centrale;* Department of Agriculture, Fisheries and Forestry (Biosecurity Queensland), Australia) on d -18. Animals were also treated for internal and external parasites (Cydectin, 5g/L moxidectin solvent, 150 g/L hydrocarbon liquid; Fort Dodge Australia P/L, Baulkham Hills, NSW, Australia) on d -19 and 63. Hormonal growth promotants were not used in the experiment.

The cattle were weighed (non-fasted) at approximately 0830 h on feedlot induction (d -18), and then at 7 day intervals for the duration of the experiment. The same pen sequence was used at each data collection point, i.e. pen 1 was assessed first through to pen 8. The steers were walked from their respective pens as a pen group for a distance of 300 to 500 m depending on feedlot pen location to a handling facility.

3.2.2 Feedlot Description

Eight pens, 162 m^2 (27 m × 6 m), were utilised within the UQ research feedlot. Steers were allocated to pens based on initial non-fasted live weight, so to equalise total pen weight across each of the 8 pens utilised within the experiment. The feedlot pens are situated in a north-south alignment. The surfaces of the pens were soil with a 2 % slope from the feed bunks towards the rear of the pens. Concrete feed bunks with a 3 m concrete apron were located at the front of each pen (facing towards the west). Each feed bunk provided a linear area of 0.42 m^2/animal and linear water trough area was 0.10 m^2/animal. Stocking density was 16.2 m^2/animal. Shade was provided by shade-cloth (black, 90 % solar block, Darling Downs Tarpaulins, Toowoomba, Queensland, Australia) attached to a 4 m high structure. The shade structure provided a shade footprint of 1.8 m^2/animal at midday.

3.2.3 Nutritional Management

Feed bunks were read at 0600 h and 1200 h each day using a modified 'clean bunk at midday' feed intake management program, as described by Lawrence (1998). Cattle entered the feedlot and commenced backgrounding in d -18, commencing on a starter ration through to d -2, then transitioned to a finisher ration over 10 d. Steers were then fed a finisher ration for the remainder of the experiment (Table 3.1). Refusals were removed and weighed daily with average consumption per pen and per animal calculated. Cattle were fed twice daily at approximately 0700 h and 1630 h daily. Mean feed intake was 11.08 ± 0.07 kg/animal.

Table 3.1: Diet and Nutrient Composition

Item	Starter[1]	Finisher[1]
Ingredient, kg (as fed)		
Barley	150	250
Sorghum	350	354
Millrun	100	100
Cottonseed Meal	25	20
Molasses	20	20
Limestone	13.45	14.7
Sodium bicarbonate	8	8
Urea	7	6.95
Sulphur (dusting)	0.29	0.29
Moneco® 200[2]	0.10	0.10
Sodium bentonite	25	25
Mineral – vitamin supplement[3]	1	1
Chickpea shell	300	200
Nutrient Composition (as fed)		
DM, %	89.15	89.11
NE_g, Mcal/kg	1.63	1.68
Crude Fat, %	2.32	2.31
CP, %	12.02	12.03
RDP; %	8.14	8.23
UDP; %	3.84	3.76
Crude Fibre, %	11.72	9.48
NDF, %	27.44	23.93
ADF, %	15.99	12.77
ME, MJ/kg (Mcal)	12.08 (2.89)	12.33 (2.95)

[1] Values are indicative of ingredient composition within the diet used, kg/ tonne
[2]Contained 200 g/kg monensin sodium (International Animal Health, Huntingwood, NSW, Australia) and provided 20 mg/kg of monensin sodium to the final diet.
[3] Contained (on a DM basis): 8000 IU/g of vitamin A; 2000 IU/g of vitamin D; 16000 mg/kg of vitamin E; 12000 mg/kg of copper; 400 mg/kg of selenium; 200 mg/kg of cobalt; 1000 mg/kg of iodine; 10000 mg/kg iron; 50000 mg/kg of zinc; 30000 mg/kg of manganese; and 15000 mg/kg antioxidant.

3.2.4 Climatic Data

Onsite climatic data were collected at 30 minute intervals using an automated weather station (GRWS100 Weather Station, Campbell Scientific, Logan, UT, USA) located beside the feedlot

(northern end). Weather data collected included ambient temperature (**T_A**; °C); relative humidity (**RH**; %); wind speed (**WS**; m/s) and direction; solar radiation (**SR**; W/m^2); black globe temperature (**BGT**; °C); and 24 hour daily rainfall, measured at 0900 h. From these data temperature humidity index (**THI**), heat load index (**HLI**) and accumulated heat load for shaded Angus (**AHL_{91}**) were calculated (Table 3.2).

Temperature humidity index was calculated by using the following equation as adapted from Thom (1959);

$$THI = 0.8 \times T_A \left\lfloor (\frac{RH}{100} \times (T_A - 14.4) \right\rfloor + 46.4$$

Where RH = Relative Humidity (%); and T_A = wet bulb or dew point temperature

Heat Load Index and accumulated heat load for shaded Angus (**AHL_{91}**) were calculated based on equations presented by Gaughan et al. (2008b) where the HLI is calculated based on two BGT thresholds where BGT i) ≤ 25 °C or ii) ≥ 25 °C where the index takes the following forms;

i) A nonlinear regression which applies when BGT is greater than 25 °C

$$HLI_{BGT>25} = 8.62 + (0.38 \times RH) + (1.55 \times BGT) - (0.5 \times WS) + [e^{2.4-WS}]$$

Where RH = Relative Humidity (%); BGT = Black Globe Temperature (°C); WS = wind speed (m/s); and e = the base of the natural logarithm (approximate value of e = 2.71828)

ii) A linear model which applies when BGT falls below 25 °C;

$$HLI_{BGT<25} = 10.66 + (0.28 \times RH) + (1.3 \times BGT) - WS$$

Gaughan et al. (2008b) established the following equations to calculate accumulated heat load;

iii) If $[HLI_{ACC} < HLI_{Lower\ Threshold}, (HLI_{ACC} - HLI_{Lower\ Threshold})/M]$;
iv) If $[HLI_{ACC} > HLI_{Upper\ Threshold}, (HLI_{ACC} - HLI_{Upper\ Threshold})/M, 0]$

Where HLI_{ACC} = the actual HLI value at a point in time; $HLI_{Lower\ Threshold}$ = the HLI lower threshold where cattle will dissipate heat (e.g. 77); $HLI_{Upper\ Threshold}$ = the HLI upper threshold where cattle will gain heat (for this experiment = 91); and M = number of measures per hour, i.e. number of times climatic data are collected per hour; if every 10 minutes, then M = 6 (Gaughan et al., 2008b).

3.2.5 Rumen Temperature

Rumen boluses (Smartstock, Pawnee, OK, USA) were orally administered via a custom designed bolus applicator. Individual boluses were cylindrical in shape (3.1 cm diameter by 8.3 cm in length) and weighed approximately 117 g. Boluses were calibrated using a water bath, for 24 hours at 39 °C, prior to administration to ensure no variability existed between boluses. The boluses were an active **RFID** transmitter operating within the 915 to 928 MHz frequencies range. The radio signal

could be detected up to 90 m. The radio transmissions were communicated via a yagi antenna to a base station, and were then transcribed to a database using proprietary software (TechTrol Inc., Pawnee, OK, USA). Rumen temperatures were transmitted and recorded at 10 minute intervals over 128 days. At each transmission of rumen temperature the previous 11 data points (110 minutes) were also enumerated, thereby minimizing potential data loss. Rectal temperatures were obtained from all 80 animals at 7 day intervals by inserting a digital thermometer (BD™, Becton, Dickinson and Company, USA), into the rectal cavity. This was done when cattle were weighed. Rectal temperatures were obtained on these occasions so that the relationship between rectal temperature and rumen temperature could be determined.

3.2.6 Statistical Analysis

Ten minute individual rumen temperature data were converted to an hourly average for each individual steer. The relationship between rumen temperature and rectal temperature was determined by a partial correlation coefficient (Minitab® 16.2.0, 2010 Minitab, Inc.), allowing for the effects of day, using both real time and mean hourly rumen temperature. As the true value of core body temperature is unknown, rumen temperature and rectal temperature both become an estimated measure of core body temperature; therefore a relationship between the two measures would be anticipated. Consequently it must be determined whether rumen temperature and rectal temperature are comparable and also to assess the degree of agreement between rumen temperature and rectal temperature (Bland and Altman, 1986). Thus rumen temperature and rectal temperature data were also analysed using the Bland-Altman method of comparison (Altman and Bland, 1983; Bland and Altman, 1986) where the difference between rumen temperature minus rectal temperature were assessed against the mean of both measures. The mean of both measures becomes the best functional estimate of core body temperature. The Bland-Altman method of comparison was conducted using both real time and mean hourly rumen temperature.

3.3 Results

3.3.1 Weather

Overall the weather conditions throughout the duration of the experiment were average for the region (Table 3.2) with some intermittent hot days above 35 °C (n = 18). The weather conditions were sufficient to incite heat load responses, i.e. an increased panting score, in the steers on most days (Figure 3.1). During the experiment there were 93 days with a maximum HLI ≥ 86. Of these 93 days, 67 days had a HLI ≥ 90, 35 days where HLI ≥ 95 and 8 day where HLI ≥ 100. There were 109 nights where HLI ≤ 60, 57 of these nights HLI ≤ 55, and 10 nights where HLI ≤ 50. These data

indicates that on 75.2 % of nights these animals were able to dissipate accumulated heat load throughout night time hours.

Table 3.2: Mean (± SEM) hourly ambient temperature (T_A,°C), relative humidity (RH, %), wind speed (WS, m/s), solar radiation (SR, W/m^2), black globe temperature (BGT, °C), temperature humidity index (THI), heat load index (HLI) and accumulated heat load for shaded Angus steers (AHL_{91}) over 128 days

Hour	Item							
	T_A (°C)	RH (%)	WS (m/s)	SR (W/m^2)	BGT (°C)	THI	HLI[1]	AHL_{91}[2]
0000	21.2 ± 0.1	80.2 ± 0.6	1.0 ± 0.0	0.0 ± 0.0	20.1 ± 0.1	68.8 ± 0.2	58.8 ± 0.4	0.8 ± 0.2
0100	20.7 ± 0.1	82.6 ± 0.6	0.9 ± 0.0	0.0 ± 0.0	19.7 ± 0.2	68.2 ± 0.2	58.7 ± 0.3	0.4 ± 0.2
0200	20.2 ± 0.1	84.9 ± 0.5	0.9 ± 0.0	0.0 ± 0.0	19.2 ± 0.2	67.6 ± 0.2	58.9 ± 0.3	0.3 ± 0.1
0300	19.9 ± 0.1	86.5 ± 0.5	0.8 ± 0.0	0.0 ± 0.0	18.8 ± 0.2	67.0 ± 0.2	58.7 ± 0.3	0.2 ± 0.1
0400	19.6 ± 0.2	87.7 ± 0.6	0.8 ± 0.0	0.0 ± 0.0	18.6 ± 0.2	66.6 ± 0.3	58.7 ± 0.3	0.2 ± 0.1
0500	19.4 ± 0.2	88.3 ± 0.6	0.8 ± 0.0	7.4 ± 0.9	18.6 ± 0.2	66.3 ± 0.3	58.9 ± 0.3	0.2 ± 0.1
0600	20.1 ± 0.1	85.5 ± 0.6	0.9 ± 0.0	79.7 ± 4.5	21.5 ± 0.2	67.3 ± 0.2	63.7 ± 0.6	0.1 ± 0.1
0700	22.0 ± 0.1	76.7 ± 0.6	1.4 ± 0.0	276.5 ± 8.7	27.7 ± 0.3	69.9 ± 0.2	78.8 ± 0.8	0.2 ± 0.1
0800	24.0 ± 0.1	68.0 ± 0.7	1.8 ± 0.1	456.9 ± 12.0	31.1 ± 0.3	72.1 ± 0.2	82.2 ± 0.6	0.7 ± 0.1
0900	25.7 ± 0.1	60.8 ± 0.7	2.0 ± 0.1	614.3 ± 14.1	34.3 ± 0.3	73.7 ± 0.2	84.2 ± 0.6	1.4 ± 0.2
1000	27.1 ± 0.2	54.5 ± 0.7	2.1 ± 0.0	731.2 ± 15.7	36.3 ± 0.3	74.9 ± 0.2	84.7 ± 0.5	2.5 ± 0.3
1100	28.4 ± 0.2	49.7 ± 0.7	2.3 ± 0.1	807.7 ± 16.1	37.9 ± 0.3	75.9 ± 0.2	85.3 ± 0.5	3.4 ± 0.5
1200	29.6 ± 0.2	45.5 ± 0.7	2.4 ± 0.1	818.7 ± 16.8	38.9 ± 0.3	76.7 ± 0.2	84.9 ± 0.5	4.4 ± 0.6
1300	30.5 ± 0.2	42.4 ± 0.7	2.5 ± 0.1	806.6 ± 15.2	40.0 ± 0.3	77.3 ± 0.2	85.1 ± 0.6	5.3 ± 0.7
1400	30.9 ± 0.2	40.6 ± 0.7	2.7 ± 0.1	681.4 ± 15.0	39.6 ± 0.3	77.5 ± 0.2	83.4 ± 0.6	6.1 ± 0.8
1500	30.8 ± 0.2	41.3 ± 0.8	2.8 ± 0.0	546.0 ± 13.2	38.5 ± 0.4	77.4 ± 0.2	81.6 ± 0.5	6.6 ± 0.8
1600	30.1 ± 0.2	43.2 ± 0.9	2.9 ± 0.1	374.1 ± 10.2	36.4 ± 0.4	76.9 ± 0.2	78.9 ± 0.5	6.6 ± 0.8
1700	28.9 ± 0.2	47.0 ± 0.9	3.0 ± 0.1	189.7 ± 6.3	32.7 ± 0.3	75.9 ± 0.2	74.0 ± 0.4	6.3 ± 0.8
1800	26.9 ± 0.2	54.0 ± 0.9	2.8 ± 0.1	41.5 ± 2.4	27.7 ± 0.3	74.3 ± 0.2	67.2 ± 0.5	5.6 ± 0.8
1900	25.0 ± 0.2	61.3 ± 0.8	2.3 ± 0.1	0.9 ± 0.2	24.3 ± 0.2	72.7 ± 0.2	61.4 ± 0.5	4.6 ± 0.7
2000	23.6 ± 0.2	67.1 ± 0.7	1.8 ± 0.0	0.0 ± 0.0	22.8 ± 0.2	71.4 ± 0.2	59.2 ± 0.5	3.5 ± 0.6
2100	22.8 ± 0.1	71.6 ± 0.6	1.5 ± 0.0	0.0 ± 0.0	21.9 ± 0.2	70.6 ± 0.2	59.2 ± 0.5	2.6 ± 0.5
2200	22.2 ± 0.1	75.0 ± 0.6	1.3 ± 0.0	0.0 ± 0.0	21.2 ± 0.2	69.9 ± 0.2	59.3 ± 0.4	2.0 ± 0.4
2300	21.7 ± 0.1	77.9 ± 0.6	1.1 ± 0.0	0.0 ± 0.0	20.6 ± 0.2	69.4 ± 0.2	59.0 ± 0.4	1.4 ± 0.3

[1] HLI Categories: 1) cool, ≤ 70; 2) moderate, 70.1 ≤ 77; 3) hot, 77.1 ≤ 86; 4) very hot, ≥ 86

[2] Accumulated heat load Categories: 1) low; 2) mild, 1.1 < 10; 3) moderate, 10.1 < 20; 4) hot, 20.1 < 50; 5) extreme, ≥ 50.1

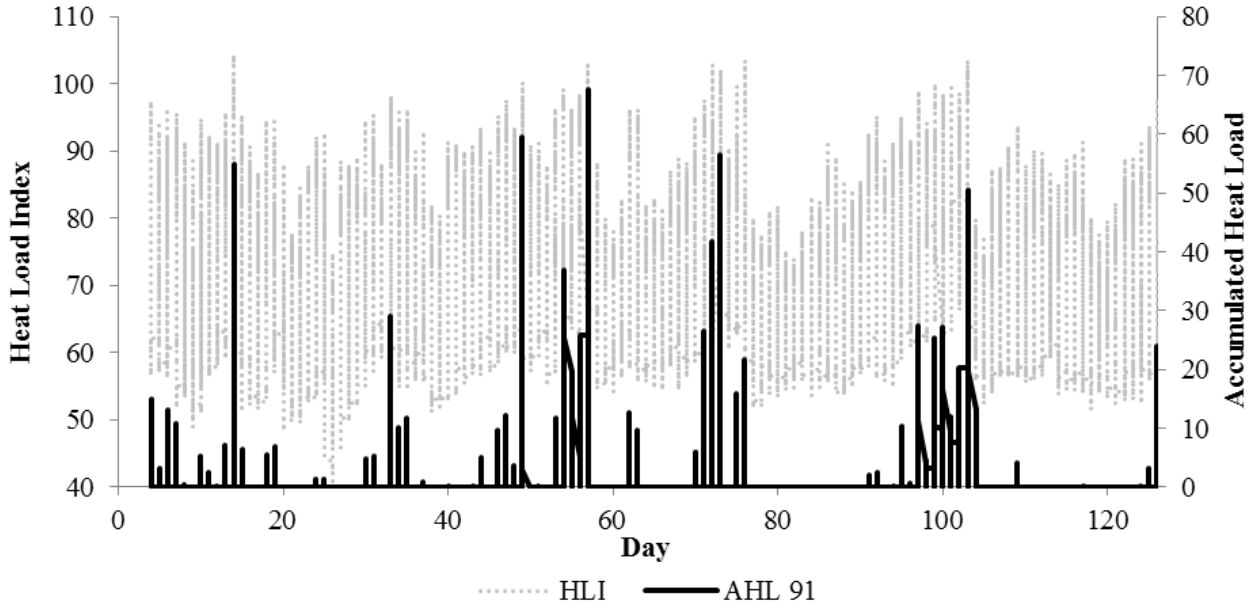


Figure 3.1: Heat load index (HLI) and accumulated heat load for shaded Angus (AHL91) over 128 days

3.3.2 Animal Performance

As feed intake was not measured individually, it was not possible to separate individual differences for dry matter intake (**DMI**). Mean DMI for all steers was 9.86 ± 0.06 kg/animal. There were no pen effects on mean average daily gain (**ADG**) for all steers (1.30 ± 0.04 kg/day) or initial (388.8 ± 2.1 kg) and final live weight (non-fasted; 577.5 ± 37 kg). Gain to feed ratio was 0.13:1.

3.3.3 Rumen Temperature versus Rectal Temperature

Individual mean hourly rumen temperatures were used to calculate an overall mean hourly rumen temperature (Minitab® 16.2.0, 2010 Minitab, Inc.) defining the diurnal rhythm of rumen temperature (Figure 3.2). A partial correlation coefficient (Minitab® 16.2.0, 2010 Minitab, Inc.) indicated that there was a moderate to strong association between rumen temperature and rectal temperature using both real time ($r = 0.55$; $P < 0.0001$) and mean hourly ($r = 0.51$; $P < 0.001$) rumen temperature. Given the type of data presented, the linear fit of real time ($R^2 = 0.35$, $P < 0.001$; Figure 3.3a) and mean hourly ($R^2 = 0.32$, $P < 0.001$; Figure 3.3b) data were also assessed.

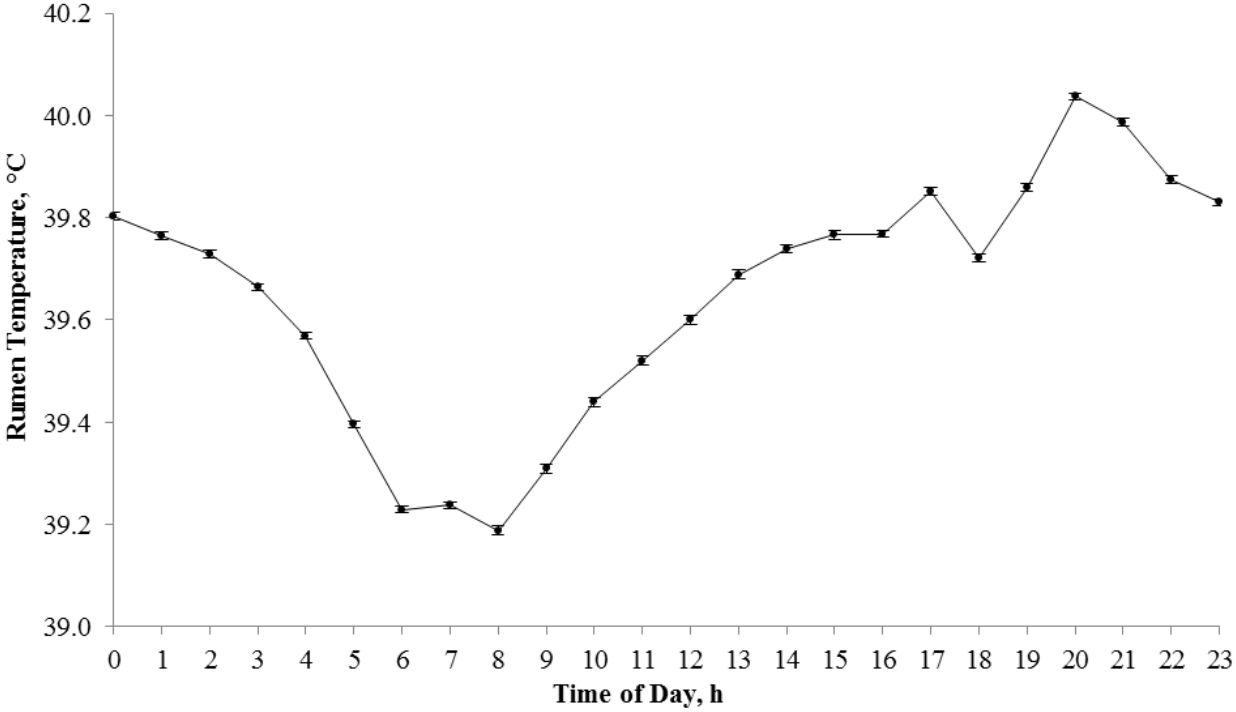


Figure 3.2: Diurnal rhythm using hourly (h) rumen temperature of shaded Angus steers over 128 days

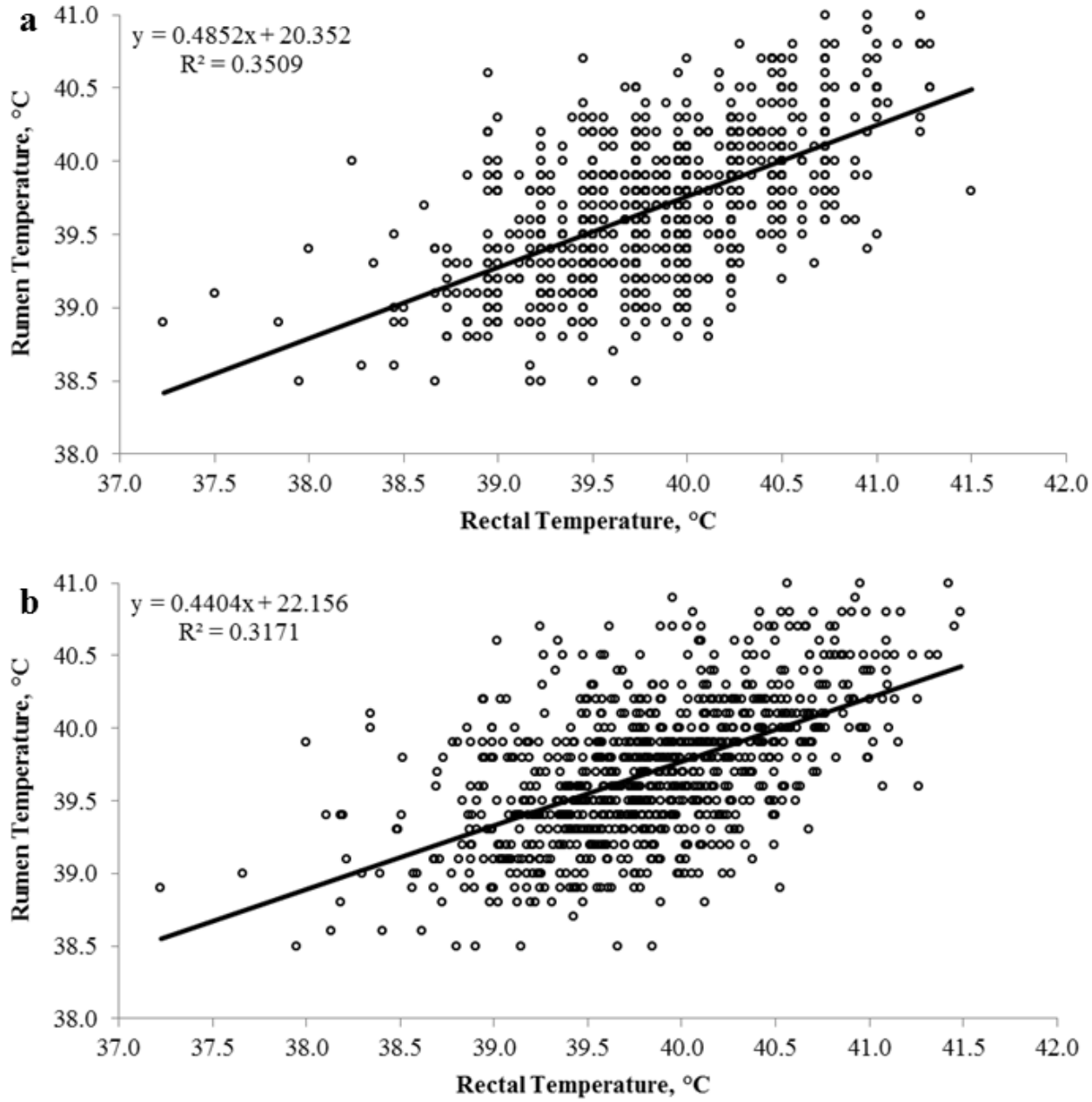


Figure 3.3: Linear relationship between rectal temperate and rumen temperature using a) real time and b) mean hourly data

In this experiment, both rumen temperature and rectal temperature were measured to estimate core body temperature; therefore data were also analysed using the Bland-Altman method of comparison (Bland and Altman, 1986). Using the Bland-Altman method mean differences between rectal temperature and rumen temperature using both real time (0.16 ± 0.02 °C; Figure 3.4a) and hourly mean rumen temperature (0.13 ± 0.02 °C; Figure 3.4b) were small.

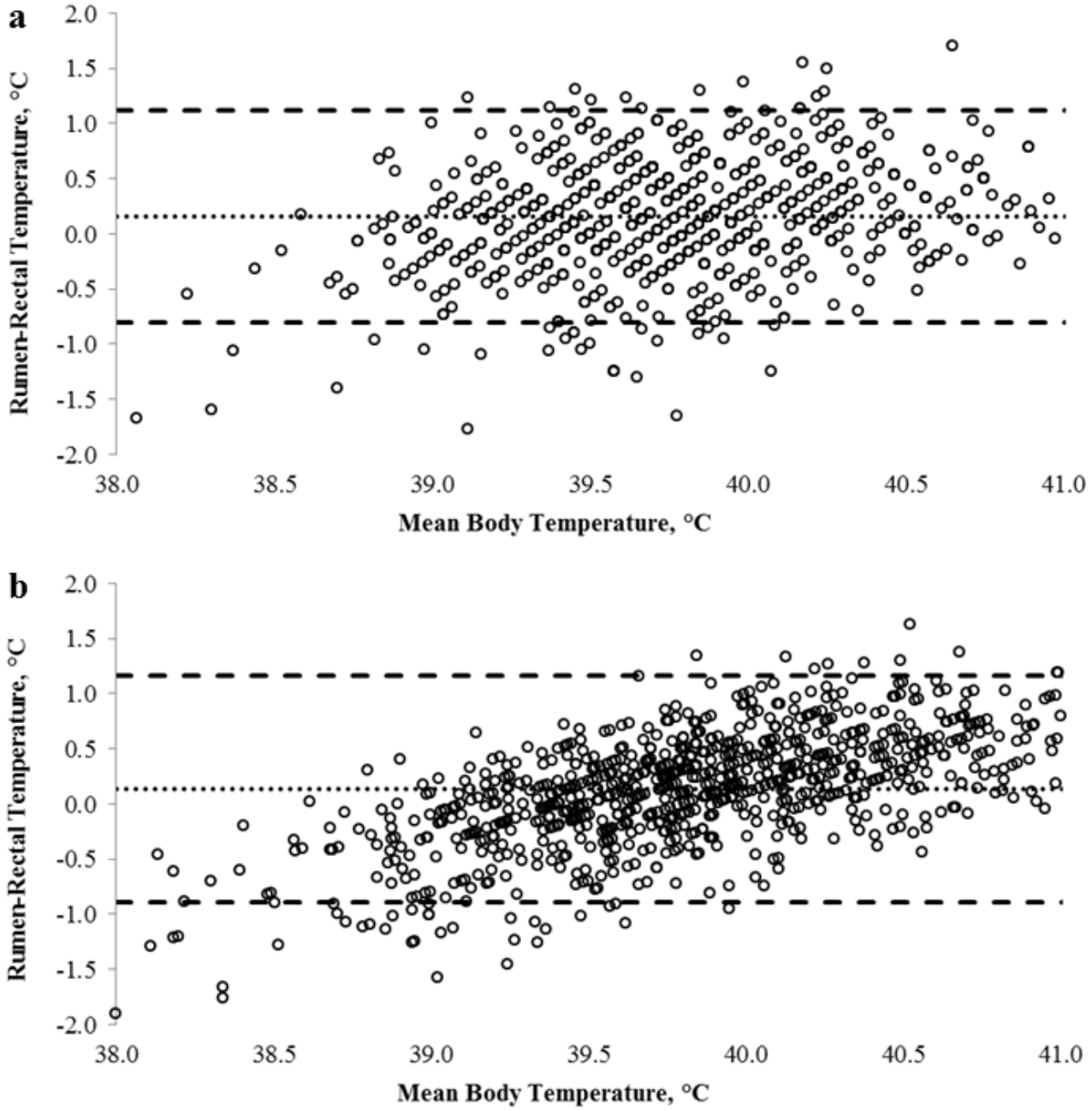


Figure 3.4: Bland Altman plot assessing the use rumen temperature as an assessment of core body temperature using a) real time and b) mean hourly rumen temperature, where the differences between rectal temperature and rumen temperature were evaluated against the combined mean of rectal temperature and rumen temperature, also showing the mean difference (dotted line) and confidence intervals (95 % = mean ± 1.96 × Standard Deviation; dashed line)

3.4 Discussion

Cattle are able to regulate and maintain a near constant body temperature despite variability in surrounding climatic conditions to maintain homeostasis (Robertshaw, 1985). However body temperature is not absolute with all animals exhibiting a diurnal variation, even under constant thermoneutral conditions (Robertshaw, 1985). These diurnal variations in body temperature can be considered a state of equilibrium between heat accumulation and dissipation (Legates et al., 1991).

Normal metabolic processes, i.e. digestion and locomotion, all contribute to metabolic heat production increasing body temperature and therefore contributing to the diurnal pattern of body temperature. Dissipation of thermal heat from the body during hot summer conditions can be ineffective as heat accumulation is often greater than heat dissipation (Bertipaglia et al., 2007). As heat load increases, cattle will partition energy away from growth and development and towards heat dissipation in order to maintain homeostasis (Kadzere et al., 2002; Ravagnolo and Misztal, 2002). Therefore core body temperature is considered to be a reliable indicator of thermal balance and estimates of core body temperature have previously been measured at several locations including tympanic (Davis et al., 2003; Mader et al., 2010a), abdominal (Lefcourt and Adams, 1996; Gaughan et al., 2010a), rectal (Gaughan et al., 1999; Gaughan et al., 2008a) and rumen (Ipema et al., 2008; Rose-Dye et al., 2011; Mohammed et al., 2014). However the true measure of core body temperature is difficult to quantify; therefore many measures of body temperature become an estimation of core body temperature.

There have been few studies that have utilized techniques that allow for continuous recording of body temperature over prolonged periods of time, ≥ 10 days (Lefcourt and Adams, 1996; Nienaber et al., 1999; Gaughan et al., 2010a). Methodologies within this experiment used RFID rumen boluses for the collection of real time continuous rumen temperature data over a prolonged period, totalling 128 days. In previous studies data loggers collect and store body temperature data that is downloaded at the completion of the data collection phase, typically ≤ 10 d; thus assessing the true impact of heat load on an animal's thermal status post event. The collection of real time data allows for the impact of hot ambient conditions on the thermal status of animals to be conducted throughout the duration of the event. Previous studies have used rumen boluses to assess the impact of controlled hot ambient conditions on rumen temperature over shorter periods of time, ≤ 25 days (Hahn et al., 1990; Beatty et al., 2008), and the changes in rumen temperature as influenced by a disease challenge (Rose-Dye et al., 2011).

The methodology used within the current experiment to obtain rumen temperature negated the need to move cattle to handling facilities, thus reducing the confounding effects of increased body temperature due to locomotion and activation of the stress response from handling. Additionally this experiment also utilised techniques that did not require the surgical implementation of radiotelemetry devices (Lefcourt and Adams, 1996; Gaughan et al., 2010a). This reduced the likelihood of production of scar tissue around data logger, whereby the production of scar tissue around the data logger may potentially falsify body temperature or reduce the sensitivity of the data

logger to alterations in body temperature due to the potential of scar tissue to have insulative properties.

Using rumen temperature as an indicator of core body temperature may present some difficulties as metabolic heat partitioned from digestion and other metabolic functions (Czerkawski, 1980), i.e. locomotion and growth, may result in an increase in rumen temperature. Metabolic heat generated during microbial fermentation (Beatty et al., 2008), accounts for 3 to 8 % of the total heat produced by the animal (Czerkawski, 1980). Failure to dissipate the heat produced within the rumen may result in an increase in rumen temperature (Beatty et al., 2008). However it is difficult to determine the proportion of the increase in rumen temperature that is due to metabolism, and body heat accumulated from the environment. Rumen temperature is considered to be 1 °C to 2 °C higher than rectal temperature (Dale et al., 1954), and stabilised between 38 °C to 42 °C ensuring a stable environment for microbial populations (Yokoyama and Johnson, 1993). Regardless, heat production within the rumen itself is likely to have an influence on body temperature regulation in cattle, where rumen temperature is suspected to contribute to the overall accumulated heat load of the whole body during periods of hot weather (Dale et al., 1954). However knowledge is limited regarding the impact of climatic conditions on rumen temperature over extended periods of time (≥ 25 d). Results from the current experiment indicated that there was a moderate to strong association between rumen temperature and rectal temperature using both real time ($r = 0.55$; $P < 0.0001$) and mean hourly ($r = 0.51$; $P < 0.001$) data.

Previous studies have also identified small differences between rectal temperature and rumen temperature (Hicks et al., 2001; Beatty et al., 2008; Bewley et al., 2008a; Bewley et al., 2008b; Rose-Dye et al., 2011). Bewley et al. (2008a) concluded that the difference in rumen temperature to core body temperature was small, being about 0.5 °C higher in the rumen. Rose-Dye et al. (2011) identified a strong relationship between rectal temperature and rumen temperature ($r = 0.89$; $P < 0.01$) reporting that rectal temperature was on average 0.13 ± 0.38 °C higher than rumen temperature. However in that study rumen boluses were used to assess the change in rumen temperature as an assessment of core body temperature throughout a disease challenge where no data of climate conditions were reported. Nevertheless the study by Rose-Dye et al. (2011) showed that rumen temperature did increase with disease challenge and presented similar changes to body temperature as identified by other methods of determining body temperature e.g. tympanic, peritoneal and rectal temperature. Further to this Hicks et al. (2001) reported that rumen temperature and rectal temperature were comparable where their study showed that over a 1 hour period mean rumen temperature and rectal temperature were 38.7 ± 0.05 °C and 38.7 ± 0.24 °C

respectively. However the study lacks animal numbers (n = 1) to validate the use of rumen temperature as a suitable estimator of core body temperature. Additionally these results may have been confounded by ruminal cannulation, given that within the rumen, under both fasting and non-fasting conditions, there is a temperature gradient between the ventral and dorsal regions of the rumen (Dale et al., 1954). Therefore the influence of a rumen cannula on heat dissipation and accumulation within the rumen becomes difficult to quantify. Furthermore it must be noted that the comparison between rumen temperature and rectal temperature within this experiment was conducted under non-fasting conditions where the cattle were fed at approximately 0700 h and rectal temperature measurements were recorded between 0730 h and 1200 h. Dale et al. (1954) concluded that under fasting conditions the difference between rumen temperature and rectal temperature reduced to 0.7 °C. However a study by Beatty et al. (2008), concluded that the difference between rumen temperature and core body temperature (abdominal) in Angus heifers was constant, approximately 1 °C, despite variations in feed and water intake in conjunction with heat load conditions, over a 25 day experiment. However the authors noted that all (n = 6) animals had rapid decreases in rumen temperature and these events were assumed to be associated with drinking events, although drinking behaviours were not recorded (Beatty et al., 2008).

It is well known that water intake results in a dramatic, but temporary, decrease in rumen temperature (Bewley et al., 2008b). However there is little literature regarding the time required for rumen temperature to return to a normal baseline temperature, where most of the literature is focused on grazing cattle and sheep, as well as dry and lactating dairy cattle. Furthermore time required to recover would depend on i) the amount of water consumed at each intake and ii) the temperature of the water ingested. Bewley et al. (2008b) concluded that ingestion of warm water (34.3 ± 1.0 °C) had minimal impact on rumen temperature. However Beede and Collier (1986) indicated that drinking water may influence thermal exchange by reducing the temperature within the reticulum thus reducing overall thermal load on the animal. Additionally the influence of water consumption on rumen temperature would depend on the frequency of intake along with water temperature and amount of water consumed at each time point and is worthy of further investigation.

Nevertheless using correlations and/or regression models to define the relationship between the two measures used to estimate core body temperature may be misleading, as the correlation coefficient is not a measure of the agreement between rumen temperature and rectal temperature, it is a measure of the association between the two variables (Altman and Bland, 1983). In the current experiment the true value of core body temperature is unknown and given that rumen temperature and rectal

temperature were both measured as an estimation of core body temperature, it would be unusual if no relationship was observed. Therefore using a correlation coefficient to define the relationship between rumen temperature and rectal temperature may not be the best method of assessing the relationship between the two measures. The Bland-Altman method of comparison determined whether rumen temperature and rectal temperature are comparable and evaluates the degree of agreement between rumen temperature and rectal temperature (Bland and Altman, 1986). The Bland-Altman method of comparison was designed to compare estimates of the same measure, i.e. core body temperature, as measured by different methodologies (Bland and Altman, 1986), i.e. rectal temperature and rumen temperature. As body temperature is typically maintained within a small dynamic range, usually within ± 1 °C (Robertshaw, 1985), the Bland-Altman method of comparison (Bland and Altman, 1986) assesses the relationship between the two measures by using rumen temperature minus rectal temperature.

Within this experiment, rectal temperature was considered as the 'gold standard' measurement of core body temperature. Therefore the degree of agreement between rumen temperature and rectal temperature was determined by comparing rumen temperature against the mean of both measures recorded at that time point. Mean data is used as the 'true' value of core body temperature because the actual value of core body temperature is unknown; thus within this experiment the functional relationship between the mean of rumen temperature and rectal temperature becomes the best estimate of core body temperature (Bland and Altman, 1986). By using this method, results from this study indicated that the mean difference between rumen temperature and rectal temperature is small using both real time (0.16 ± 0.02 °C) and hourly mean (0.13 ± 0.02 °C) rumen temperature, demonstrating that rumen temperature can be used as a functional indicator of core body temperature.

3.5 Conclusion

Results from this experiment indicate that rumen temperatures are variable and show a diurnal trend similar to what is observed from other body temperature (tympanic, abdominal and rectal) data sets. Thus rumen temperatures observed within this experiment appear to trend with increasing and decreasing ambient conditions, therefore providing a functional and somewhat reliable estimation of core body temperature. Even though there are small differences between rectal temperature and rumen temperature the assessment of an animal's thermal status can be undertaken through the remote assessment of rumen temperature, using real time or mean hourly rumen temperature. In future studies, given that body temperature was assessed in all animals via the same methodologies, i.e. rumen temperature, variability in the temperature changes are relative to the individuals and

groups within the studies thereby supporting that particular methodology. Thus it may be concluded that rumen temperature appears to be a functional estimate of core body temperature and therefore rumen temperature can be used to measure and quantify heat load in feedlot cattle.

Chapter 4

Using Infrared Thermography as an Assessment of Body Temperature

Experimental Hypothesis: There is a correlation between body surface temperature and rumen temperature, whereby body surface temperature can be used as a predictor of rumen temperature.

4.1 Introduction

Infrared thermography is a non-invasive measurement of body surface temperature. Infrared thermography measures the infrared radiation emitted from an animal, which then allows for the determination of body surface temperature (McCafferty, 2007). Infrared thermography has diagnostic applications in veterinary medicine (Schaefer et al., 2004; Metzner et al., 2014), particularly inflammation (McCafferty, 2007) and disease detection (Schaefer et al., 2004; Schaefer et al., 2012). Due to increasing animal welfare concerns non-invasive methods of obtaining body temperature that are fast, efficient and reliable need to be investigated. Recently there have been studies to investigate the use of infrared thermography as a measure of body temperature (Clerc and González, 2012; George et al., 2014; Metzner et al., 2014). Furthermore there have been some studies utilizing infrared thermography to determine thermal balance in poultry (Nascimento et al., 2014), pigs (Brown-Brandl et al., 2012), cattle (Montanholi et al., 2008) and wildlife (Weissenböck et al., 2010; Rowe et al., 2013).

Evaluating animal health and measuring thermal balance in livestock is typically determined by body temperature. However traditional methods of measuring body temperature involve relocating animals to specifically designed handling facilities (Rose-Dye et al., 2011). Relocation of animals may result in an increase in body temperature, potentially masking illness and disrupting the thermal balance. The use of non-invasive methods, such as infrared thermography, negates the need to restrain or relocate animals to measure body temperature (McCafferty, 2007; George et al., 2014), thereby reducing animal stress. It has been suggested that infrared thermography has the potential to become a non-invasive and rapid determination of core body temperature (George et al., 2014).

To be considered as an alternative method for determining body temperature, infrared thermography needs to be not only rapid and reliable but also must have a strong association with other validated measures of body temperature (Johnson et al., 2011), i.e. tympanic (Davis et al., 2003; Mader et al., 2010a), abdominal (Lefcourt and Adams, 1996; Gaughan et al., 2010a), rumen (Ipema et al., 2008; Rose-Dye et al., 2011; Mohammed et al., 2014) and rectal (Gaughan et al., 1999; Gaughan et al., 2008a). However there have been limited studies investigating the relationship between body surface temperature and validated measures of core body temperature. The objective of this study was *to determine the relationship between body surface temperature and body temperature as measured by rumen temperature.*

4.2 Materials and Methods

This experiment was conducted with the approval of The University of Queensland (**UQ**) animal ethics committee (SAFS/210/13/MLA). The experiment was undertaken in southeast Queensland, Australia, at UQ (27.54^0S, 152.34^0E; 100 m above mean sea level) large animal research facility during a southern hemisphere summer (December to February). During summer the location is characterised by a hot, humid sub-tropical climate.

4.2.1 Animal Housing and Experimental Design

The cattle used within this experiment were a part of a larger 130 day study. Steers were vaccinated against clostridial diseases (enterotoxaemia (pulpy kidney disease), tetanus, blacks disease, malignant oedema and blackleg; Pfizer Animal Health, Australia) on d -19; bovine respiratory disease (Bovillis MH, inactivated *Mannheimia haemolytica*; Coopers Animal Health, Australia) on d -7 and d -28; and trivalent tick fever (chilled, 3 germ; *Babesia. bovis, Babesia bigemina and Anaplasma centrale;* Department of Agriculture, Fisheries and Forestry (Biosecurity Queensland), Australia) on d -18. Hormonal growth promotants were not used in the experiment. Cattle were weighed, non-fasted, on d 0 and d 6 at approximately 0830 h.

In the experiment presented here 36 Angus steers were used in a repeated experiment; 3 observational periods of 12 steers. Steers had an initial non-fasted live weight of 392.3 ± 5.1 kg, 427.5 ± 6.3 kg and 392.7 ± 3.7 kg for each observational period respectively. Twelve individual animal pens (10 m × 3.4 m) were used within the Queensland Animal Science Precinct at UQ. Steers were randomly allocated to each individual pen. The individual pens were situated in a north-south alignment, where only pens on the northern side were used. Pen surfaces were soil. Concrete feed bunks were situated at the front of the pens with a 2.5 m concrete apron located at the front of each pen (southern). Shade was provided by shade-cloth (cream, 90 % solar block, Darling Downs Tarpaulins, Toowoomba, Queensland, Australia) attached to a 4 m steel framed roof structure, covering approximately 5 m of the pen at midday. Animals were relocated from grouped housing to the individual pens on d 0.

Prior to the commencement of each observational period, d -28 to d 0, cattle were housed as a group (n = 12) in a singular feedlot pen, 162 m^2 (27 m × 6 m), within the research feedlot at UQ. Stocking density was 13.5 m^2/animal. The feed bunk provided a linear area of 0.35 m^2/animal and the linear water trough area was 0.08 m^2/animal. Shade was provided by shade-cloth (black, 90 % solar block, Darling Downs Tarpaulins, Toowoomba, QLD, Australia) attached to a 4 m high structure. The shade structure provided a shade footprint of 1.5 m^2/animal at midday.

Each observational period was conducted over a 6 day period where data collection occurred at 3 h intervals, commencing at 0600 h on d 1 and concluding at 0600 h on d 6. At each data collection time point the same pen sequence was used, i.e. commenced at pen 1 and was completed at pen 12.

4.2.2 Nutritional Management

Each observational period entered the research feedlot as a group on d -28, commencing on a starter ration through to d -13, then transitioned to a finisher ration over 10 d (d -3) for the remainder of the study (Table 4.1). Refusals were removed and weighed daily with average consumption per pen and per animal calculated, whilst the animals were housed as a group. Individual feed intake was calculated once animals were housed in individual pens on d 0. Cattle were fed twice daily at approximately 0700 h and 1630 h. Mean feed intake was 9.15 ± 0.26 kg/animal, 10.56 ± 0.12 kg/animal and 10.95 ± 0.03 kg/animal during each observational period respectively.

Table 4.1: Diet and Nutrient Composition

Item	Starter[1]	Finisher[1]
Ingredient, kg (as fed)		
Barley	150	250
Sorghum	350	354
Millrun	100	100
Cottonseed Meal	25	20
Molasses	20	20
Limestone	13.45	14.7
Sodium bicarbonate	8	8
Urea	7	6.95
Sulphur (dusting)	0.29	0.29
Moneco® 200[2]	0.10	0.10
Sodium bentonite	25	25
Mineral – vitamin supplement[3]	1	1
Chickpea shell	300	200
Nutrient Composition (as fed)		
DM, %	89.15	89.11
NE_g, Mcal/kg	1.63	1.68
Crude Fat, %	2.32	2.31
CP, %	12.02	12.03
RDP; %	8.14	8.23
UDP; %	3.84	3.76
Crude Fibre, %	11.72	9.48
NDF, %	27.44	23.93
ADF, %	15.99	12.77
ME, MJ/kg (Mcal)	12.08 (2.89)	12.33 (2.95)

[1] Values are indicative of ingredient composition within the diet used, kg/ tonne

[2]Contained 200 g/kg monensin sodium (International Animal Health, Huntingwood, NSW, Australia) and provided 20 mg/kg of monensin sodium to the final diet.

[3] Contained (on a DM basis): 8000 IU/g of vitamin A; 2000 IU/g of vitamin D; 16000 mg/kg of vitamin E; 12000 mg/kg of copper; 400 mg/kg of selenium; 200 mg/kg of cobalt; 1000 mg/kg of iodine; 10000 mg/kg iron; 50000 mg/kg of zinc; 30000 mg/kg of manganese; and 15000 mg/kg antioxidant.

4.2.3 Climatic Data

Weather data were collected at 30 minute intervals using an automated weather station (GRWS100 Weather Station, Campbell Scientific, Logan, UT, USA), located at the northern end of the feedlot (≈ 100 m from individual animal pens). Weather data collected included ambient temperature (**T_A**; °C); relative humidity (**RH**; %); wind speed (**WS**; m/s) and direction; solar radiation (**SR**; W/m^2); black globe temperature (**BGT**; °C); and 24 hour daily rainfall, measured at 0900 h. From these data temperature humidity index (**THI**), heat load index (**HLI**) and accumulated heat load for shaded Angus (**AHL_{91}**) were calculated as described in Chapter 3 section 3.2.4 for each observational period from the equations adapted from Thom (1959) and Gaughan et al. (2008b).

4.2.4 Rumen Temperature

Rumen temperatures were recorded as described in Chapter 3 section 3.2.5. Briefly rumen boluses (Smartstock, Pawnee, OK, USA) were orally administered via a custom designed bolus applicator. The boluses were an active RFID transmitter operating within the 915 to 928 MHz frequencies range. The radio transmissions were communicated via a yagi antenna to a base station, and were then transcribed to a database using proprietary software (TechTrol Inc., Pawnee, OK, USA). Rumen temperatures were transmitted and recorded at 10 minute intervals during each observational period. At each transmission of rumen temperature the previous 11 data points (110 minutes) were also enumerated.

4.2.5 Infrared Thermography

Infrared thermography images of the head and body of each steer were obtained using an infrared thermal image camera (Fluke Ti25, Fluke Corporation, Everett, WA, USA). Infrared thermography images were taken at a distance no greater than 2 m from the animal. Infrared thermography images were obtained where the animal was located within the pen; therefore in shaded and un-shaded areas. Each individual infrared thermography images were analysed using proprietary software (Fluke Smart View Software, version 3.0, Fluke Corporation, Everett, WA, USA). As infrared thermography measures radiant energy from the animal the usefulness of infrared thermography images is dependent on capturing the object perpendicular to the infrared thermography camera. Usable infrared thermography images were evaluated using an emissivity value of 0.98 as recommended by Steketee (1973); non-usable images were excluded from analysis (Figure 4.1). Additionally a correction was made for T_A by the software package.

Mean, minimum and maximum body surface temperatures were determined using a zone analysis where temperature was determined from the i) head (zone 1; Figure 4.2a) and ii) 3 defined zones on the trunk of the animal (zone 2, shoulder; zone 3, trunk; and zone 4, rump; Figure 4.2b). Mean, minimum and maximum body surface temperatures were also determined from the medial line on the i) head, between the poll and the nose (Figure 4.3a), and ii) body, between the transverse medial plane between the point of the shoulder (greater tubercle of humerus) and the hind limb of the animal (Figure 4.3b).

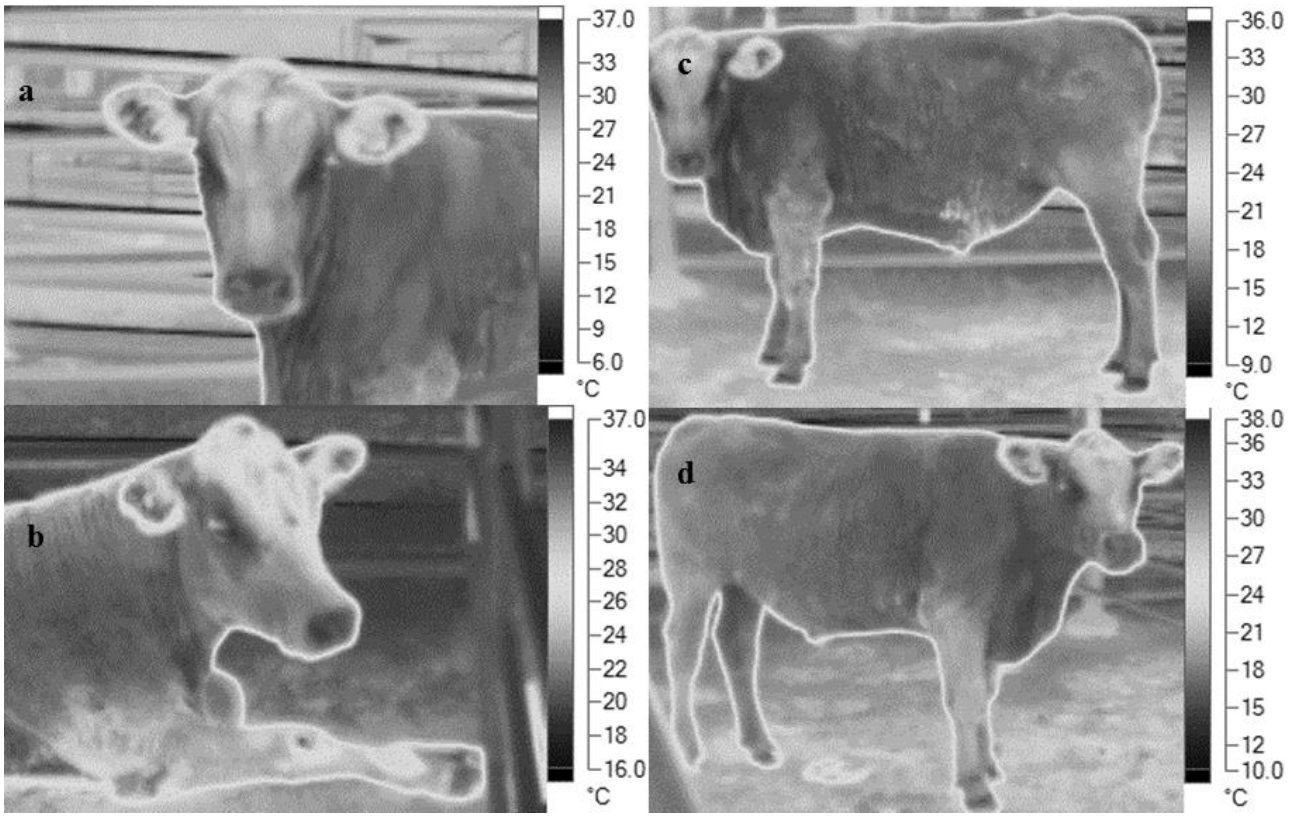


Figure 4.1: Usable and non-useable infrared thermography images of the head (a, usable; b, non-usable) and body (c, useable; d, non-usable)

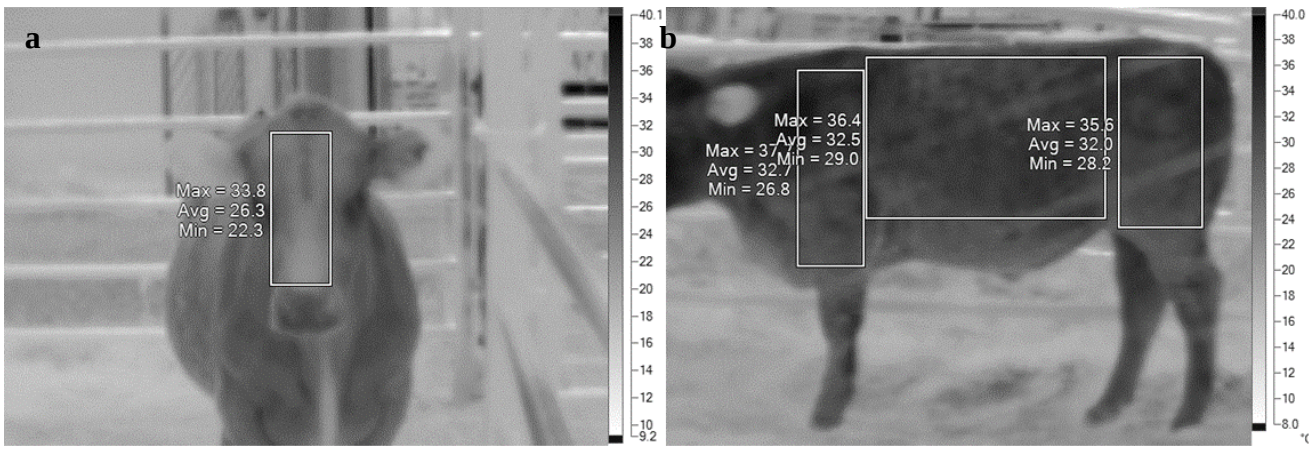


Figure 4.2: a) zone analysis of the head and b) shoulder, trunk and rump

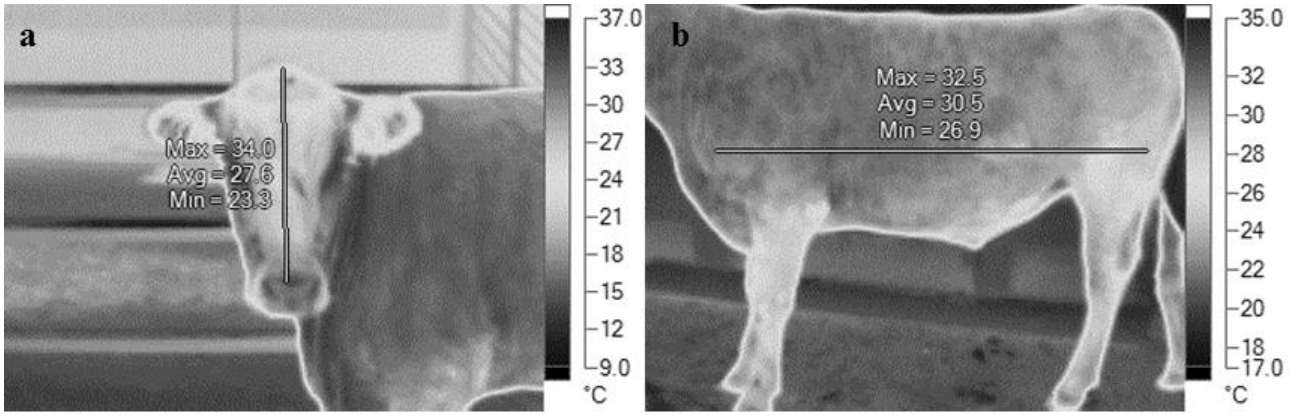


Figure 4.3: Infrared thermography image analysis for a) the head using the medial line between the poll and the nose and b) the body using the transverse medial plane between the point of the shoulder and the hind limb of the animal

4.2.6 Statistical Analysis

Ten minute individual rumen temperature data were converted to an hourly average for each individual steer. The relationship between rumen temperature and body surface temperature was determined using a Pearson's correlation coefficient (Minitab® 16.2.0, 2010 Minitab, Inc.). Rumen temperature and body surface temperature from the same data collection time points were used, i.e. body surface temperature obtained at 0600 h were assessed against 0600 h mean rumen temperature, body surface temperature obtained at 0900 h were assessed against 0900 h mean rumen temperature and so on for each hour. Additionally to assess the degree of agreement between rumen temperature and body surface temperature the relationship between the two measures was analysed using the Bland-Altman method of comparison (Altman and Bland, 1983; Bland and Altman, 1986). To determine whether rumen temperature and body surface temperature are comparable, the difference between rumen temperature and body surface temperature was assessed against the mean of both measures. The mean of both measures becomes the functional estimate of the agreement between rumen temperature and body surface temperature (Bland and Altman, 1986), within this experiment.

4.3 Results

4.3.1 Weather

Overall the weather conditions throughout the observational periods were moderate. Throughout the duration of the summer the overall weather conditions were average for the region with some intermittent hot days above 35 °C. Mean hourly HLI and accumulated heat load for shaded Angus (**AHL_{91}**) were calculated throughout the duration of each observational period (Table 4.2, Period 1; Table 4.3, Period 2; Table 4.4, Period 3). During observational period 1 and 2, steers were exposed to conditions where the average duration of HLI ≥ 86 were 7 hours and 8 hours respectively,

corresponding with exposure to moderate accumulated heat load (10.1 ≤ 20). Furthermore conditions during observational period 2 night time conditions were warmer than observational period 1 and 3, whereby accumulated heat load remained moderate (10.1 ≤ 20), mild (1.1 ≤ 10) and low (≤ 1) for 12, 11 and 1 hours respectively. However night time conditions were sufficient for heat dissipation to occur during observational period 1 and 2, whereby animals were able to dissipate any accumulated heat load throughout the night time hours. Weather conditions during observational period 3 were mild; however conditions were sufficient for low accumulated heat load (≤ 1) for 7 hours.

Table 4.2: Mean (± SEM) hourly ambient temperature (T_A,°C), relative humidity (RH, %), wind speed (WS, m/s), solar radiation (SR, W/m^2), black globe temperature (BGT, °C), temperature humidity index (THI), heat load index (HLI) and accumulated heat load for shaded Angus steers (AHL_{91}) during period 1

Hour	Item							
	T_A (°C)	RH (%)	WS (m/s)	SR (W/m^2)	BGT (°C)	THI	HLI[1]	AHL_{91}[2]
0000	20.2 ± 0.2	88.1 ± 2.2	0.9 ± 0.1	0.0 ± 0.0	19.4 ± 0.2	67.6 ± 0.3	59.7 ± 0.8	0.0 ± 0.0
0100	20.1 ± 0.2	88.2 ± 1.8	0.8 ± 0.1	0.0 ± 0.0	19.3 ± 0.3	67.5 ± 0.3	59.6 ± 0.7	0.0 ± 0.0
0200	19.8 ± 0.2	89.1 ± 1.6	0.9 ± 0.1	0.0 ± 0.0	18.8 ± 0.3	67.0 ± 0.4	59.2 ± 0.7	0.0 ± 0.0
0300	19.0 ± 0.4	88.9 ± 2.0	0.9 ± 0.1	0.0 ± 0.0	18.2 ± 0.5	65.8 ± 0.7	58.4 ± 1.1	0.0 ± 0.0
0400	18.6 ± 0.5	89.2 ± 2.2	0.9 ± 0.1	0.0 ± 0.0	17.9 ± 0.6	65.1 ± 0.9	58.0 ± 1.2	0.0 ± 0.0
0500	18.4 ± 0.5	90.3 ± 1.8	0.7 ± 0.1	13.7 ± 4.0	17.8 ± 0.6	64.8 ± 1.0	58.4 ± 1.1	0.0 ± 0.0
0600	19.0 ± 0.6	87.2 ± 1.7	0.8 ± 0.1	126.8 ± 20.9	21.5 ± 0.9	65.6 ± 1.0	63.8 ± 2.4	0.0 ± 0.0
0700	21.4 ± 0.4	78.5 ± 2.6	1.2 ± 0.1	389.6 ± 35.9	28.9 ± 1.1	69.1 ± 0.7	81.9 ± 3.6	0.1 ± 0.1
0800	23.5 ± 0.2	69.5 ± 3.0	1.4 ± 0.1	565.8 ± 44.3	32.8 ± 0.8	71.5 ± 0.5	87.2 ± 1.7	0.7 ± 0.3
0900	25.2 ± 0.2	62.3 ± 3.3	1.8 ± 0.2	732.8 ± 39.0	35.7 ± 0.5	73.2 ± 0.4	88.2 ± 1.9	1.7 ± 0.7
1000	26.8 ± 0.2	55.7 ± 3.1	2.0 ± 0.2	925.9 ± 42.4	37.9 ± 0.7	74.8 ± 0.5	88.4 ± 2.7	3.1 ± 1.3
1100	28.1 ± 0.2	51.1 ± 3.2	1.9 ± 0.2	1038.5 ± 29.5	40.0 ± 0.8	75.9 ± 0.5	90.3 ± 2.6	5.3 ± 2.6
1200	29.0 ± 0.1	48.4 ± 3.3	2.0 ± 0.2	820.8 ± 86.7	39.1 ± 0.9	76.7 ± 0.5	87.5 ± 2.1	7.9 ± 3.8
1300	30.1 ± 0.2	45.7 ± 3.4	1.9 ± 0.1	873.2 ± 45.3	41.2 ± 0.9	77.7 ± 0.5	89.6 ± 2.1	9.1 ± 4.5
1400	30.7 ± 0.3	43.1 ± 3.6	2.1 ± 0.1	697.1 ± 48.7	40.5 ± 1.1	77.9 ± 0.5	87.0 ± 2.0	10.8 ± 5.5
1500	31.0 ± 0.5	40.2 ± 3.5	2.1 ± 0.1	516.9 ± 66.5	38.5 ± 1.5	77.7 ± 0.5	82.8 ± 1.7	11.6 ± 5.9
1600	31.0 ± 0.5	39.1 ± 3.1	2.4 ± 0.2	355.1 ± 39.8	37.4 ± 1.2	77.6 ± 0.5	80.2 ± 1.5	11.6 ± 5.9
1700	29.8 ± 0.7	42.5 ± 4.5	3.0 ± 0.3	163.4 ± 27.8	33.3 ± 1.2	76.5 ± 0.5	74.3 ± 1.0	11.5 ± 5.9
1800	27.5 ± 0.7	50.3 ± 5.7	2.5 ± 0.3	21.7 ± 7.0	27.6 ± 0.8	74.6 ± 0.4	67.7 ± 1.6	10.0 ± 5.8
1900	24.9 ± 0.7	61.3 ± 5.8	2.1 ± 0.3	0.0 ± 0.0	23.7 ± 0.6	72.3 ± 0.5	59.7 ± 1.5	7.3 ± 4.7
2000	22.6 ± 0.6	70.7 ± 6.4	1.7 ± 0.3	0.0 ± 0.0	21.4 ± 0.4	69.8 ± 0.4	56.5 ± 1.2	4.0 ± 2.9
2100	21.6 ± 0.4	77.1 ± 4.7	1.4 ± 0.2	0.0 ± 0.0	20.6 ± 0.3	69.0 ± 0.3	57.6 ± 1.2	0.3 ± 0.3
2200	20.8 ± 0.2	80.7 ± 4.0	1.2 ± 0.1	0.0 ± 0.0	19.8 ± 0.2	68.2 ± 0.2	57.8 ± 1.2	0.0 ± 0.0
2300	20.5 ± 0.2	86.0 ± 2.2	1.1 ± 0.1	0.0 ± 0.0	19.7 ± 0.2	68.0 ± 0.3	59.2 ± 0.8	0.0 ± 0.0

[1] HLI Categories: 1) cool, ≤ 70; 2) moderate, 70.1 ≤ 77; 3) hot, 77.1 ≤ 86; 4) very hot, ≥ 86

[2] Accumulated heat load Categories: 1) low; 2) mild, 1.1 < 10; 3) moderate, 10.1 < 20; 4) hot, 20.1 < 50; 5) extreme, ≥ 50.1

Table 4.3: Mean (± SEM) hourly ambient temperature (T_A,°C), relative humidity (RH, %), wind speed (WS, m/s), solar radiation (SR, W/m^2), black globe temperature (BGT, °C), temperature humidity index (THI), heat load index (HLI) and accumulated heat load for shaded Angus steers (AHL_{91}) during period 2

Hour	Item							
	T_A (°C)	RH (%)	WS (m/s)	SR (W/m^2)	BGT (°C)	THI	HLI[1]	AHL_{91}[2]
0000	24.0 ± 0.5	82.2 ± 1.6	0.8 ± 0.1	0.0 ± 0.0	23.0 ± 0.7	73.5 ± 0.9	64.4 ± 2.3	8.4 ± 3.3
0100	23.4 ± 0.6	84.6 ± 1.3	0.7 ± 0.1	0.0 ± 0.0	22.5 ± 0.7	72.8 ± 1.0	66.5 ± 3.2	5.1 ± 2.7
0200	22.8 ± 0.7	86.3 ± 0.6	0.7 ± 0.1	0.0 ± 0.0	21.9 ± 0.8	72.0 ± 1.2	66.3 ± 3.3	4.1 ± 2.7
0300	22.4 ± 0.8	88.3 ± 0.5	0.6 ± 0.1	0.0 ± 0.0	21.5 ± 0.9	71.4 ± 1.3	66.0 ± 3.1	4.1 ± 2.7
0400	22.1 ± 0.8	89.4 ± 0.6	0.5 ± 0.1	0.0 ± 0.0	21.1 ± 0.9	71.0 ± 1.3	66.2 ± 3.3	4.1 ± 2.7
0500	21.9 ± 0.7	90.7 ± 0.8	0.5 ± 0.1	6.3 ± 2.6	21.1 ± 0.8	70.8 ± 1.3	64.8 ± 2.5	4.1 ± 2.7
0600	22.5 ± 0.6	86.1 ± 1.7	0.8 ± 0.2	90.3 ± 15.3	24.6 ± 0.8	71.4 ± 1.0	74.0 ± 3.6	1.4 ± 1.4
0700	24.8 ± 0.7	73.0 ± 1.5	1.4 ± 0.2	297.3 ± 30.7	31.3 ± 1.4	73.8 ± 1.0	85.1 ± 3.2	0.4 ± 0.3
0800	27.0 ± 0.8	63.8 ± 1.2	1.7 ± 0.2	462.9 ± 45.3	34.9 ± 1.5	76.0 ± 1.0	87.7 ± 2.5	2.1 ± 0.9
0900	29.4 ± 1.1	53.7 ± 2.5	1.6 ± 0.2	664.8 ± 52.7	39.3 ± 1.6	77.8 ± 1.1	90.7 ± 1.8	4.2 ± 1.8
1000	31.8 ± 1.2	45.5 ± 2.8	1.9 ± 0.2	897.2 ± 30.0	42.7 ± 1.1	79.5 ± 1.0	92.1 ± 1.5	6.4 ± 2.4
1100	33.6 ± 1.2	40.3 ± 2.6	2.1 ± 0.2	989.9 ± 23.5	44.8 ± 0.9	80.8 ± 1.1	92.9 ± 1.2	9.1 ± 2.9
1200	35.4 ± 1.3	35.8 ± 3.0	2.4 ± 0.2	937.9 ± 56.4	45.5 ± 1.2	81.9 ± 1.1	91.7 ± 1.2	11.7 ± 3.4
1300	37.1 ± 1.3	31.2 ± 3.0	2.7 ± 0.3	910.8 ± 54.6	47.1 ± 1.1	82.9 ± 1.0	91.9 ± 0.8	13.5 ± 3.8
1400	37.6 ± 1.4	29.0 ± 2.9	3.0 ± 0.4	742.4 ± 74.5	46.3 ± 1.4	82.9 ± 1.0	89.3 ± 1.9	15.1 ± 4.0
1500	37.3 ± 1.3	29.0 ± 2.9	3.1 ± 0.3	558.6 ± 72.0	44.7 ± 1.4	82.6 ± 0.9	86.6 ± 2.1	16.8 ± 4.0
1600	36.9 ± 1.3	29.3 ± 2.8	2.6 ± 0.3	387.9 ± 63.3	43.4 ± 1.6	82.2 ± 0.9	85.4 ± 2.5	17.8 ± 4.1
1700	34.7 ± 1.5	36.0 ± 4.9	2.9 ± 0.4	218.1 ± 36.3	38.4 ± 1.9	80.9 ± 1.0	80.0 ± 2.1	18.5 ± 4.1
1800	32.6 ± 1.5	44.0 ± 5.7	2.7 ± 0.3	74.0 ± 14.6	34.3 ± 1.8	79.9 ± 1.0	76.8 ± 1.7	17.9 ± 3.9
1900	30.6 ± 1.4	50.7 ± 5.5	2.2 ± 0.3	2.4 ± 1.3	29.8 ± 1.3	78.5 ± 1.0	72.4 ± 2.2	17.3 ± 3.9
2000	28.5 ± 1.1	58.4 ± 4.4	1.5 ± 0.2	0.0 ± 0.0	27.7 ± 1.0	77.2 ± 1.0	72.5 ± 2.8	15.8 ± 3.9
2100	26.7 ± 0.8	69.3 ± 2.6	1.3 ± 0.2	0.0 ± 0.0	25.8 ± 0.8	76.2 ± 1.1	74.2 ± 3.1	15.0 ± 3.9
2200	26.0 ± 0.7	73.0 ± 1.9	1.5 ± 0.3	0.0 ± 0.0	25.1 ± 0.8	75.6 ± 1.0	74.2 ± 3.1	14.2 ± 4.1
2300	24.7 ± 0.6	79.2 ± 1.4	1.0 ± 0.1	0.0 ± 0.0	23.8 ± 0.7	74.4 ± 1.0	69.0 ± 3.1	13.2 ± 4.1

[1] HLI Categories: 1) cool, ≤ 70; 2) moderate, 70.1 ≤ 77; 3) hot, 77.1 ≤ 86; 4) very hot, ≥ 86

[2] Accumulated heat load Categories: 1) low; 2) mild, 1.1 < 10; 3) moderate, 10.1 < 20; 4) hot, 20.1 < 50; 5) extreme, ≥ 50.1

Table 4.4: Mean (± SEM) hourly ambient temperature (T_A,°C), relative humidity (RH, %), wind speed (WS, m/s), solar radiation (SR, W/m^2), black globe temperature (BGT, °C), temperature humidity index (THI), heat load index (HLI) and accumulated heat load for shaded Angus steers (AHL_{91}) during period 3

Hour	Item							
	T_A (°C)	RH (%)	WS (m/s)	SR (W/m^2)	BGT (°C)	THI	HLI[1]	AHL_{91}[2]
0000	20.9 ± 0.5	78.8 ± 1.4	0.9 ± 0.3	0.0 ± 0.0	19.9 ± 0.7	68.3 ± 0.8	57.6 ± 0.9	0.0 ± 0.0
0100	20.8 ± 0.5	80.5 ± 1.5	0.8 ± 0.1	0.0 ± 0.0	19.9 ± 0.6	68.1 ± 0.8	58.3 ± 0.8	0.0 ± 0.0
0200	20.5 ± 0.5	82.0 ± 1.4	0.7 ± 0.1	0.0 ± 0.0	19.9 ± 0.6	67.9 ± 0.8	58.8 ± 0.9	0.0 ± 0.0
0300	20.3 ± 0.6	82.8 ± 1.5	0.7 ± 0.1	0.0 ± 0.0	19.4 ± 0.8	67.5 ± 1.0	58.4 ± 1.0	0.0 ± 0.0
0400	19.9 ± 0.7	85.1 ± 2.2	0.6 ± 0.1	0.0 ± 0.0	18.8 ± 0.9	66.9 ± 1.1	58.4 ± 1.0	0.0 ± 0.0
0500	19.9 ± 0.7	85.5 ± 2.6	0.6 ± 0.1	0.1 ± 0.1	19.1 ± 0.8	66.9 ± 1.1	58.8 ± 0.9	0.0 ± 0.0
0600	19.8 ± 0.5	84.4 ± 2.5	0.6 ± 0.1	51.8 ± 12.0	20.5 ± 0.8	66.7 ± 0.8	61.9 ± 2.1	0.0 ± 0.0
0700	21.7 ± 0.4	73.2 ± 1.7	1.4 ± 0.1	269.1 ± 25.2	26.9 ± 0.8	69.0 ± 0.6	77.1 ± 3.0	0.0 ± 0.0
0800	23.9 ± 0.5	63.6 ± 1.9	2.0 ± 0.1	472.6 ± 42.0	29.9 ± 0.7	71.6 ± 0.6	78.6 ± 1.0	0.0 ± 0.0
0900	25.4 ± 0.4	56.8 ± 1.8	2.3 ± 0.2	575.4 ± 51.9	32.9 ± 0.7	73.0 ± 0.5	80.2 ± 1.2	0.0 ± 0.0
1000	26.7 ± 0.5	50.5 ± 1.6	2.4 ± 0.2	676.8 ± 51.4	34.8 ± 0.9	74.0 ± 0.6	80.6 ± 1.6	0.0 ± 0.0
1100	27.6 ± 0.5	47.2 ± 1.4	2.5 ± 0.2	700.3 ± 51.8	35.9 ± 0.7	74.7 ± 0.6	80.8 ± 1.3	0.0 ± 0.0
1200	28.3 ± 0.4	45.2 ± 1.0	2.4 ± 0.1	623.3 ± 48.1	36.5 ± 1.0	75.4 ± 0.6	81.0 ± 2.0	0.1 ± 0.1
1300	29.1 ± 0.4	43.9 ± 1.3	2.3 ± 0.1	710.3 ± 76.8	38.1 ± 1.1	76.2 ± 0.5	83.2 ± 1.8	0.2 ± 0.1
1400	29.5 ± 0.5	42.8 ± 1.7	2.5 ± 0.1	581.5 ± 71.7	37.5 ± 1.3	76.4 ± 0.6	81.5 ± 1.8	0.3 ± 0.2
1500	29.1 ± 0.6	44.6 ± 2.6	2.9 ± 0.2	505.6 ± 80.5	36.2 ± 1.5	76.2 ± 0.6	79.3 ± 1.9	0.4 ± 0.3
1600	28.3 ± 0.8	48.6 ± 2.7	3.1 ± 0.2	340.6 ± 48.8	33.6 ± 1.7	75.6 ± 0.8	76.7 ± 2.2	0.4 ± 0.3
1700	27.1 ± 0.7	53.6 ± 2.4	3.3 ± 0.1	203.6 ± 43.8	31.2 ± 1.3	74.8 ± 0.7	73.4 ± 2.2	0.4 ± 0.3
1800	25.4 ± 0.6	60.7 ± 2.9	2.9 ± 0.1	45.1 ± 7.6	25.9 ± 0.8	73.2 ± 0.7	65.1 ± 2.3	0.3 ± 0.2
1900	23.9 ± 0.5	65.4 ± 1.7	2.5 ± 0.2	0.4 ± 0.4	23.5 ± 0.5	71.7 ± 0.6	58.2 ± 1.7	0.0 ± 0.0
2000	22.9 ± 0.3	68.9 ± 1.1	1.9 ± 0.2	0.0 ± 0.0	22.3 ± 0.4	70.6 ± 0.5	57.1 ± 0.6	0.0 ± 0.0
2100	22.4 ± 0.4	71.4 ± 0.6	1.6 ± 0.3	0.0 ± 0.0	21.8 ± 0.4	70.1 ± 0.6	57.4 ± 0.7	0.0 ± 0.0
2200	21.9 ± 0.4	74.4 ± 0.8	1.5 ± 0.3	0.0 ± 0.0	21.2 ± 0.5	69.5 ± 0.7	57.6 ± 0.7	0.0 ± 0.0
2300	21.4 ± 0.5	76.9 ± 1.2	1.6 ± 0.3	0.0 ± 0.0	20.6 ± 0.6	68.8 ± 0.7	57.4 ± 0.8	0.0 ± 0.0

[1] HLI Categories: 1) cool, ≤ 70; 2) moderate, 70.1 ≤ 77; 3) hot, 77.1 ≤ 86; 4) very hot, ≥ 86

[2] Accumulated heat load Categories: 1) low; 2) mild, 1.1 < 10; 3) moderate, 10.1 < 20; 4) hot, 20.1 < 50; 5) extreme, ≥ 50.1

4.3.2 Rumen Temperature

A diurnal trend in rumen temperature existed (Figure 4.4); however there were differences in the rhythm of the diurnal trend in rumen temperature across the observational periods. Although there were differences in rumen temperature between the observational periods, minimum rumen temperatures occurred between 0800h and 0900 h and maximum rumen temperatures occurred between 1900 h and 2100 h for all observational periods respectively.

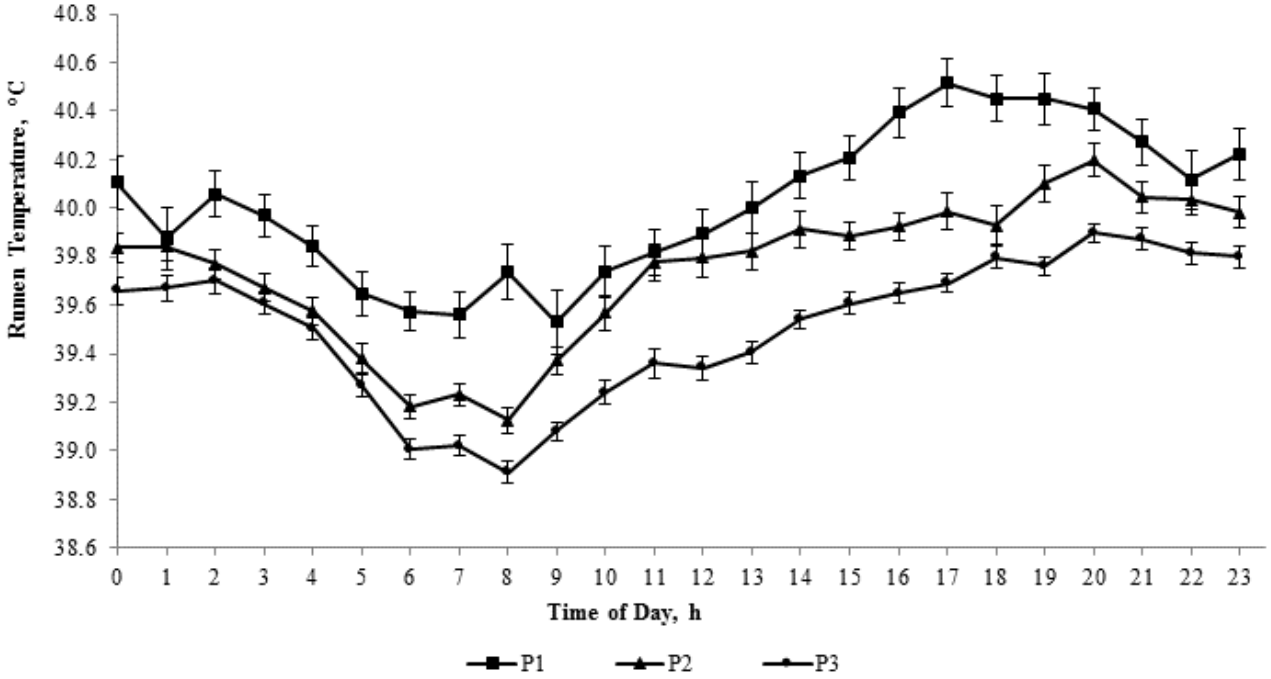


Figure 4.4: Mean hourly (± SEM) rumen temperature of Angus steers during period 1 (P1), period 2 (P2) and period 3 (P3)

4.3.3 Rumen Temperature versus Infrared Thermography

There were no linear trends between mean hourly rumen temperature and mean body surface temperature as determined by zone (Figure 4.5) or medial (Figure 4.6) analysis of infrared thermography images. Pearson's correlation coefficient (Minitab® 16.2.0, 2010 Minitab, Inc.) indicated that there were weak associations ($r \leq 0.1$; $P < 0.003$) between rumen temperature and body surface temperature; however there were strong associations between body surface temperature determined from the medial and zone analysis of infrared thermography images (Table 4.5).

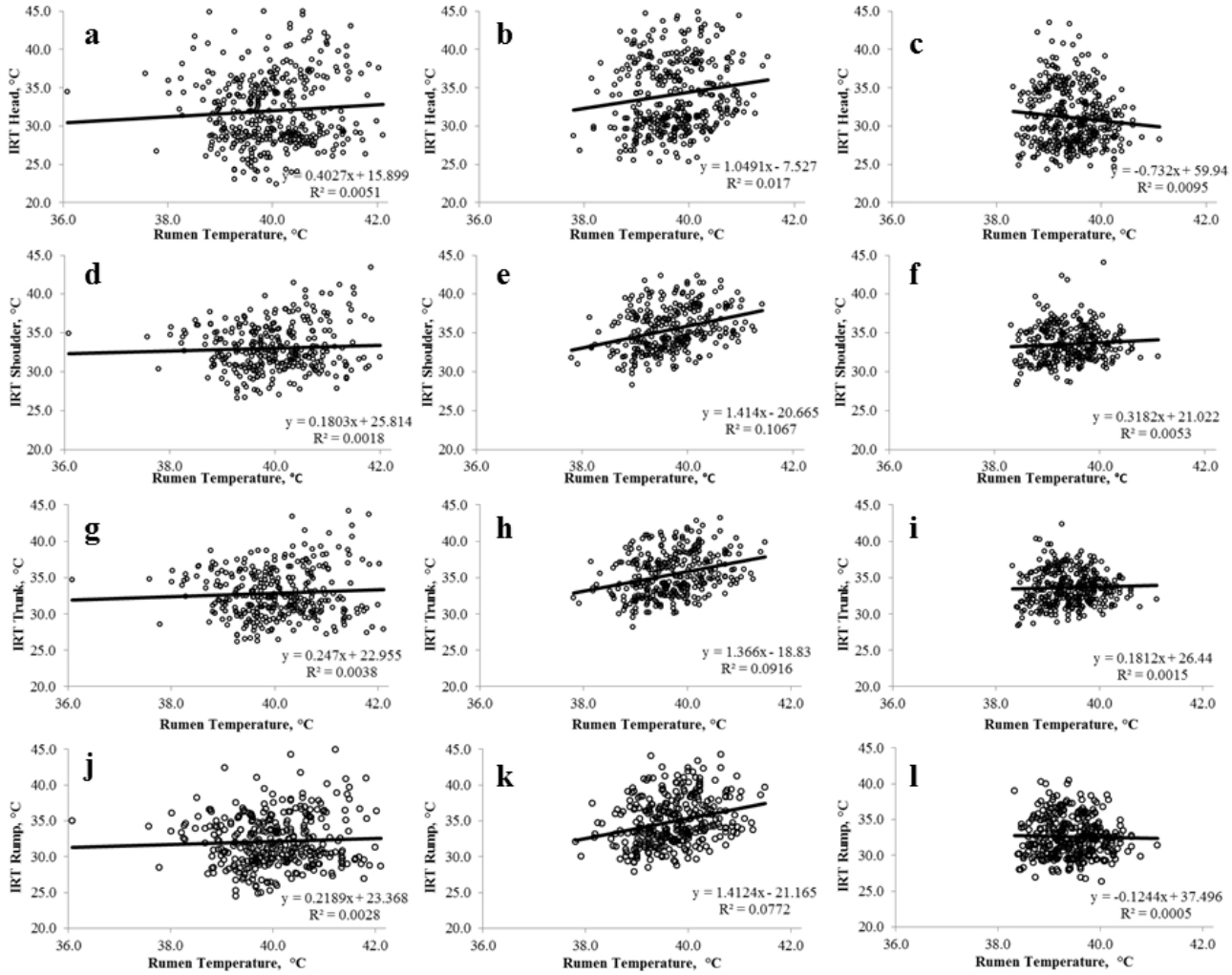


Figure 4.5: Linear relationship between rumen temperature and body surface temperature of the head, shoulder, trunk and rump zones during period 1 (a, head (IRT Head, °C); d, shoulder (IRT Shoulder, °C), g) trunk (IRT Trunk, °C), j) rump (IRT Rump, °C)); period 2 (b, head (IRT Head, °C); e, shoulder (IRT Shoulder, °C), h) trunk (IRT Trunk, °C), k) rump (IRT Rump, °C)); and period 3 (c, head (IRT Head, °C); f, shoulder (IRT Shoulder, °C), i) trunk (IRT Trunk, °C), l) rump (IRT Rump, °C))

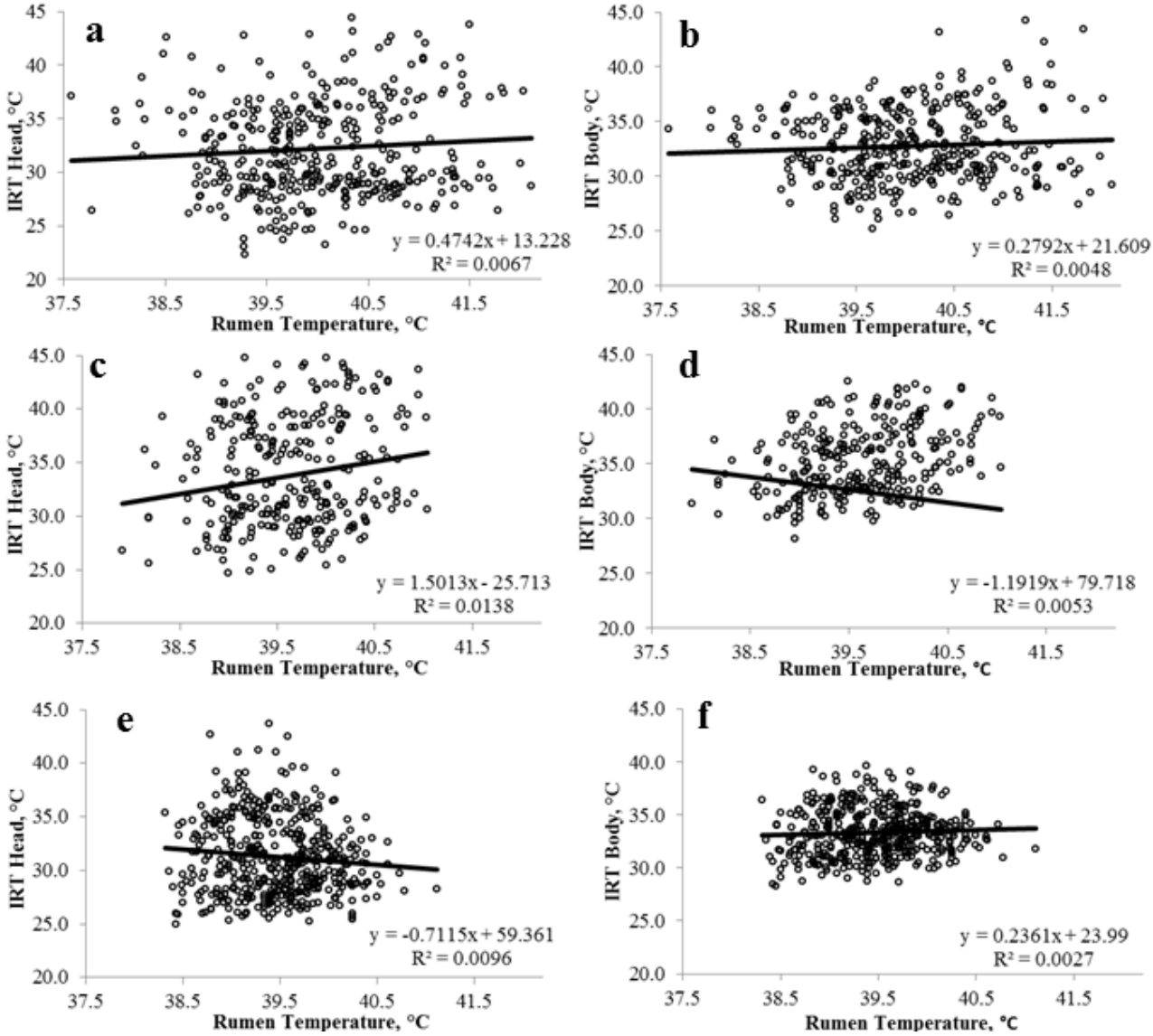


Figure 4.6: Linear relationship between rumen temperature and body surface temperature of the head and body during period 1 (a, head (IRT Head, °C); b, body (IRT Body, °C)); period 2 (c, head (IRT Head, °C); d, body (IRT Body, °C)); and period 3 (e, head (IRT Head, °C); f, f, body (IRT Body, °C))

Table 4.5: Pearson's correlation coefficients between maximum, average and minimum body surface temperatures determined from zone and medial analysis of infrared thermography images

Item	Head[1]	Shoulder[2]	Trunk[2]	Rump[2]
Maximum	0.512*	0.899*	0.902*	0.889*
Average	0.553*	0.977*	0.983*	0.974*
Minimum	0.964*	0.904*	0.932*	0.920*

[1] correlation between body surface temperatures determined from the medial line of the head and zone analysis of the head

[2] correlation between body surface temperatures determined from the medial line of the body and zone analysis of the shoulder, trunk and rump

* denotes $P < 0.001$

The Bland-Altman method of comparison indicated that the mean difference between rumen temperature and body surface temperature was substantial across sites analysed for body surface

temperature determined from zone (Figure 4.7) and medial (Figure 4.8) infrared thermography image analyses. Mean temperature (pooled) differences, determined by zone analysis, were -7.32 ± 0.13 °C, -5.65 ± 0.09 °C, -5.79 ± 0.10 °C and -6.49 ± 0.11 °C for the head, shoulder, trunk and rump respectively.

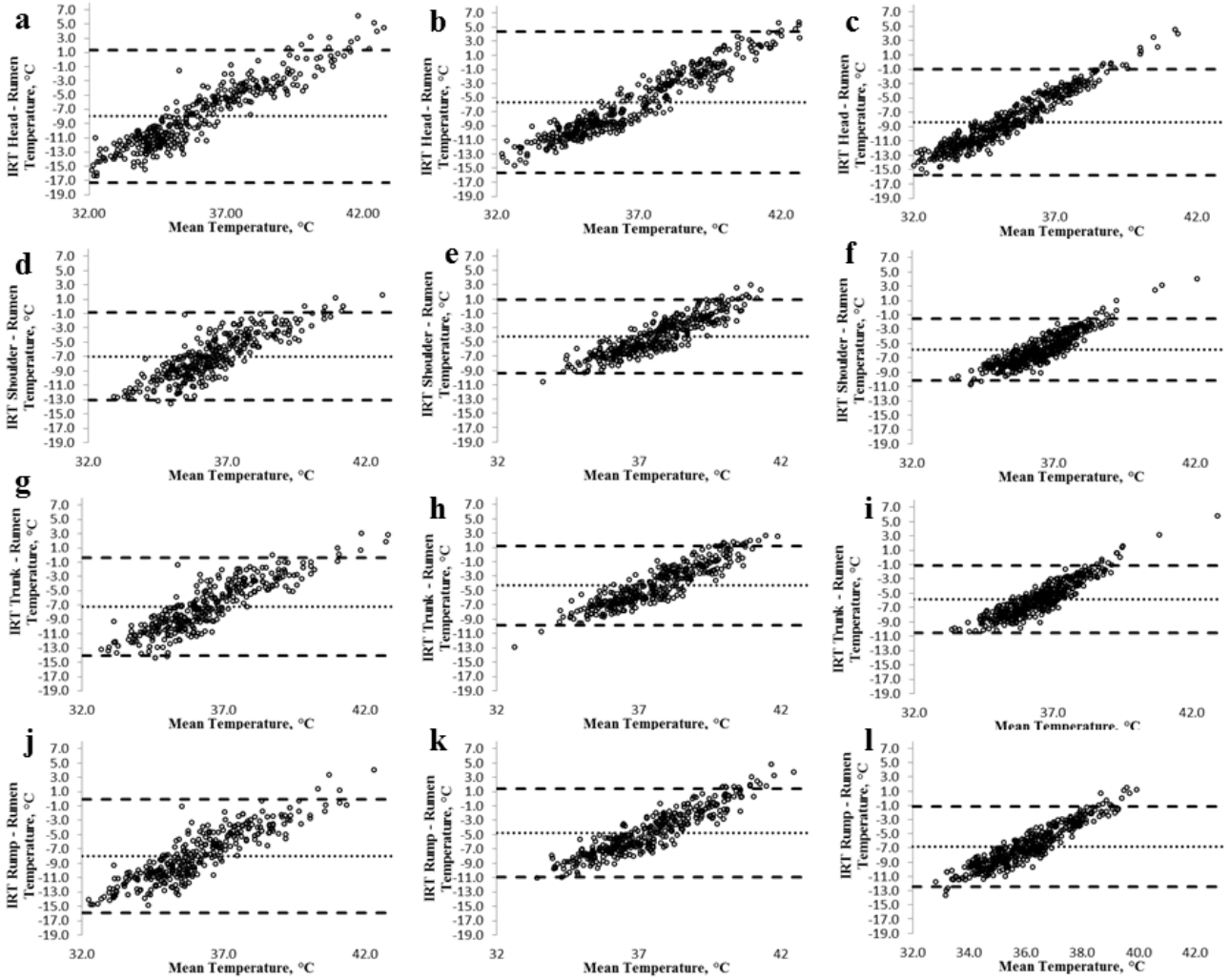


Figure 4.7: Bland-Altman method of comparison between rumen temperature and body surface temperature of the head, shoulder, trunk and rump zones during period 1 (a, head (IRT Head, °C); d, shoulder (IRT Shoulder, °C), g) trunk (IRT Trunk, °C), j) rump (IRT Rump, °C)); period 2 (b, head (IRT Head, °C); e, shoulder (IRT Shoulder, °C), h) trunk (IRT Trunk, °C), k) rump (IRT Rump, °C)); and period 3 (c, head (IRT Head, °C); f, shoulder (IRT Shoulder, °C), i) trunk (IRT Trunk, °C), l) rump (IRT Rump, °C)) also showing the mean difference (dotted line) and confidence intervals (95 % = mean ± 1.96 × SD; dashed line)

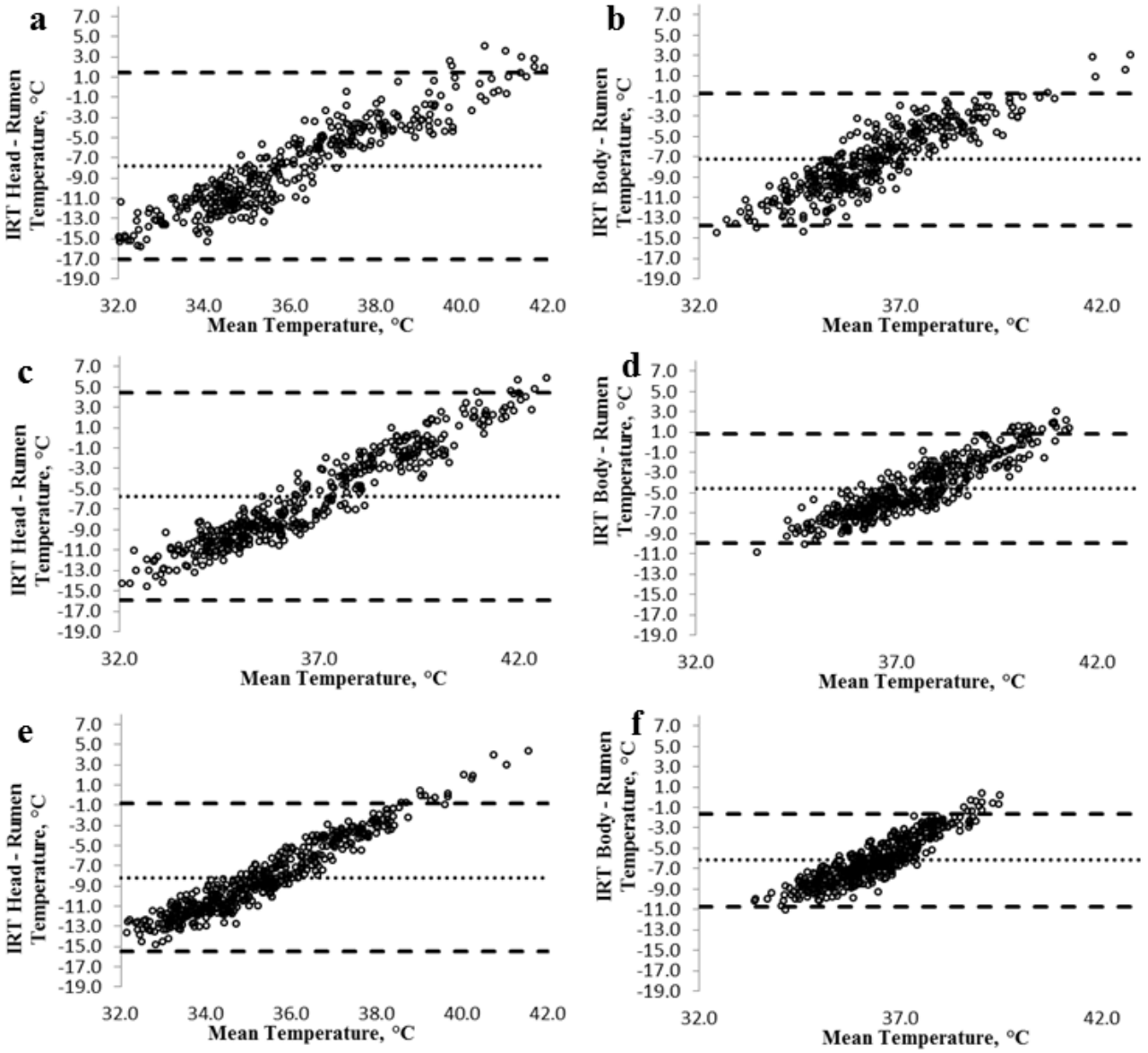


Figure 4.8: Bland-Altman method of comparison between rumen temperature and body surface temperature of the head and body during period 1 (a, head (IRT Head, °C); b, body (IRT Body, °C)); period 2 (c, head (IRT Head, °C); d, body (IRT Body, °C)); and period 3 (e, head (IRT Head, °C); f, body (IRT Body, °C)) also showing the mean difference (dotted line) and confidence intervals (95 % = mean ± 1.96 × SD; dashed line)

4.4 Discussion

Infrared thermography can be used to examine many aspects of animal wellbeing (McCafferty, 2007). Numerous studies have investigated the diagnostic applications of infrared thermography in veterinary medicine (Schaefer et al., 2004; Metzner et al., 2014) particularly in detecting inflammation (McCafferty, 2007) and disease (Schaefer et al., 2004; Schaefer et al., 2012). Furthermore some studies suggest that infrared thermography can potentially evaluate an animal's thermal balance (Brown-Brandl et al., 2012; Giloh et al., 2012; Nascimento et al., 2014). Infrared thermography measures the radiated electromagnetic energy, within the 3 µm to 12 µm wavelength range (Schaefer et al., 2004), emitted from the animal's surface (McCafferty, 2007). Images were

evaluated using an emissivity value of 0.98 as described by Steketee (1973) for biological tissues. However the emissivity of the coat surface can be influenced by environmental contaminants (McCafferty, 2007), such as dirt (emissivity between 0.93 and 0.96) and water (emissivity = 0.96; Campbell and Norman (1998)). Therefore determining the correct emissivity value where infrared thermography is used in field conditions becomes difficult. However McCafferty (2007) indicated that due to the linear relationship between radiative heat transfer and emissivity, the differences in body surface temperature due to environmental contaminants would result in a temperature difference of less than 0.5 °C, for the typical mammalian coat.

A potential confounding issue within the current experiment was that there are inconsistencies within the literature regarding infrared thermography image analysis. Specifically there is no defined standard for the assessment of infrared thermography images. A study by Kotrba et al. (2007) analysed images of eland and dairy cows using 7 zones across the body (1, neck; 2, dewlap; 3, shoulder; 4, barrel; 5, rump; 6, foreleg; and 7, hind leg). Montanholi et al. (2008) identified 4 zones in lactating dairy cows (1, triangle in the paralumbar fossa; 2, rectangle between sacrum and ischiatic tuber; rectangle below the vulva, and rectangle on the caudal palmer, metacarpus). Furthermore Brown-Brandl et al. (2012) used 2 zones (1, whole body; and 2, barrel) in finishing pigs. Therefore a part of this experiment was to investigate the necessity of generating zones on animals to determine the best measure of body surface temperature. i.e. body surface temperature from defined zones compared with the body surface temperature determined from the medial line. Results from this experiment suggest that there is a strong association between the body surface temperatures determined from defined zones compared with the medial line (Table 4.5). However there was no association between body surface temperature and rumen temperature. Additionally results of the Bland-Altman method of comparison indicate that there is no agreement between body surface temperature and rumen temperature, even though Figures 4.7 and 4.8 highlight a strong linear trend. These data have been calculated to create a relationship between body surface temperature and rumen temperature, whereby if there were an agreement between the two measures, the data would form a horizontal line at a 0 °C temperature difference, i.e. body surface temperature minus rumen temperature = 0 °C and remain within a 95 % confidence interval of approximately ± 1 °C.

The data collated in the current experiment, suggests that there was little relationship between the body surface temperature and rumen temperature. In the current experiment there does not appear to be a relationship between the two measures, therefore body surface temperature is unable to be used as a predictor for rumen temperature. However shade availability is also likely to impact on body

surface temperature, although this may change in climate controlled chamber studies where the impact of solar radiation on body surface temperature is removed.

4.5 Conclusion

Whilst infrared thermography technology appears to be useful in highly controlled circumstances, i.e. detection of inflammation within clinical environments, this technology is unpredictable in field conditions. The data from the current experiment suggests that infrared thermography may not be a predictor of rumen temperature, and as such cannot be used as a proxy to predict core body temperature. However infrared thermography may have application in the development of a thermal balance model. For infrared thermography imaging to become a useful tool in commercial situations, the identification of a surface on the body that has a strong correlation with core body temperature is necessary (Giloh et al., 2012), thus further analysis is required to determine whether a relationship exists between body surface temperature and rumen temperature, and/or other measures of core body temperature.

Chapter 5

Responses of *Bos taurus* and *Bos indicus* Feedlot Cattle to Heat Load

Experimental Hypothesis: Responses of Bos indicus and Bos taurus cattle to heat load will differ. Additionally the responses of Bos indicus and Bos taurus cattle to heat load will be further influenced by shade availability.

This chapter is divided into four sections each of which focuses on a specific component of the experiment. The first section is a general introduction to the experiment and an overview of the materials and methods and general results for the experiment, namely weather conditions and animal performance. The following three sections focus on the rumen temperature, behavioural and haematological responses of *Bos indicus* and *Bos taurus* cattle to heat load. Within these sections specific materials and methods, statistical analysis, results, discussion and conclusions are outlined in detail. This Chapter has been presented in this format to reduce the repetition of materials and methods relevant to each subsection.

5.1 Introduction

The most significant effect of high heat load on homoeothermic animals is an increase in core body temperature (Bianca, 1963). An increase in core body temperature is recognised as a key indicator of the severity of heat load an animal is experiencing (Spiers et al., 2004; Gaughan et al., 2010a). The ability to regulate core body temperature, within a physiologically acceptable range, during periods of increasing heat load is referred to as heat tolerance (Johnson et al., 2012).

An animal's genotype is a major factor contributing to its susceptibility or tolerance to heat load, and it is widely accepted that *Bos indicus* breeds have a greater heat tolerance compared to *Bos taurus* (Brody, 1956; Olbrich et al., 1971; Hansen, 2004). *Bos taurus* cattle have a reduced capacity to regulate increases in rectal temperature compared to purebred *Bos indicus* and *Bos indicus* derived breeds (McManus et al., 2009). The identification of heat tolerant cattle breeds is certainly not a new concept (Bianca, 1961, 1963; Hammond et al., 1996; Hammond et al., 1998; Gaughan et al., 1999; Brown-Brandl et al., 2006b; McManus et al., 2009; Gaughan et al., 2010b; Scharf et al., 2010). Additionally it is well supported that the heat tolerance of tropically adapted breeds, i.e. *Bos indicus*, extends to the cellular level (Hansen, 2004; Basiricò et al., 2011; Romero et al., 2013). Studies have suggested that there is a genetic linkage between heat tolerance at the cellular level; however there is considerable variation not only between breeds but also within breeds (Basiricò et al., 2011).

Feedlot cattle are particularly susceptible to heat load, which is partially due to the nature of the diets they are fed (Blackshaw and Blackshaw, 1994), i.e. high energy concentrate feeds. High energy dense feeds have the potential to increase core body temperature (Cho et al., 2014). The heat increment for feedlot cattle is high, 35 to 70 % of metabolisable energy (**ME**), however this is dependent on the balance of nutrients within the diet (Blackshaw and Blackshaw, 1994). As heat load conditions increase, the energy required for maintenance increases. Increase in energy requirements is associated with the behavioural and physiological responses, i.e. panting (Beede and Collier, 1986). Cattle responses to heat load can be categorised into three sections i) physiological; ii) behavioural; and iii) haematological (DeShazer et al., 2009); therefore the objectives of this experiment were;

i) to determine the differences in the responses of Bos taurus and Bos indicus breeds to heat load; and

ii) to further determine the effect of shade on Bos taurus and Bos indicus responses to heat load

5.1.1 Materials and Methods

This experiment was conducted with the approval of The University of Queensland (**UQ**) animal ethics committee (SAFS/335/11/MLA). The experiment was undertaken in Southeast Queensland, Australia, at UQ (27.54 °S, 152.34 °E; 100 m above mean sea level) research feedlot during the southern hemisphere summer (October to April). During the summer the location is characterized by a hot, humid sub-tropical climate.

Thirty-six steers (12 Angus, 12 Charolais, and 12 Brahman) with an initial non-fasted live weight of 318.5 ± 6.7 kg were used in a 154 day feedlot study consisting of two treatments: un-shaded and shaded (3 m^2/animal). Treatments were replicated with 3 pens per treatment (see below for pen details). There were 6 steers per pen, and each pen consisted of 2 Angus, 2 Brahman and 2 Charolais.

Bos taurus cattle were sourced approximately 80 km south-west of UQ, whilst *Bos indicus* were sourced approximately 380 km north-west of UQ. The area from which the *Bos taurus* cattle were obtained is tick free, has a mild climate, and improved pasture, whereas the *Bos indicus* were from an area with cattle ticks (*Rhipicephalus (Boophilus) microplus*), and un-improved pastures. The *Bos taurus* cattle were purchased from the area to the south-west to reduce negative effects, i.e. low post weaning growth, associated with tick burdens, heat and poor nutrition that can arise in areas to the north-west. At the completion of the experiment the cattle were slaughtered.

5.1.1.1 Animal Management

Steers were vaccinated against bovine ephemeral fever (Webster's bovine ephemeral fever vaccine; Live; Fort Dodge Australia P/L, Baulkham Hills, NSW, Australia); bovine respiratory disease (Bovillis MH, in-activated *Mannheimia haemolytica*; Coopers Animal Health, Australia); and clostridial diseases (enterotoxaemia (pulpy kidney disease), tetanus, blacks disease, malignant oedema and blackleg; Pfizer Animal Health, Australia) on d 0 and d 14. Animals were also treated for internal and external parasites (Cydectin, 5g/L moxidectin solvent, 150 g/L hydrocarbon liquid; Fort Dodge Australia P/L, Baulkham Hills, NSW, Australia) on d -14, d 0, d 106 and d 148. Hormonal growth promotants were not used in the study.

The cattle were weighed (non-fasted) at approximately 0800 h on feedlot induction (d -14), and then at 7 day intervals for the duration of the study. Body condition scores (Table 2.3) were assessed on d -14 and then at 30 day intervals using a 1 to 5 scale, in which 1 = emaciated and 5 = obese (Houghton et al., 1990; Department of Agriculture Fisheries and Forestry, 2009). The same pen

sequence was used for each weighing and body condition score data collection time point, i.e. cattle from pen 1 were assessed first through to pen 6. The steers were walked from their respective pens as a pen group for a distance of 300 to 450 m, depending on feedlot pen location, to a handling facility.

5.1.1.2 Feedlot Description

Six pens, 162 m^2 (27 m × 6 m), were utilized within the UQ research feedlot. The feedlot pens were situated in a north-south alignment. Pen surface was soil and pens had a 2 % slope from the feed bunks towards the rear of the pens (east). Concrete feed bunks with a 3 m concrete apron were located at the front of each pen (west). Each feed bunk provided a linear area of 0.7 m^2/animal and the linear water trough area was 0.17 m^2/animal. Stocking density was 27 m^2/animal. Three shaded pens and three un-shaded pens were used. The shaded pens and un-shaded pens were located side by side. The un-shaded and shaded treatment pens were separated by a single unused un-shaded pen. This was done to ensure that the shade footprint from the shaded pens did not encroach on un-shaded treatment pens. Shade was provided by shade-cloth (black, 90 % solar block, Darling Downs Tarpaulins, Toowoomba, Queensland, Australia) attached to a 4 m steel framed high structure. The shade structure provided a shade footprint of 3.0 m^2/animal (6 m × 3 m) at midday.

5.1.1.3 Nutritional Management

Feed bunks were read at 0700 h and 1200 h each day using a modified ‘clean bunk at midday’ feed intake management program (Lawrence, 1998). Cattle were backgrounded from d -14 until d 8. Cattle were fed a starter diet until d 37, and then from d 37 they transitioned to a finisher ration over 35 days. They remained on the finisher ration for the remainder of the study (Table 5.1.1). Refusals were removed and weighed daily with average consumption per pen and per animal calculated. Cattle were fed once daily at approximately 1430 h. Feeding schedule was modified during hot weather conditions i.e. heat wave events, in accordance with experimental protocols.

Table 5.1.1: Diet and Nutrient Composition

Item	Starter[1]	Finisher[1]
Ingredient, kg (as fed)		
Barley	165	250
Sorghum	399	339
Wheat	82.5	-
Millrun	100	100
Peanut hulls	160	-
Cottonseed meal	17.5	-
Molasses	20	20
Limestone	11	14.45
Sodium bicarbonate	8	8
Potassium chloride	3.42	-
Urea	7	6.95
Sulphur (dusting)	0.47	0.23
Moneco® 200[2]	0.10	0.10
Sodium bentonite	25	25
Mineral – vitamin supplement[3]	1	1
Chickpea shell	-	200
Sunflower meal	-	35
Nutrient Composition (as fed)		
DM, %	89.30	89.20
NE_g, Mcal/kg	1.44	1.66
Crude fat, %	2.25	2.29
CP, %	11.90	12.01
RDP; %	8.49	8.39
UDP; %	3.62	3.58
Crude Fibre, %	14.73	10.05
NDF, %	23.74	24.16
ADF, %	14.62	13.31
Mcal (ME, MJ/kg)	2.68 (11.20)	2.93 (12.27)

[1]Values are indicative of ingredient composition within the diet used, kg/ tonne

[2]Contained 200 g/kg monensin sodium (International Animal Health, Huntingwood, NSW, Australia) and provided 20 mg/kg of monensin sodium to the final diet.

[3]Contained (on a DM basis): 8000 µIU/g of vitamin A; 2000 µIU/g of vitamin D; 16000 mg/kg of vitamin E; 12000 mg/kg of copper; 400 mg/kg of selenium; 200 mg/kg of cobalt; 1000 mg/kg of iodine; 10000 mg/kg iron; 50000 mg/kg of zinc; 30000 mg/kg of manganese; and 15000 mg/kg antioxidant.

During heat wave events, feed offered was reduced to 95 % of the previous 5 day mean feed intake and feeding delayed until 1530 h. Heat wave events were defined as 3 or more consecutive days where maximum accumulated heat load for un-shaded Angus (threshold = 86, **AHL_{86}**) were ≥ 30 for 3 consecutive days and did not completely abate ($AHL_{86} \neq 0$) at night and/or maximum heat load index (**HLI**) were ≥ 90 as described by Gaughan et al. (2008b).

5.1.1.4 Climatic Data

Weather data were collected at 10 minute intervals using an automated weather station (Davis Pro V2, Davis Weather Station, Hayward, CA, USA) located at the front of the feedlot (western side).

Weather data collected included ambient temperature (**T_A**; °C); relative humidity (**RH**; %); wind speed (**WS**; m/s) and direction; solar radiation (**SR**; W/m^2); and 24 hour daily rainfall (measured at 0900 h each day). From these data, black globe temperature (**BGT**; °C), temperature humidity index (**THI**), HLI and accumulated heat load were calculated. Black globe temperature was calculated as described by (Hahn et al., 2009) using the following equation;

$$BGT = 1.33 \times T_{db} - 2.65 \times T_{db}^{0.5} + 3.21 \times \log_{10}(SR + 1) + 3.5$$

Where T_{db} = air temperature (°C) and SR = solar radiation (W/m^2)

Additionally THI and HLI were calculated as described in Chapter 3 section 3.2.4 from the equations adapted from Thom (1959) and Gaughan et al. (2008b). Accumulated heat load was also calculated for each breed × treatment combination based on the threshold adjustments described by Gaughan et al. (2008b).

For this experiment HLI was divided into four categories based on the reference animal as described by Gaughan et al. (2008b), a clinically healthy black Angus steer < 100 days on feed. The four categories are described as;

1. Cool (thermoneutral), HLI ≤ 70;
2. Moderate, HLI 70.1 ≤ 77;
3. Hot, HLI 77.1 ≤ 86; and
4. Very hot, HLI ≥ 86

Further to this accumulated heat load was divided into five stress categories as described by Gaughan et al. (2008b) and Gaughan et al. (2010b), where the accumulated heat load stress categories are described as;

1. Low, accumulated heat load ≤ 1;
2. Mild, accumulated heat load 1.1 ≤ 10;
3. Moderate, accumulated heat load 10.1 ≤ 20;
4. Hot, accumulated heat load 20.1 ≤ 50; and
5. Extreme, accumulated heat load ≥ 50.1

5.1.1.5 Heat Waves

For the purpose of this experiment a heat wave was defined as 3 or more consecutive days where the maximum HLI for the reference animal, a un-shaded Angus < 100 days on feed HLI threshold

for heat accumulation = 86, was ≥ 86 combined with a maximum accumulated heat load ≥ 50 during daylight hours (0600 and 1800 h). Using this definition there were 4 heat wave events throughout this study; event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147.

5.1.1.6 Body Temperature

Rumen temperatures were recorded as described in Chapter 3 section 3.2.5. Briefly rumen boluses (Smartstock, Pawnee, OK, USA) were orally administered via a bolus applicator on d 23. The boluses were an active RFID transmitter operating within the 915 to 928 MHz frequencies range. The radio transmissions were communicated via a yagi antenna to a base station, and were then transcribed to a database using proprietary software (TechTrol Inc., Pawnee, OK, USA). Rumen temperatures were transmitted and recorded at 10 minute intervals over 130 days. All 36 boluses were recovered from the animals post slaughter.

Additionally rectal temperatures were recorded from all 36 animals on 5 occasions at 30 day intervals by inserting a digital thermometer (BD™, Becton, Dickinson and Company, USA), into the rectal cavity. This was done when cattle were weighed. Rectal temperatures were obtained on these occasions so that the relationship between rectal temperature and rumen temperature could be determined.

5.1.1.7 Behavioural Observations

Observation data was obtained for each animal at 2 hour intervals between 0600 h and 1800 h daily from d 1 to d 154. Night time observation data were obtained for each animal at 2 hour intervals between 2000 h and 0400 h on d 13; d 27; d 35; d 41; d 54; d 69; d 75; d 83; d 97; d 125; d 139; and d 153. During heat wave event 2, behavioural observation data were collected at 1 hour intervals during day (0600 h to 1800 h) and night time (2000 h to 0400 h) hours. At each observation individual data were recorded for panting score, shade utilisation (under shade, in sun), activity (feeding, drinking, ruminating) and posture (standing, lying).

All behavioural observations were visually assessed from the feed bunk. During night time observations, night vision binoculars (NVA 5 X 42 LT Digital Binocular, Night Vision Australia Pty Ltd, Sydney, New South Wales, Australia) were used to assist in the determination of individual behaviours.

For steers in the shaded pens, location within pen was described as under shade or in sun, where shade utilisation was defined as ≥ 60 % of the body covered by shade. Feeding was defined as the animal standing with their head in the feed bunk actively eating (Mitlöhner et al., 2001a). Drinking was defined as the animal standing with their head in the water trough actively drinking. Rumination was classified where the steer were actively ruminating. When assessing the posture of the steers, standing was defined as the animals standing in an inactive upright position, alternatively laying was defined as sternal recumbency as described by Mitlöhner et al. (2001a).

Panting scores were visually determined based on the open and closed mouth panting of cattle using a 0 to 4.5 scale (Table 2.5) as described by Brown-Brandl et al. (2006a), Mader et al. (2006) and Gaughan et al. (2008b) between d 1 and 63. However during this time it was observed that these descriptions were not accounting for subtle changes in respiratory dynamics between panting score 1 and 2; therefore between d 64 and 154 panting scores were determined using a modified panting score system (Table 5.1.2).

Table 5.1.2: Modified assessment of panting score and description of breathing/panting condition

Panting Score	Breathing Condition
0	No panting
1	Slight panting, mouth closed, no drool, slight chest movement
1.5	Fast panting, mouth closed, no drool, fast easily observed chest movements
2	Fast panting, drool present, no open mouth (Figure 5.1.1a)
2.5	As for 2, but occasional open mouth panting, tongue not extended (Figure 5.1.1b)
3	Open mouth and excessive drooling, neck extended, head (Figure 5.1.1c)
3.5	As for 3, but with tongue out slightly and occasionally fully extended for short periods (Figure 5.1.1d)
4	Open mouth with tongue fully extended for prolonged periods with excessive drooling. Neck extended and head up (Figure 5.1.1e)
4.5	As for 4, but head held down. Cattle "breath" from flank (Figure 5.1.1f). Drooling may cease.

Adapted from Brown-Brandl et al. (2006); Mader et al. (2006) and Gaughan et al. (2008b)

Figure 5.1.1: Panting score a) 2; b) 2.5; c) 3; d) 3.5; e) 4; f) 4.5

5.1.1.8 Blood Sampling

Blood samples were collected at weighing. Samples were collected from 18 steers (1 × each breed/ pen i.e. 3 animals/ pen) on days 8, 36, 64, 99 and 127, whilst samples from the remaining 18 steers (1 × each breed/ pen i.e. 3 animals/ pen) occurred on days 9, 37, 65, 100 and 128.

Heat wave events occurred throughout the study, however for the purpose of blood sample collection, samples were collected based on the HLI and accumulated heat load forecast provided by Katestone Environmental (Cattle Heat Load Toolbox; http://chlt.katestone.com.au/). Heat wave blood samples were collected from 18 steers during heat wave event 1 on d 29, 32 and 36; event 2

on d 71, 74 and 78; and event 3 on d 120, 123 and 127. Blood samples were collected in an attempt to represent haematological parameters prior, during and post heat wave event.

Blood was obtained for each steer via jugular venepuncture into four 10 mL vacuum tubes (BD Vacutainer®, Franklin Lakes, USA). Three tubes contained the anticoagulant lithium heparin (120 IU) and the remaining vacutainer contained no anticoagulant. Immediately following collection, the lithium heparin (anti-coagulant) vacutainers samples were chilled on ice before centrifugation, with plasma separated within 2 hours of collection. Samples collected in the no anticoagulant vacutainer, were allowed to coagulate at room temperature (24 °C) prior to centrifugation. All samples were centrifuged at 1575 × g (3500 rpm) at 4 °C for 10 minutes (Eppendorf 5810R, Eppendorf South Pacific Pty Ltd, North Ryde, NSW). Plasma samples were then frozen (-20 ˚C) within 8 hours, and stored at -80 ˚C until assayed.

5.1.2 Statistical Analysis

Ten minute weather data were converted to an hourly average for each individual climatic variable, including HLI and accumulated heat load. Mean climatic variables were then calculated by hour for the duration of the study, monthly and heat wave data periods.

As feed intake was not measured individually it was not possible to separate out breed differences for dry matter intake (**DMI**), however feed intake for each pen was recorded daily. Average DMI per pen and then per animal were calculated. Average daily gains (**ADG**) were calculated for each individual steer at 7 day intervals; for live weight (non-fasted) obtained on d 15, ADG were calculated using the following formula;

$$Average\ Daily\ Gain\ (kg) = \frac{d\ 15\ Weight\ (kg) - d\ 8\ Weight\ (kg)}{7\ (number\ of\ days\ between\ weighings)}$$

From these data feed efficiency was determined by calculating the gain to feed ratio. The gain to feed ratio was used to determine the live weight gain (kg) per kilogram of DMI.

The method used to determine differences in mean weather conditions and animal performance was determined using the t test function in Minitab® (Minitab® 16.2.0, 2010 Minitab, Inc.).

5.1.3 Results and Discussion

5.1.3.1 Weather

The weather conditions during the study period were similar to long-term averages for the location with some intermittent hot days above 35 °C (n = 15). Overall there were sufficient hot conditions to elicit a heat load response, in the un-shaded Angus steers on most days (HLI ≥ 86; n = 127; Figure 5.1.2). There were a number of high rainfall events during the study, two of which lead to widespread flooding between d 86 to 89 and d 118 to 125. Furthermore during d 86 to 89 there was thick cloud cover which resulted in low solar load (mean 136.5 ± 6.5 W/m^2) during daylight (0600 h to 1800 h), and low T_A (mean 24.2 ± 0.1 °C) during daylight (0600 h to 1800 h). The low solar load and T_A combined with steady rainfall resulting in conditions that were conducive to cold stress. During this period Brahman steers in both shaded and un-shaded treatment pens were observed shivering at 1400 h on d 88. Whole study mean T_A (28.7 ± 0.2 °C) and SR (532.9 ± 7.7 W/m^2) at 1400 h were greater (P < 0.05) compared to the cold rain period over d 86 (25.5 ± 0.2 °C; 103.2 ± 9.9 W/m^2); d 87 (24.9 ± 0.4 °C; 273.2 ± 40.8 W/m^2); d 88 (24.3 ± 0.1 °C; 131.8 ± 12.3 W/m^2); and d 89 (22.6 ± 0.1 °C; 53.3 ± 5.1 W/m^2). The days immediately following the flooding events were hot (T_A ≥ 27.1 °C and RH ≥ 89 %), and the feedlot pens were wet and muddy. Mud depth varied between 5 cm and 35 cm depending on location within each pen.

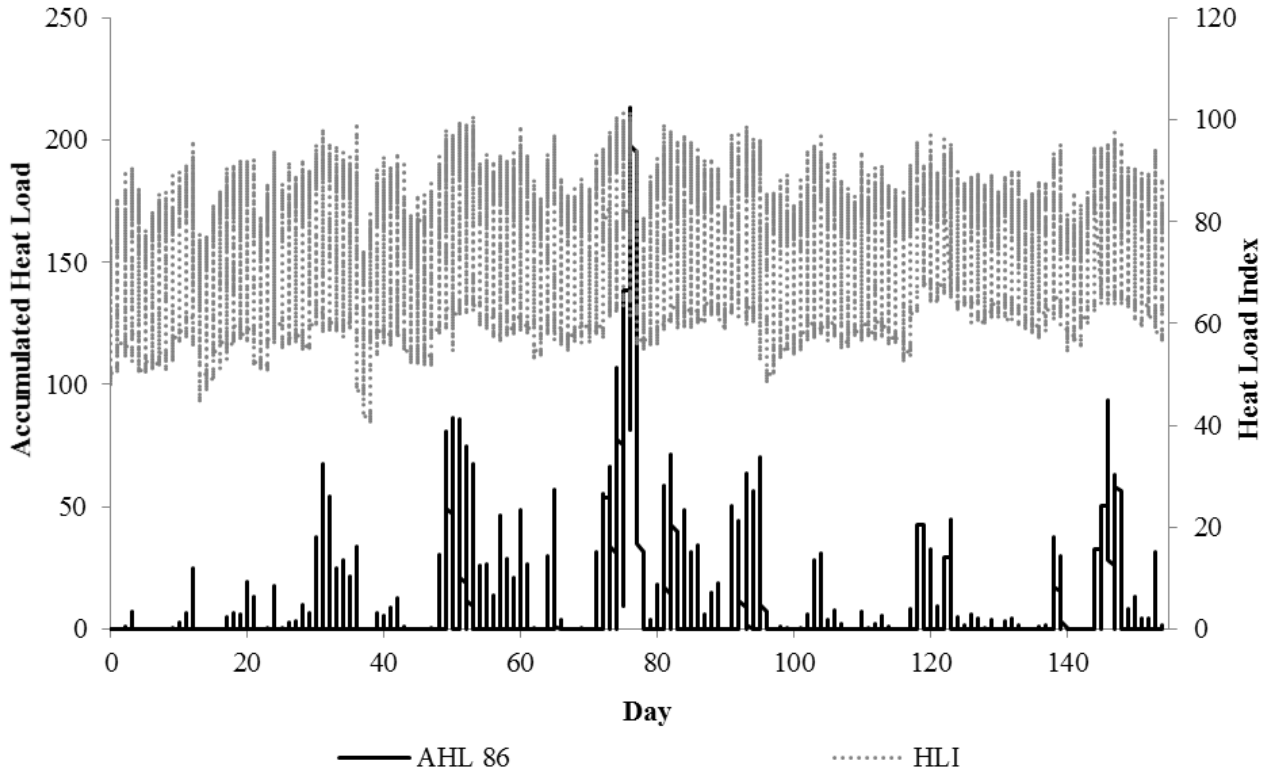


Figure 5.1.2: Accumulated heat load (primary axis; solid black line) and heat load index (HLI; secondary axis; dashed line) for the reference animal, a black Angus steer < 100 days on feed, heat accumulation threshold 86, over the duration of the study (154 d)

Mean hourly HLI and accumulated heat load for un-shaded Angus (AHL_{86}) were calculated throughout the duration of each heat wave (Event 1, Table 5.1.3; Event 2, Table 5.1.4; Event 3, Table 5.1.5; Event 4, Table 5.1.6). Mean HLI through the 4 heat wave events were 78.37 ± 2.78, 78.27 ± 2.50, 78.10 ± 2.86 and 80.37 ± 1.87 respectively. During each day of the heat wave events cattle were exposed to conditions where HLI ≥ 86 for 9 (event 4) to 12 (event 2) hours.

Table 5.1.3: Mean hourly (± SEM) heat load index[1] (HLI) and accumulated heat load[2] (AHL86) over a heat wave event 1 lasting 5 days (d 48 to 52)

HOUR	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5	
	HLI	AHL_{86}	HLI	AHL_{86}	HLI	AHL_{86}	HLI	AHL_{86}	HLI	AHL_{86}
0000	59.9 ± 0.1	0.0 ± 0.0	62.9 ± 0.1	41.3 ± 1.8	62.8 ± 0.1	0.0 ± 0.0	62.4 ± 0.1	12.6 ± 1.9	64.4 ± 0.1	4.4 ± 1.5
0100	60.0 ± 0.1	0.0 ± 0.0	63.8 ± 0.2	27.5 ± 1.7	63.4 ± 0.1	0.0 ± 0.0	62.4 ± 0.1	1.0 ± 0.7	64.0 ± 0.1	0.0 ± 0.0
0200	59.7 ± 0.1	0.0 ± 0.0	63.6 ± 0.2	14.2 ± 1.7	63.9 ± 0.2	0.0 ± 0.0	62.5 ± 0.1	0.0 ± 0.0	64.0 ± 0.1	0.0 ± 0.0
0300	58.9 ± 0.0	0.0 ± 0.0	64.1 ± 0.1	2.1 ± 1.1	63.9 ± 0.1	0.0 ± 0.0	62.7 ± 0.1	0.0 ± 0.0	64.0 ± 0.0	0.0 ± 0.0
0400	59.1 ± 0.1	0.0 ± 0.0	64.5 ± 0.0	0.0 ± 0.0	64.6 ± 0.2	0.0 ± 0.0	62.4 ± 0.1	0.0 ± 0.0	64.3 ± 0.0	0.0 ± 0.0
0500	73.2 ± 5.6	0.1 ± 0.1	88.1 ± 4.2	1.4 ± 0.6	85.8 ± 6.3	1.6 ± 0.9	75.2 ± 5.4	0.2 ± 0.2	74.6 ± 6.0	0.2 ± 0.2
0600	91.3 ± 0.5	4.0 ± 0.6	91.5 ± 0.3	7.5 ± 0.7	96.1 ± 1.0	10.9 ± 1.4	94.2 ± 1.1	5.1 ± 1.0	93.9 ± 0.5	5.9 ± 1.1
0700	90.8 ± 0.8	9.4 ± 0.7	92.4 ± 0.5	13.1 ± 0.7	94.5 ± 1.0	19.7 ± 1.1	95.4 ± 0.4	14.0 ± 1.1	95.2 ± 1.0	14.0 ± 1.2
0800	89.5 ± 0.6	12.9 ± 0.4	94.4 ± 0.5	20.4 ± 1.0	97.5 ± 0.8	29.6 ± 1.5	94.8 ± 0.7	23.6 ± 1.1	96.5 ± 0.8	23.7 ± 0.8
0900	91.7 ± 1.0	17.5 ± 0.8	95.8 ± 0.5	29.6 ± 1.2	96.7 ± 0.5	41.1 ± 1.3	94.0 ± 0.8	31.8 ± 1.1	97.4 ± 1.3	35.2 ± 1.6
1000	93.9 ± 0.6	24.5 ± 1.0	95.7 ± 0.4	39.6 ± 1.3	93.7 ± 0.7	50.9 ± 1.1	96.0 ± 0.2	40.8 ± 1.3	95.6 ± 1.1	46.1 ± 1.4
1100	94.3 ± 0.5	32.2 ± 1.0	94.2 ± 0.4	48.5 ± 1.0	93.7 ± 0.3	58.1 ± 1.0	91.5 ± 0.5	48.7 ± 0.7	92.5 ± 0.5	53.9 ± 0.8
1200	94.8 ± 0.4	40.9 ± 1.2	95.2 ± 0.4	57.1 ± 1.2	92.4 ± 0.6	65.2 ± 0.8	87.0 ± 0.7	52.9 ± 0.2	89.2 ± 0.5	59.1 ± 0.4
1300	95.4 ± 0.4	49.9 ± 1.2	93.8 ± 0.5	65.7 ± 1.0	92.5 ± 0.8	71.9 ± 0.9	82.8 ± 0.7	53.2 ± 0.0	89.2 ± 0.6	61.9 ± 0.3
1400	94.8 ± 0.3	59.1 ± 1.1	95.3 ± 0.4	74.0 ± 1.2	90.3 ± 0.7	77.5 ± 0.5	83.3 ± 0.6	53.2 ± 0.0	88.6 ± 0.4	65.3 ± 0.4
1500	94.3 ± 1.2	68.0 ± 1.2	92.9 ± 1.1	82.9 ± 1.0	90.2 ± 0.5	81.9 ± 0.5	85.6 ± 1.1	53.2 ± 0.0	83.5 ± 1.8	67.1 ± 0.1
1600	93.0 ± 1.1	76.1 ± 1.0	70.5 ± 5.3	85.2 ± 0.9	87.5 ± 0.6	85.2 ± 0.3	94.1 ± 1.2	56.7 ± 0.9	67.9 ±2.5	63.9 ± 1.3
1700	82.6 ± 1.3	79.7 ± 0.0	61.2 ± 1.2	71.7 ± 1.9	85.2 ± 0.5	85.9 ± 0.0	95.8 ± 0.4	66.5 ± 1.3	68.6 ± 0.3	54.0 ± 1.1
1800	85.8 ± 1.2	80.2 ± 0.2	59.6 ± 0.2	54.6 ± 2.2	78.7 ± 1.7	85.9 ± 0.1	81.8 ± 5.5	73.2 ± 0.4	67.5 ± 0.9	45.7 ± 1.1
1900	74.3 ± 0.5	79.6 ± 0.4	60.6 ± 0.5	37.8 ± 2.1	68.1 ± 2.8	82.8 ± 1.0	65.1 ± 0.1	65.8 ± 1.5	63.4 ± 0.3	34.2 ± 1.7
2000	73.6 ± 0.3	76.8 ± 0.4	62.4 ± 0.7	21.6 ± 1.9	62.7 ± 0.2	70.1 ± 1.8	65.4 ± 0.1	54.1 ± 1.5	63.8 ± 0.3	20.5 ± 1.7
2100	72.5 ± 0.3	73.0 ± 0.5	64.2 ± 0.1	8.3 ± 1.6	62.8 ± 0.1	55.8 ± 1.8	64.6 ± 0.1	42.1 ±1.6	64.2 ± 0.1	7.6 ± 1.6
2200	68.7 ± 2.5	68.1 ± 0.8	62.2 ± 0.3	0.1 ± 0.1	62.7 ± 0.1	41.6 ± 1.8	64.1 ± 0.1	29.4 ± 1.6	63.8 ± 0.1	0.0 ± 0.0
2300	62.2 ± 0.3	55.6 ± 1.9	61.4 ± 0.2	0.0 ± 0.0	62.5 ± 0.1	27.3 ± 1.8	64.5 ± 0.1	16.8 ± 1.6	63.8 ± 0.1	0.0 ± 0.0

[1] HLI Categories: 1) cool, ≤ 70; 2) moderate, 70.1 ≤ 77; 3) hot, 77.1 ≤ 86; 4) very hot, ≥ 86

[2] Accumulated heat load Categories: 1) low; 2) mild, 1.1 < 10; 3) moderate, 10.1 < 20; 4) hot, 20.1 < 50; 5) extreme, ≥ 50.1

Table 5.1.4: Mean hourly (± SEM) heat load index[1] (HLI) and accumulated heat load[2] (AHL86) over a heat wave event 2 lasting 6 days (d 71 to 76)

HOUR	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6	
	HLI	AHL_{86}	HLI	AHL_{86}	HLI	AHL_{86})	HLI	AHL_{86}	HLI	AHL_{86}	HLI	AHL_{86}
0000	59.2 ± 0.1	0.0 ± 0.0	80.3 ± 1.5	53.7 ± 0.1	63.1 ± 0.3	25.3 ± 1.8	63.9 ± 0.1	69.6 ± 1.7	74.8 ± 4.3	137.4 ± 0.7	61.9 ± 0.1	189.0 ± 1.9
0100	59.4 ± 0.1	0.0 ± 0.0	63.7 ± 0.1	47.8 ± 1.7	63.4 ± 0.2	11.7 ± 1.7	64.5 ± 0.1	56.7 ± 1.6	64.8 ± 0.1	127.5 ± 1.5	62.4 ± 0.2	174.1 ± 1.8
0200	59.0 ± 0.1	0.0 ± 0.0	63.5 ± 0.3	34.6 ± 1.7	63.9 ± 0.2	0.9 ± 0.6	64.8 ± 0.1	44.4 ± 1.6	65.2 ± 0.1	115.5 ± 1.5	62.3 ± 0.2	159.3 ± 1.9
0300	58.0 ± 0.2	0.0 ± 0.0	61.8 ± 0.2	20.3 ± 1.9	63.4 ± 0.2	0.0 ± 0.0	65.0 ± 0.1	32.3 ± 1.5	65.6 ± 0.1	103.7 ± 1.4	62.4 ± 0.2	144.7 ± 1.9
0400	58.2 ± 0.1	0.0 ± 0.0	62.3 ± 0.1	5.4 ± 1.8	63.7 ± 0.1	0.0 ± 0.0	65.2 ± 0.1	20.4 ± 1.5	65.1 ± 0.1	92.2 ± 1.5	62.9 ± 0.1	130.3 ± 1.8
0500	62.7 ± 0.5	0.0 ± 0.0	77.5 ± 6.0	0.3 ± 0.2	80.9 ± 6.7	0.8 ± 0.5	85.7 ± 6.5	12.0 ± 0.7	85.3 ± 6.2	83.7 ± 0.6	76.0 ± 5.6	117.6 ± 1.1
0600	85.4 ± 1.9	0.5 ± 0.1	89.7 ± 0.7	4.2 ± 0.5	99.5 ± 0.5	10.3 ± 1.7	94.3 ± 19.7	19.7 ± 1.1	95.0 ± 1.0	91.9 ± 1.2	86.3 ± 0.7	117.1 ± 0.1
0700	89.2 ± 0.5	4.6 ± 0.1	93.4 ± 1.0	8.7 ± 0.8	97.4 ± 0.8	23.4 ± 1.5	93.5 ± 0.3	27.7 ± 1.0	96.6 ± 1.1	101.4 ± 1.5	86.0 ± 0.7	117.7 ± 0.1
0800	88.6 ± 0.6	7.0 ± 0.1	95.8 ± 0.6	17.7 ± 1.3	93.3 ± 0.8	33.3 ± 1.0	94.1 ± 0.4	35.4 ± 1.0	95.2 ± 0.8	110.9 ± 1.2	89.3 ± 0.7	119.1 ± 0.4
0900	89.7 ± 0.6	9.8 ± 0.2	93.1 ± 0.5	26.5 ± 1.0	94.4 ± 0.7	41.0 ± 1.1	94.2 ± 0.2	43.4 ± 1.0	95.9 ± 1.0	120.1 ± 1.3	91.5 ± 0.6	123.4 ± 0.7
1000	91.7 ± 0.3	14.2 ± 0.4	93.5 ± 0.7	33.3 ± 0.9	93.9 ± 0.4	49.0 ± 1.1	96.5 ± 0.5	52.4 ± 1.3	96.1 ± 0.4	130.2 ± 1.3	92.0 ± 0.6	129.7 ± 0.8
1100	93.1 ± 0.5	21.0 ± 0.4	93.4 ± 0.5	40.9 ± 1.0	94.2 ± 0.5	56.7 ± 1.0	97.5 ± 0.3	63.6 ± 1.5	95.8 ± 0.7	140.3 ± 1.3	90.4 ± 0.7	135.2 ± 0.6
1200	91.1 ± 0.4	27.5 ± 0.2	93.8 ± 0.8	48.5 ± 1.0	94.9 ± 0.6	65.1 ± 1.0	97.2 ± 0.4	74.9 ± 1.4	98.7 ± 0.4	151.4 ± 1.6	88.4 ± 0.4	138.4 ± 0.3
1300	90.6 ± 0.5	32.0 ± 0.4	91.6 ± 0.4	55.7 ± 0.7	94.9 ± 0.4	74.3 ± 1.2	98.5 ± 0.2	86.6 ± 1.6	98.3 ± 0.8	163.8 ± 1.5	85.9 ± 0.2	139.9 ± 0.0
1400	90.6 ± 0.4	36.7 ± 0.3	90.3 ± 0.4	60.7 ± 0.6	94.2 ± 0.5	82.8 ± 1.1	98.8 ± 0.6	99.4 ± 1.7	98.3 ± 0.5	176.3 ± 1.6	83.9 ± 0.6	140.0 ± 0.0
1500	91.5 ± 0.6	41.7 ± 0.4	88.9 ± 0.2	64.3 ± 0.4	94.5 ± 0.5	91.4 ± 1.1	100.0 ± 0.3	112.5 ± 1.8	98.2 ± 0.5	188.7 ± 1.6	81.4 ± 0.5	140.0 ± 0.0
1600	89.1 ± 0.1	46.1 ± 0.2	86.1 ± 0.8	66.5 ± 0.1	93.4 ± 0.8	99.4 ± 1.0	97.5 ± 0.8	125.9 ± 1.6	96.1 ± 1.1	200.6 ± 1.3	79.1 ± 0.7	140.0 ± 0.0
1700	90.9 ± 0.5	49.9 ± 0.4	81.5 ± 0.8	66.7 ± 0.0	89.1 ± 0.6	105.2 ± 0.5	91.2 ± 1.1	135.1 ± 0.8	92.7 ± 0.6	208.9 ± 0.9	79.2 ± 0.4	140.0 ± 0.0
1800	88.2 ± 1.1	54.2 ± 0.2	76.8 ± 1.1	66.7 ± 0.1	82.6 ± 1.0	106.7 ± 0.0	86.2 ± 0.8	138.1 ± 0.2	84.3 ± 1.5	213.2 ± 0.1	71.7 ± 3.1	139.4 ± 3.1
1900	80.3 ± 0.1	55.4 ± 0.0	72.9 ± 0.4	64.1 ± 0.5	76.9 ± 0.5	106.4 ± 0.1	81.5 ± 0.8	138.4 ± 0.0	77.9 ± 0.3	213.3 ± 0.0	56.4 ± 0.1	126.1 ± 2.6
2000	77.1 ± 0.1	55.2 ± 0.0	76.0 ± 0.7	60.8 ± 0.2	77.7 ± 0.5	106.2 ± 0.0	80.0 ± 0.6	138.4 ± 0.1	80.3 ± 1.0	213.3 ± 0.0	56.6 ± 0.2	105.6 ± 2.6
2100	77.2 ± 0.4	54.6 ± 0.0	76.4 ± 0.6	60.0 ± 0.1	73.5 ± 3.1	105.8 ± 0.4	80.1 ± 0.5	138.4 ± 0.0	78.3 ± 0.9	213.3 ± 0.0	56.0 ± 0.2	85.0 ± 2.7
2200	77.3 ± 1.2	54.3 ± 0.2	62.6 ± 0.3	53.5 ± 1.8	63.7 ± 0.1	96.2 ± 1.7	79.0 ± 0.4	138.4 ± 0.0	74.4 ± 2.5	213.0 ± 0.1	56.3 ± 0.0	64.0 ± 2.6
2300	81.9 ± 1.5	53.8 ± 0.0	63.2 ± 0.3	39.4 ± 1.7	63.7 ± 0.1	82.9 ± 1.7	84.2 ± 0.7	138.4 ± 0.0	61.9 ± 0.2	204.0 ± 1.9	56.7 ± 0.1	43.4 ± 2.6

[1] HLI Categories: 1) cool, ≤ 70; 2) moderate, 70.1 ≤ 77; 3) hot, 77.1 ≤ 86; 4) very hot, ≥ 86

[2] Accumulated heat load Categories: 1) low; 2) mild, 1.1 < 10; 3) moderate, 10.1 < 20; 4) hot, 20.1 < 50; 5) extreme, ≥ 50.1

Table 5.1.5: Mean hourly (± SEM) heat load index[1] (HLI) and accumulated heat load[2] (AHL86) over a heat wave event 3 lasting 3 days (d 92 to 94)

HOUR	DAY 1		DAY 2		DAY 3	
	HLI	AHL_{86}	HLI	AHL_{86}	HLI	AHL_{86}
0000	63.1 ± 0.3	3.5 ± 1.5	62.6 ± 0.1	0.0 ± 0.0	63.3 ± 0.1	0.0 ± 0.0
0100	62.7 ± 0.3	0.0 ± 0.0	62.9 ± 0.1	0.0 ± 0.0	63.0 ± 0.2	0.0 ± 0.0
0200	60.9 ± 0.2	0.0 ± 0.0	63.5 ± 0.1	0.0 ± 0.0	62.9 ± 0.1	0.0 ± 0.0
0300	60.7 ± 0.2	0.0 ± 0.0	63.4 ± 0.1	0.0 ± 0.0	63.4 ± 0.1	0.0 ± 0.0
0400	60.0 ± 0.0	0.0 ± 0.0	63.5 ± 0.1	0.0 ± 0.0	63.6 ± 0.1	0.0 ± 0.0
0500	62.9 ± 1.2	0.0 ± 0.0	69.7 ± 4.9	0.0 ± 0.0	68.5 ± 4.2	0.0 ± 0.0
0600	85.8 ± 1.3	0.2 ± 0.1	93.1 ± 0.9	4.7 ± 0.9	91.4 ± 0.6	3.1 ± 0.8
0700	92.0 ± 0.7	3.7 ± 0.8	89.6 ± 0.3	10.0 ± 0.5	90.6 ± 0.2	7.9 ± 0.6
0800	92.7 ± 0.8	9.5 ± 0.8	88.8 ± 0.3	13.1 ± 0.4	90.8 ± 0.5	12.7 ± 0.7
0900	96.8 ± 0.4	18.4 ± 1.4	90.3 ± 0.9	16.2 ± 0.5	92.2 ± 1.0	17.8 ± 0.8
1000	94.2 ± 0.6	27.9 ± 1.0	91.3 ± 0.6	21.2 ± 0.7	93.5 ± 0.8	24.7 ± 1.0
1100	95.2 ± 0.5	36.5 ± 1.1	90.2 ± 0.7	26.5 ± 0.6	93.2 ± 0.7	31.9 ± 0.9
1200	94.5 ± 2.4	45.5 ± 2.7	92.2 ± 1.0	31.0 ± 1.9	91.8 ± 1.5	38.6 ± 1.9
1300	93.0 ± 0.8	53.7 ± 1.0	93.4 ± 0.7	38.2 ± 0.9	93.0 ± 0.6	44.7 ± 0.8
1400	88.5 ± 0.5	58.7 ± 0.4	92.1 ± 0.5	45.0 ± 0.8	92.8 ± 0.6	51.9 ± 0.9
1500	88.7 ± 0.3	61.2 ± 0.3	90.6 ± 0.8	50.4 ± 0.7	91.5 ± 0.6	58.4 ± 0.8
1600	85.7 ± 0.3	62.8 ± 0.0	89.0 ± 0.8	54.8 ± 0.5	91.6 ± 0.4	63.8 ± 0.7
1700	86.0 ± 0.2	63.0 ± 0.0	86.2 ± 0.4	56.3 ± 0.1	89.4 ± 0.9	68.0 ± 0.1
1800	85.0 ± 1.4	63.6 ± 0.2	78.4 ± 3.6	56.5 ± 0.0	83.3 ± 1.4	70.5 ± 0.0
1900	67.9 ± 1.8	58.8 ± 2.4	67.9 ± 1.8	58.8 ± 2.4	67.9 ± 1.8	58.8 ± 2.4
2000	64.3 ± 0.1	45.9 ± 1.6	61.5 ± 0.3	31.4 ± 2.0	66.8 ± 2.7	66.9 ± 1.4
2100	64.3 ± 0.1	33.2 ± 1.6	62.3 ± 0.2	16.3 ± 1.9	57.5 ± 1.8	52.3 ± 2.6
2200	64.4 ± 0.1	20.5 ± 1.6	62.6 ± 0.1	2.8 ± 1.3	60.2 ± 0.1	33.4 ± 2.1
2300	63.0 ± 0.4	7.5 ± 1.8	62.8 ± 0.1	0.0 ± 0.0	60.6 ± 0.3	16.5 ± 2.1

[1] HLI Categories: 1) cool, ≤ 70; 2) moderate, 70.1 ≤ 77; 3) hot, 77.1 ≤ 86; 4) very hot, ≥ 86

[2] Accumulated heat load Categories: 1) low; 2) mild, 1.1 < 10; 3) moderate, 10.1 < 20; 4) hot, 20.1 < 50; 5) extreme, ≥ 50.1

Table 5.1.6: Mean hourly (± SEM) heat load index[1] (HLI) and accumulated heat load[2] (AHL 86) over a heat wave event 4 lasting 4 days (d 144 to 147)

HOUR	DAY 1		DAY 2		DAY 3		DAY 4	
	HLI	AHL_{86}	HLI	AHL_{86}	HLI	AHL_{86}	HLI	AHL_{86}
0000	75.8 ± 4.4	30.8 ± 0.5	84.4 ± 0.3	50.6 ± 0.0	66.0 ± 0.1	21.7 ± 1.4	65.8 ± 0.1	51.5 ± 1.4
0100	66.1 ± 0.2	22.6 ± 1.4	88.1 ± 0.5	51.5 ± 03	65.5 ± 0.2	10.6 ± 1.5	65.5 ± 0.2	40.2 ± 1.5
0200	66.2 ± 0.0	11.7 ± 1.4	77.7 ± 4.5	52.4 ± 0.4	64.4 ± 0.3	0.9 ± 0.6	65.2 ± 0.1	28.6 ± 1.5
0300	66.2 ± 0.2	1.9 ± 0.9	71.3 ± 3.4	46.5 ± 0.9	65.1 ± 0.3	0.0 ± 0.0	65.4 ± 0.1	16.9 ± 1.5
0400	64.8 ± 0.2	0.0 ± 0.0	81.0 ± 4.1	40.8 ± 0.2	65.5 ± 0.1	0.0 ± 0.0	65.4 ± 0.1	5.3 ± 1.5
0500	64.0 ± 0.0	0.0 ± 0.0	75.9 ± 4.9	36.5 ± 0.9	65.8 ± 0.2	0.0 ± 0.0	64.0 ± 0.2	0.0 ± 0.0
0600	66.1 ± 0.3	0.0 ± 0.0	89.7 ± 0.6	37.2 ± 0.5	67.7 ± 0.3	0.0 ± 0.0	66.5 ± 0.8	0.0 ± 0.0
0700	78.0 ± 4.6	0.1 ± 0.1	90.9 ± 0.8	40.9 ± 0.6	85.5 ± 3.4	0.7 ± 0.3	85.9 ± 5.2	1.3 ± 0.7
0800	91.6 ± 0.6	3.4 ± 0.7	91.6 ± 0.5	46.9 ± 0.8	90.8 ± 0.3	4.2 ± 0.6	93.1 ± 0.6	8.7 ± 1.0
0900	92.9 ± 0.2	9.5 ± 0.9	92.0 ± 0.5	52.5 ± 0.7	94.3 ± 0.6	10.8 ± 1.1	89.7 ± 0.3	14.1 ± 0.5
1000	93.2 ± 0.4	16.7 ± 0.9	92.1 ± 0.9	58.0 ± 0.7	94.1 ± 0.7	18.8 ± 1.0	88.0 ± 0.6	17.3 ± 0.3
1100	92.4 ± 0.5	23.3 ± 0.8	93.1 ± 0.5	65.1 ± 0.9	94.4 ± 1.2	26.4 ± 1.1	85.8 ± 1.0	18.4 ± 0.1
1200	92.2 ± 0.5	29.9 ± 0.8	95.0 ± 0.3	72.7 ± 1.1	94.0 ± 0.4	35.2 ± 1.0	85.8 ± 0.4	19.2 ± 0.0
1300	91.1 ± 0.3	35.6 ± 0.7	91.9 ± 0.8	81.1 ± 0.8	94.3 ± 0.4	43.3 ± 1.1	86.7 ± 0.6	19.7 ± 0.1
1400	91.5 ± 0.3	40.8 ± 0.7	91.6 ± 0.4	86.4 ± 0.8	92.5 ± 0.4	51.1 ± 0.9	85.7 ± 0.8	20.6 ± 0.1
1500	91.4 ± 0.6	46.0 ± 0.7	89.7 ± 0.7	91.6 ± 0.5	90.1 ± 0.5	56.7 ± 0.5	83.5 ± 0.9	21.0 ± 0.0
1600	85.5 ± 0.3	49.4 ± 0.0	85.5 ± 0.3	93.4 ± 0.0	88.5 ± 0.2	59.9 ± 0.3	81.6 ± 0.5	21.0 ± 0.0
1700	84.6 ± 0.7	49.5 ± 0.0	80.4 ± 0.8	93.4 ± 0.0	87.2 ± 0.3	61.9 ± 0.2	79.6 ± 0.3	21.0 ± 0.0
1800	82.7 ± 0.3	49.5 ± 0.0	67.6 ± 2.8	90.2 ± 1.3	86.3 ± 0.8	62.9 ± 0.1	77.5 ± 0.5	20.8 ± 0.0
1900	85.8 ± 0.6	49.7 ± 0.1	64.9 ± 0.1	78.1 ± 1.5	80.7 ± 0.4	63.4 ± 0.0	80.6 ± 0.4	20.8 ± 0.0
2000	85.5 ± 0.6	50.2 ± 0.1	65.5 ± 0.1	66.4 ± 1.5	81.8 ± 0.3	63.4 ± 0.0	79.6 ± 0.4	20.8 ± 0.0
2100	85.3 ± 0.4	50.3 ± 0.0	65.9 ± 0.2	55.1 ± 1.4	82.7 ± 0.4	63.4 ± 0.0	73.2 ± 3.7	19.7 ± 0.7
2200	85.1 ± 0.5	50.4 ± 0.0	65.9 ± 0.1	44.0 ± 1.4	82.4 ± 0.4	63.4 ± 0.0	65.0 ± 0.1	9.7 ± 1.5
2300	85.6 ± 0.3	50.5 ± 0.0	65.8 ± 0.1	32.8 ± 1.4	72.0 ± 3.6	61.6 ± 0.9	64.9 ± 0.1	0.6 ± 0.5

[1] HLI Categories: 1) cool, ≤ 70; 2) moderate, 70.1 ≤ 77; 3) hot, 77.1 ≤ 86; 4) very hot, ≥ 86

[2] Accumulated heat load Categories: 1) low; 2) mild, 1.1 < 10; 3) moderate, 10.1 < 20; 4) hot, 20.1 < 50; 5) extreme, ≥ 50.1

Heat wave event 2 (d 71 to 76) was the most significant, in terms of hot ambient conditions and was further confounded by duration of the event. During this heat wave event maximum HLI recorded was 101.5 (1600 h, d 75) and maximum accumulated heat load for un-shaded Angus (AHL_{86}) recorded was 213 (1800 h, d 75). Furthermore night time conditions remained abnormally warm, where HLI and subsequently accumulated heat load (AHL_{86}) remained high (Table 5.1.4), providing limited night-time recovery. During this heat wave event excessive heat load responses, i.e. panting score 4 and 4.5 and head wetting behaviours (Figure 5.1.3), were observed in un-shaded Angus steers on d 74. Persistent observation of these high panting scores over a 3 hour period resulted in the un-shaded treatment pens being relocated to shaded pens, in accordance with the heat load management plan, until heat wave conditions abated (HLI ≤ 60; AHL_{86} = 0).

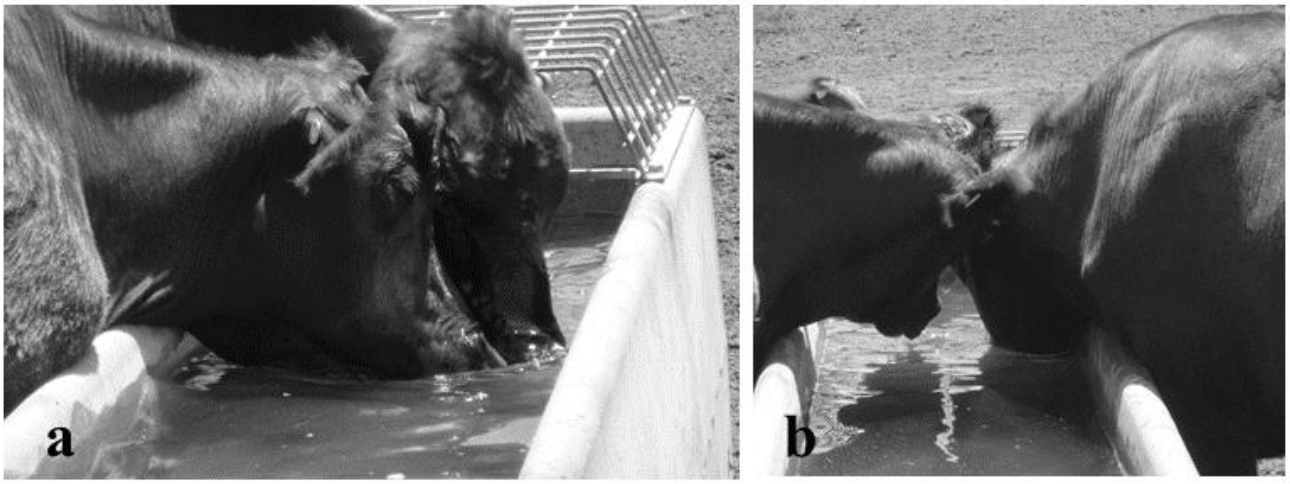


Figure 5.1.3 a and b: Un-shaded Angus steers displaying head wetting behaviours during heat wave event 2

During the study there were 127 days with a maximum HLI ≥ 86. Of these 127 days, 91 days had a HLI ≥ 90, 37 days had a HLI ≥ 95 and 4 days had a HLI ≥ 100. Throughout most of the study there was sufficient night time cooling to allow the cattle to dissipate any accumulated heat load, i.e. accumulated heat load returned to 0 overnight, excluding heat wave events where conditions were not always sufficient to allow accumulated heat load to return to 0. Overall there were 117 nights where HLI was ≤ 60, and 43 of these nights HLI was ≤ 55. These data indicate that on 76 % of nights these animals were able to dissipate accumulated heat load throughout night time hours, returning to a thermal equilibrium.

5.1.3.2 Animal Performance

There were no differences in the mean DMI of shaded (7.67 ± 0.12 kg/animal) and un-shaded (7.85 ± 0.11 kg/animal) treatment pens ($P > 0.05$). Feed offered within this experiment was regulated to i)

reduce excessive feed intake during high heat load periods, i.e. reducing metabolic heat load and ii) ensure that cattle were growing to comply with Australian domestic market specifications.

There were also no differences in initial live weight (non-fasted) of un-shaded (317.6 ± 11.0) and shaded (319.4 ± 8.1) treatments, however Brahman (365.5 ± 7.16 kg) steers had a higher initial live weight ($P < 0.001$) than the Angus (303.3 ± 5.12 kg) and Charolais (286.7 ± 6.47 kg). Final live weight was obtained on d 155. There were no treatment ($P = 0.17$) or breed ($P = 0.15$) weight differences, where live weight were 451.0 ± 10.0 kg and 469.3 ± 7.9 kg for un-shaded and shaded cattle respectively. Final body condition scores were not ($P > 0.05$) influenced by shade availability, 3.9 ± 0.06 and 3.7 ± 0.11 for un-shaded and shaded cattle respectively.

Mean ADG for all breed × treatment groups was 0.85 ± 0.17 kg/day. There were no differences ($P > 0.05$) in ADG within breed: Angus (un-shaded 0.97 ± 0.18 kg/day; shaded 1.08 ± 0.18 kg/day), Charolais (un-shaded 0.97 ± 0.46 kg/day; shaded 1.11 ± 0.19 kg/day) and Brahman (un-shaded 0.62 ± 0.19 kg/day; shaded 0.51 ± 0.18 kg/day) steers nor were there breed × treatment ($P > 0.05$) differences. The ADG of the cattle was lower than expected, and this was largely due to the hot conditions encountered during the study, which had a negative impact on feed intake.

A heat wave ration (decreased concentrate component by 10 % and added roughage) was introduced between d 73 and d 76. There were large variations in feed intake across pens (within and between treatments). Cattle were adversely affected by the heat wave and rain event in January and it took until mid-February for the shaded cattle to fully recover feed intake. From mid-February feed intakes remained above 10 kg/animal. The un-shaded cattle took longer to recover and even by mid-March intakes remained variable. Surprisingly the un-shaded Angus had the highest average final weight (481 kg), which may be reflective of compensatory growth, when the hot conditions abated.

There were no treatment differences in feed usage and feed efficiency. Gain to feed ratio was numerically higher ($P = 0.10$) for the shaded cattle (0.11:1) compared with the un-shaded cattle (0.10:1).

5.2 Changes in Rumen Temperature of Feedlot Cattle

Experimental Hypothesis: Rumen temperature will be influenced by breed and shade availability.

5.2.1 Introduction

One of the predicted consequences of global warming is the increased prevalence and intensity of heat waves (Solomon et al., 2007). The number and intensity of heat wave events differ from summer to summer and in the future it is likely that there will continue to be large variability in the climatic behaviour of these climatic events (Robinson, 2001; Westcott, 2011). Heat waves, more so, the subject of climatic extremes, are of great interest worldwide due to their substantial economic and physical impact on both people and livestock (Perkins and Alexander, 2012). St-Pierre et al. (2003) reported that heat load has an annual economic burden of > $300 million in the US beef sector alone. Throughout the summer season, feedlot cattle may be exposed to numerous high heat load events which compromise animal welfare and performance. Feedlot cattle are exposed to high heat load, not only during extreme conditions but also when exposed to sudden and rapid changes to local ambient conditions (Mader, 2003).

Numerous authors have reported on the impact of heat waves on feedlot cattle (Bushby and Loy, 1997; Hahn and Mader, 1997; Hahn, 1999; Entwistle et al., 2000; Gaughan, 2002; Brown-Brandl et al., 2006a; Brown-Brandl et al., 2006b) whereby most of the effects are associated with the regulation of body temperature. However body temperature is not an absolute measure, as a diurnal variation exists. Heat produced and dissipated from the body influences the regulation of body temperature (Legates et al., 1991) which must be maintained within a physiologically acceptable range. The amount of heat accumulation and dissipation from the body is constantly adjusting; however as ambient heat load increases above a given threshold which is largely species specific, heat accumulation becomes greater than dissipation resulting in an increase to core body temperuatre. These diurnal variations in body temperature can be considered a state of equilibrium between heat accumulation and dissipation (Legates et al., 1991). Therefore core body temperature is considered a reliable indicator of thermal balance.

There have been numerous studies investigating the effects of hot ambient conditions on thermoregulation in livestock; however few studies have utilized techniques that have allowed for continuous recording of body temperature periods of time ≥ 10 days (Nienaber et al., 1999). Traditional methods of obtaining body temperature rely on portable data loggers. However the use of data loggers to collate rectal (Gaughan et al., 1999; Gaughan et al., 2008a) and tympanic (Davis

et al., 2003; Gaughan et al., 2010a) temperatures are restricted to short term use typically 7 to 10 day periods (Gaughan et al., 2010b). Studies that have allowed for continuous recording of body temperature for periods of time ≥ 10 days have utilised remote sensing technology, although these studies required surgical implantation (Lefcourt and Adams, 1996; Gaughan et al., 2010a). Remote sensing technology is a potential method of obtaining body temperature over long periods of time (months, potentially years) without compromising animal welfare (Gaughan et al., 2010b). It is well known that *Bos indicus* and *Bos taurus* cattle regulate body temperature differently (Brody, 1956; Olbrich et al., 1971; Hansen, 2004), therefore the objectives of this section of the experiment were to;

i) *determine if differences exist in the regulation of rumen temperature between Bos indicus and Bos taurus breeds;*

ii) *identity differences between the rumen temperature of Bos indicus and Bos taurus steers with and without access to shade during periods of high heat load;*

iii) *determine the relationship between rectal temperature and rumen temperature; and*

iv) *determine the suitability of rumen temperature as an estimation of core body temperuatre in shaded and un-shaded Bos indicus and Bos taurus cattle housed in outside feedlot pens.*

5.2.2 Materials and Methods

5.2.2.1 Statistical Analysis

Individual 10 minute rumen temperature data were collated and converted to an hourly mean rumen temperature for each individual steer for each day. Hourly rumen temperatures were then converted to a mean within hour rumen temperature across all daily observations, i.e. 130 d. Mean hourly rumen temperature for each animal was analysed using a first order autoregressive repeated measures model (PROC MIXED; SAS Inst. Inc. Cary, NC), where each individual steer was considered as a subject. Each individual steer × hour (time of day; **h**) was considered as an experimental unit, weighted by the number of hourly observations. The model included fixed effects for breed, treatment, h, breed × treatment, breed × h, treatment × h and breed × treatment × h. The model then used pen nested within treatment, and individual animal nested within pen, as random effects, with correlation between hourly observations on each steer modelled using a first order autoregressive procedure. These data were used to identify differences in rumen temperature over the duration of the study (130 d). Data were also separated into monthly (December 2012, January 2013, February 2013 and March 2013) and heat wave data periods (event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147).

The relationship between rumen temperature and rectal temperature was determined by a partial correlation coefficient (Minitab® 16.2.0, 2010 Minitab, Inc.), allowing for the effects of treatment, breed and day, using mean rumen temperature from within the hour rectal temperature was collected. As the true value of core body temperuatre is unknown, rumen temperature and rectal temperature have been considered as estimates of core body temperuatre, and a relationship between the two measures would be anticipated. The degree of agreement between rumen temperature and rectal temperature was also analysed using the Bland-Altman method of comparison (Altman and Bland, 1983; Bland and Altman, 1986) where the difference between rumen temperature and rectal temperature was assessed against the mean of both measures. The mean of rectal temperature and rumen temperature becomes the best estimate of the value of core body temperuatre. These data were analysed based on the time rectal temperature was obtained and assessed using rumen temperature from within the hour of rectal temperature.

5.2.3 Results

5.2.3.1 Rumen Temperature

A similar diurnal rumen temperature pattern was observed between breeds and treatments with minimum rumen temperature occurring at approximately 0900 h and maximum rumen temperature at approximately 1600 h within 130 day mean (Figure 5.2.1), monthly (Figure 5.2.2) and heat wave (heat wave 1, Figure 5.2.3; heat wave 2, Figure 5.2.4; heat wave 3, Figure 5.2.5; heat wave 4, Figure 5.2.6) data periods. January was the hottest month during the study with midday (1200 h) means of, T_A 30.00 ± 0.65 °C; RH 57.55 ± 3.26 %; and HLI 89 ± 0.93. Maximum individual rumen temperature was 43.72 °C between 1300 h and 1400 h in an un-shaded Angus steer on d 50 where HLI = 98.5 (very hot) and AHL_{86} = 86.6 (extreme). The importance of shade availability for *Bos taurus* breeds was demonstrated during January, where significant differences ($P < 0.05$) in rumen temperature of the three breeds were observed. Notably rumen temperature of un-shaded Angus was ≥ 1 °C higher compared to shaded and un-shaded Brahman steers between 0900 h and 1700 h.

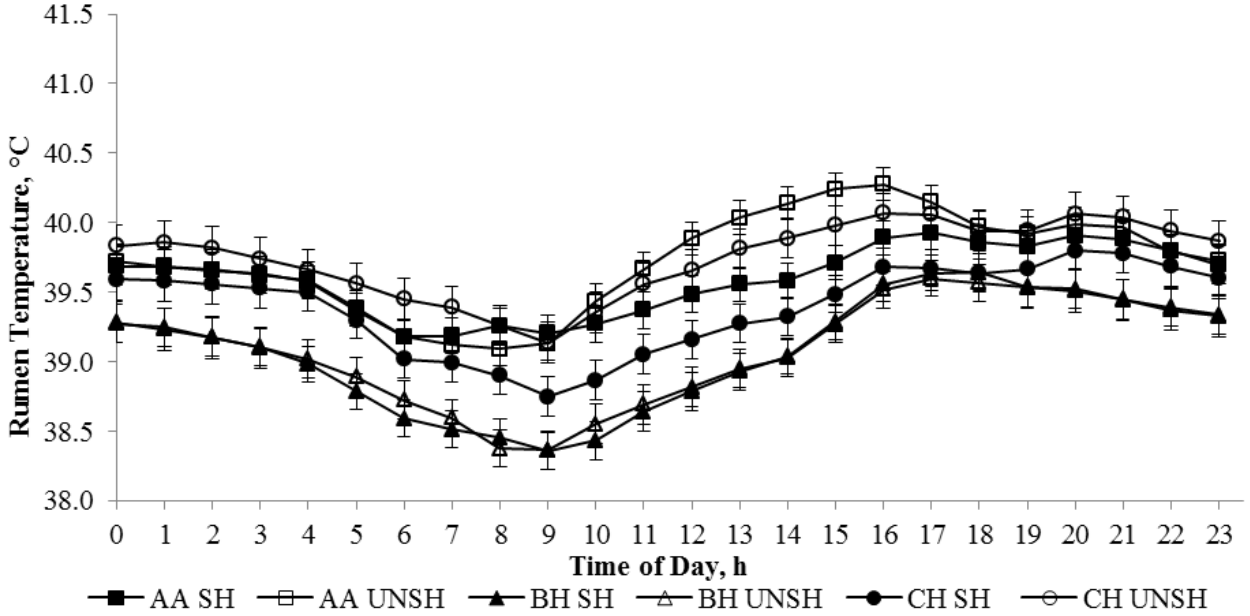


Figure 5.2.1: Mean hourly (h) rumen temperature of un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahmans (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers over 130 days

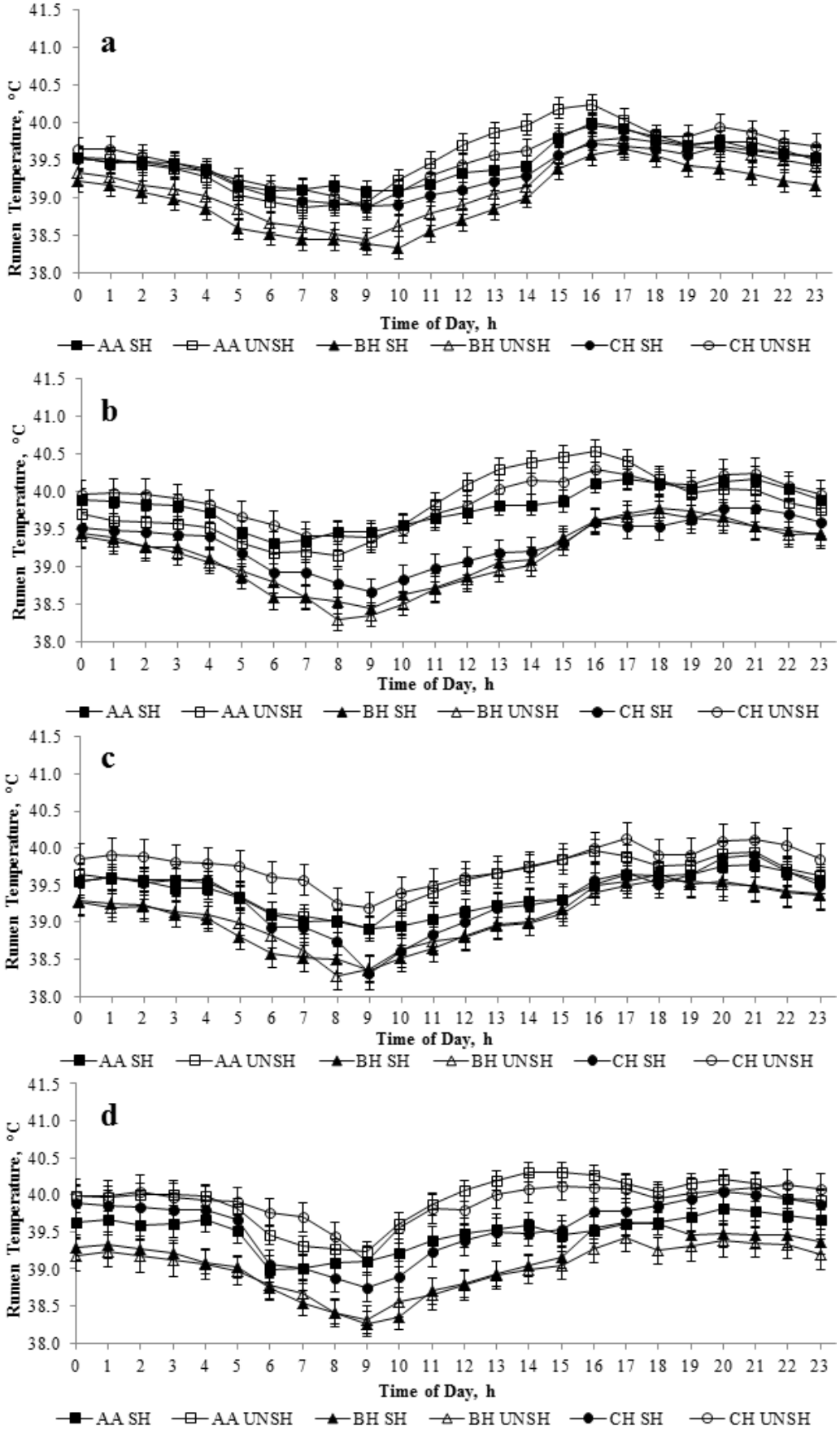


Figure 5.2.2: Mean hourly rumen temperature of un-shaded Angus (AA UNSH), un-shaded Charolais (CH UNSH), un-shaded Brahmans (BH UNSH), shaded Angus (AA SH), shaded Charolais (CH SH) and shaded Brahman (BH SH) steers during a) December, b) January, c) February and d) March

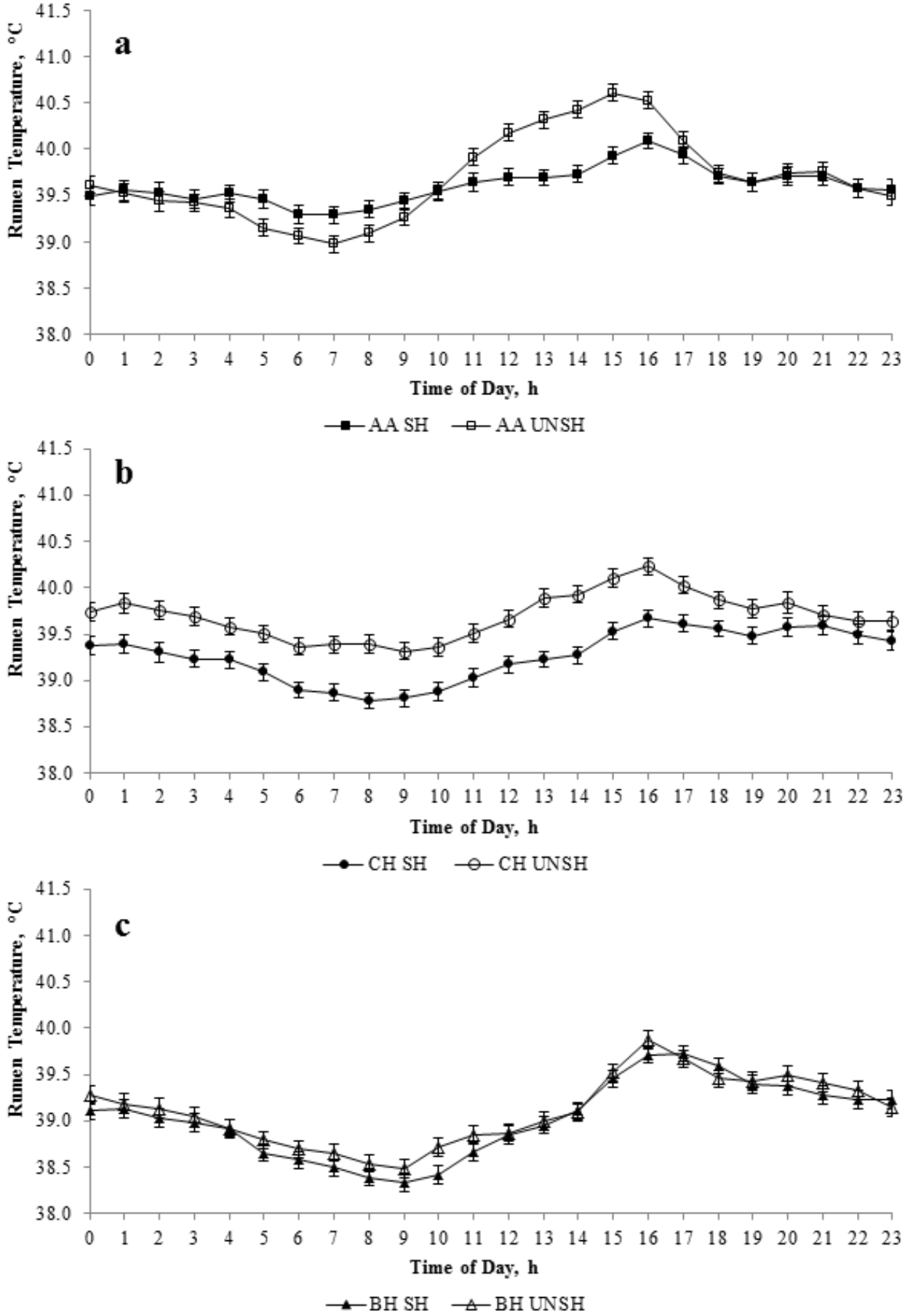


Figure 5.2.3: Mean hourly (± SEM) rumen temperature of shaded (SH) and un-shaded (UNSH) a) Angus (AA), b) Charolais (CH) and c) Brahman (BH) steers during heat wave event 1

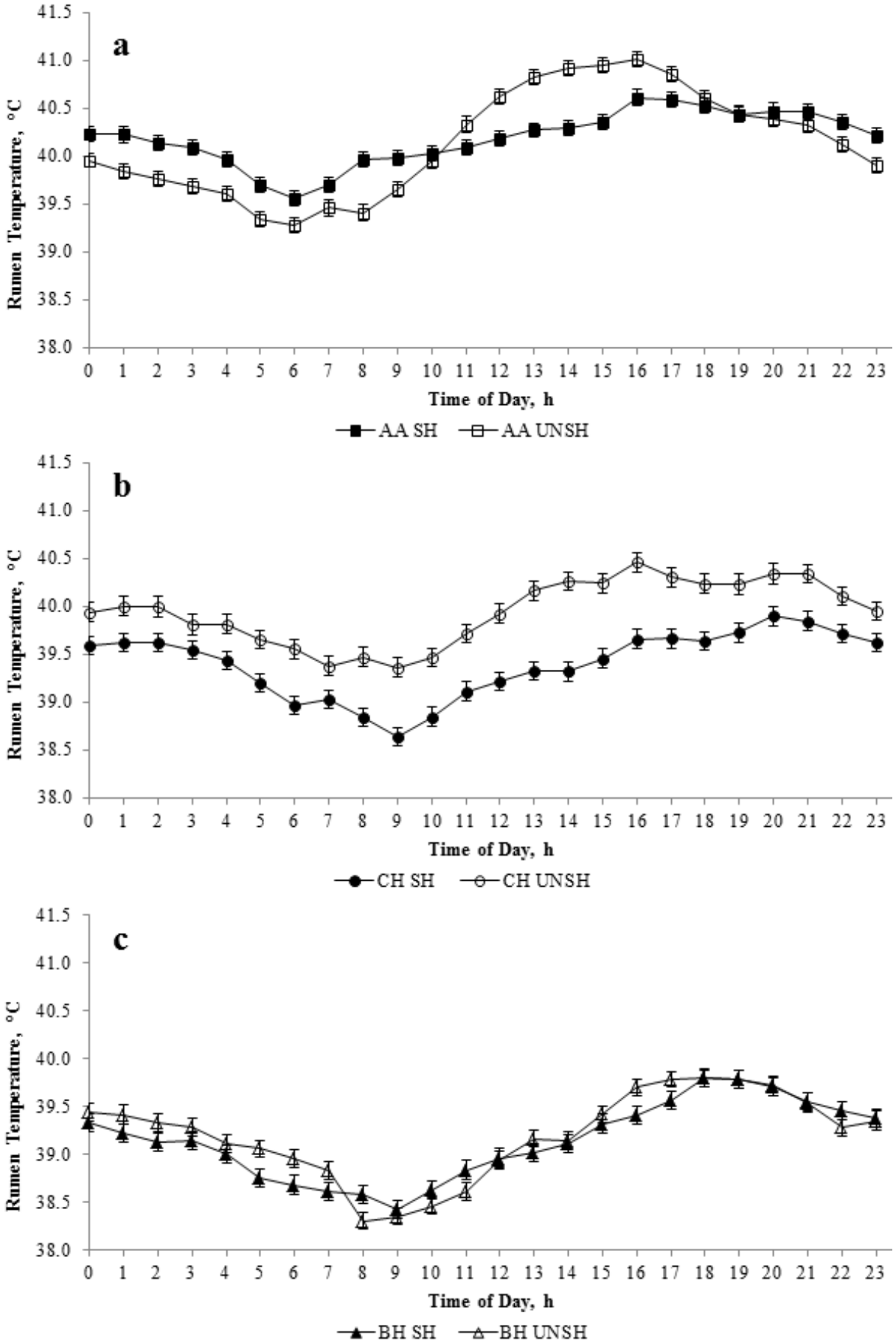


Figure 5.2.4: Mean hourly (± SEM) rumen temperature of shaded (SH) and un-shaded (UNSH) a) Angus (AA), b) Charolais (CH) and c) Brahman (BH) steers during heat wave event 2

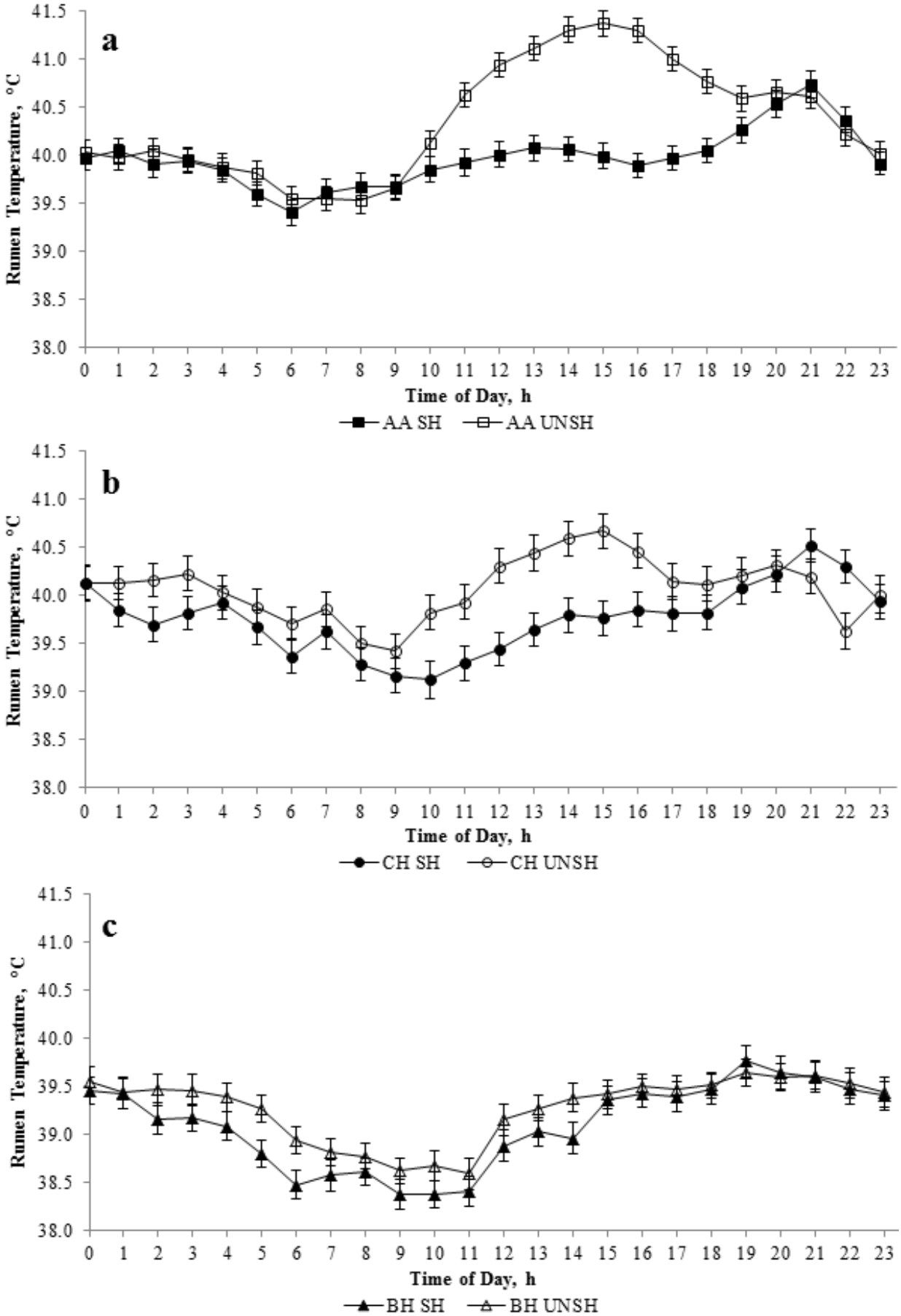


Figure 5.2.5: Mean hourly (± SEM) rumen temperature of shaded (SH) and un-shaded (UNSH) a) Angus (AA), b) Charolais (CH) and c) Brahman (BH) steers during heat wave event 3

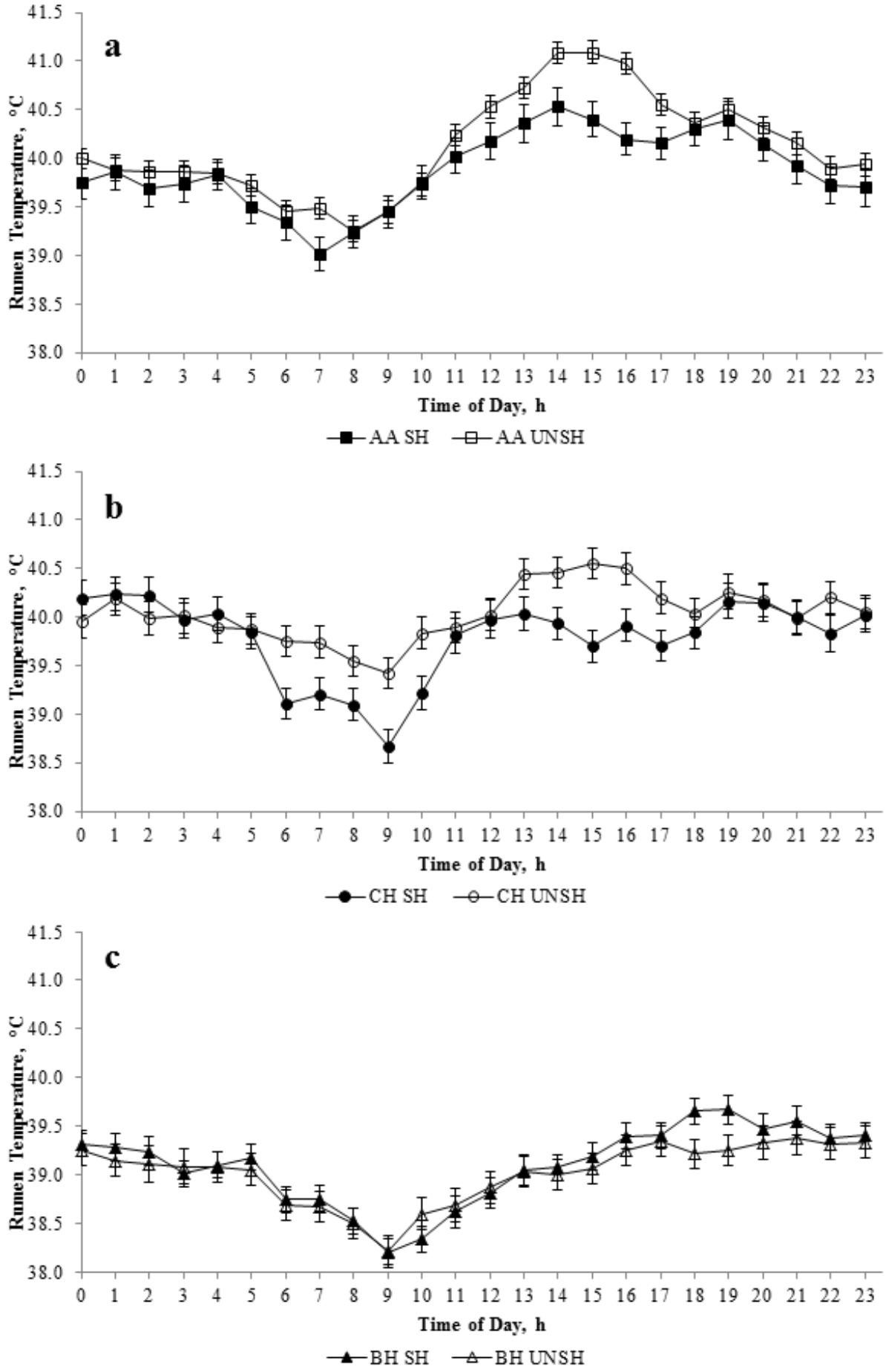


Figure 5.2.6: Mean hourly (± SEM) rumen temperature of shaded (SH) and un-shaded (UNSH) a) Angus (AA), b) Charolais (CH) and c) Brahman (BH) steers during heat wave 4

Significant interactions from the autoregressive repeated measures model (PROC MIXED; SAS Inst. Inc. Cary, NC), for mean 130 d, monthly and heat wave data periods are summarised in Table 5.2.1. Furthermore using the mean hourly rumen temperature for all breed × treatment groups over the 130 day study established a diurnal rhythm in rumen temperature (Figure 5.2.1). Deviations from the 130 day hourly means during heat wave events could be considered as the variation in rumen temperature due to environmental conditions (Table 5.2.2). Heat wave event 2 was the most significant hot period throughout the study. Duration of the heat wave event was 6 days, where accumulated heat load did not completely abate, i.e. $AHL_{86} \neq 0$, for a period greater than 2 hours (d 3), leading to cattle commencing consecutive days with an accumulated heat load. Un-shaded Angus were particularly affected during this heat wave event where maximum rumen temperature recorded was 43.72 °C between 1300 h and 1400 h in an un-shaded Angus steer on d 50 where HLI = 98.5 (very hot) and accumulated heat load for un-shaded Angus (AHL_{86}) = 86.6 (extreme). Rumen temperature deviations from 130 day hourly rumen temperature means (Table 5.2.2) had the greatest variability during heat wave event 3 (Figure 5.2.7). However climatic conditions did abate during the night resulting in the accumulated heat load for un-shaded Angus (AHL_{86}) = 0 for periods of at least 5 consecutive hours (Table 5.1.5), therefore providing an opportunity for the steers to dissipate the accumulated heat.

Table 5.2.1: Significant interactions (*P* value), for mean 130 d, monthly and heat wave data periods

Item	Mean	Month				Heat Wave			
		December	January	February	March	1	2	3	4
Breed	0.0002	0.0054	0.0002	0.0117	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment	0.3543	0.1763	0.1289	0.0965	0.0568	< 0.0001	0.0002	0.0002	0.0265
Breed × Treatment	0.3683	0.8695	0.0974	0.4103	0.1663	0.0017	< 0.0001	0.3582	0.0443
Hour	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Breed × Hour	< 0.0001	< 0.0001	< 0.0001	0.0002	0.0015	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment × Hour	< 0.0001	< 0.0001	< 0.0001	0.0013	0.0002	0.0043	< 0.0001	< 0.0001	0.0797
Breed × Treatment × Hour	0.0029	0.0373	0.0046	0.0597	0.6459	0.1017	< 0.0001	0.0335	0.2366
Day	X	X	X	X	X	0.4523	< 0.0001	0.4856	< 0.0001
Breed × Day	X	X	X	X	X	0.3796	0.0030	0.4750	< 0.0001
Treatment × Day	X	X	X	X	X	0.2513	0.2829	0.4280	0.2627
Breed × Treatment × Day	X	X	X	X	X	0.2127	0.6263	0.2283	0.5011

X Day interactions were unable to be computed within the 130 day mean and monthly data periods

Table 5.2.2: Mean (± SEM) deviation from 130 day mean[1] rumen temperature during each of the four heat wave events[2]

Item	Heat Wave 1	Heat Wave 2	Heat Wave 3	Heat Wave 4
Un-shaded Angus	-0.04 ± 0.04^{a}	0.41 ± 0.05^{b}	0.64 ± 0.06^{c}	0.40 ± 0.05^{b}
Shaded Angus	0.01 ± 0.04^{a}	0.59 ± 0.03^{b}	0.38 ± 0.04^{c}	0.29 ± 0.06^{c}
Un-shaded Charolais	-0.05 ± 0.03^{a}	0.20 ± 0.02^{b}	0.33 ± 0.05^{c}	0.30 ± 0.03^{c}
Shaded Charolais	-0.12 ± 0.02^{a}	0.00 ± 0.01^{b}	0.36 ± 0.03^{c}	0.40 ± 0.05^{c}
Un-shaded Brahman	0.03 ± 0.03^{a}	0.11 ± 0.02^{b}	0.19 ± 0.03^{c}	-0.06 ± 0.03^{d}
Shaded Brahman	-0.05 ± 0.02^{a}	0.07 ± 0.02^{b}	0.03 ± 0.03^{b}	0.03 ± 0.03^{b}

[a – d] Within a row, means without a common superscript differ ($P < 0.01$)

[1] Mean 130 day rumen temperature data includes heat wave events

[2]The heat waves occurred on d 48 to d 52; d 71 to d 76; d 92 to d 94; and d 144 to d 147

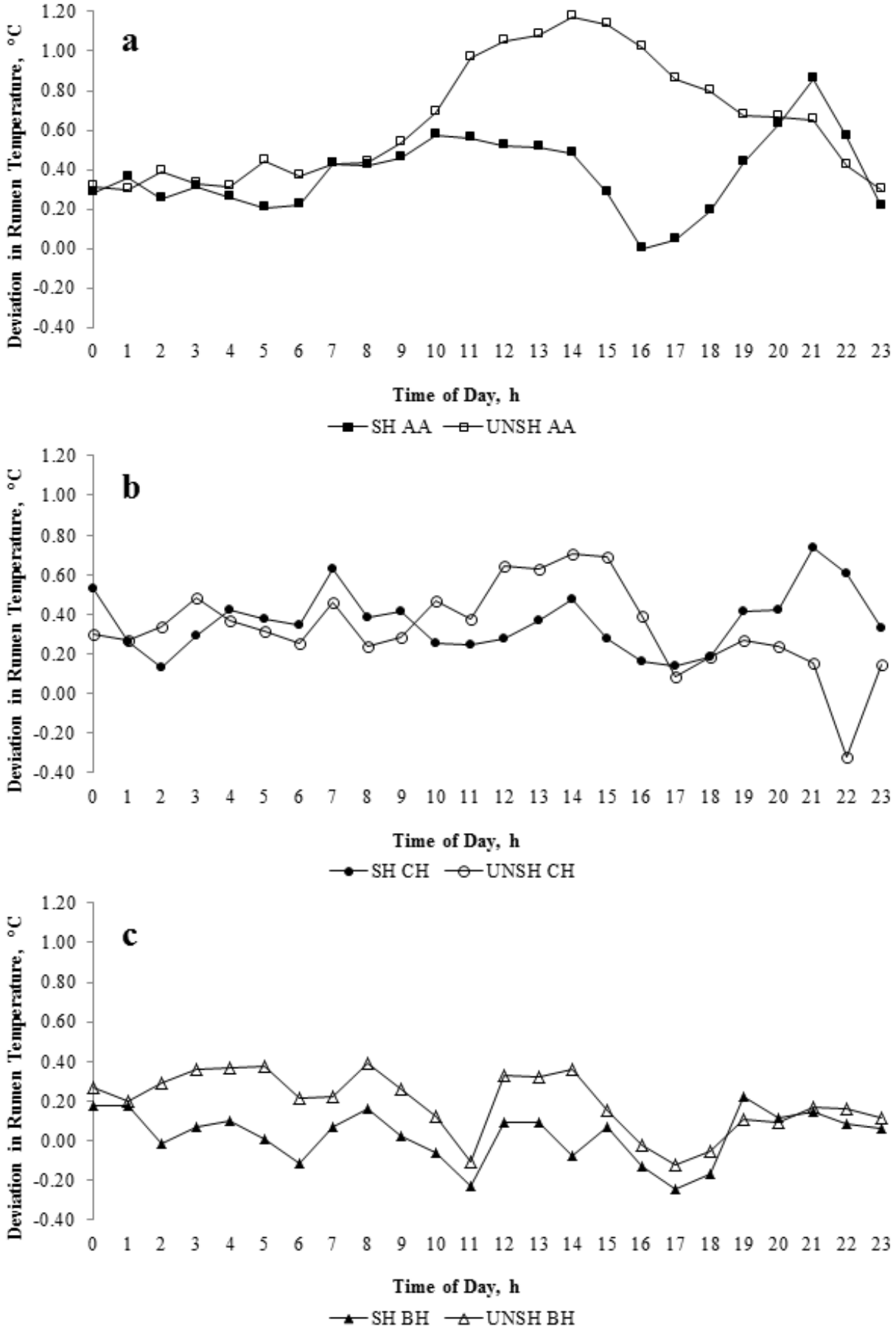


Figure 5.2.7: Deviation in rumen temperatures (°C) from 130 day hourly mean rumen temperature of shaded (SH) and un-shaded (UNSH) a) Angus (AA), b) Charolais (CH) and c) Brahman (BH) steers during heat wave event 3

5.2.3.2 Rumen Temperature versus Rectal Temperature

A linear trend was observed between rectal temperature and rumen temperature (Figure 5.2.8). A partial correlation coefficient (Minitab® 16.2.0, 2010 Minitab, Inc.) indicated that there was a moderate to strong association (r = 0.52; $P < 0.0001$) between rumen temperature and rectal temperature. As the true value of core body temperature is unknown, rectal temperature and rumen temperature become an estimation of core body temperature, therefore temperature data were also analysed using the Bland-Altman method of comparison (Figure 5.2.9; Bland and Altman, 1986). Results determined from the Bland-Altman method indicated that the mean difference between rumen temperature and rectal temperature is small, 0.06 ± 0.06 °C, where a majority of the data (n = 89) is situated within the 95 % confidence interval (upper limit +1.23 °C; lower limit -1.12 °C). However it was acknowledged that there was a limited number (n = 93) data points available for this analysis, due to missing rumen temperature data within the hour of obtaining rectal temperature at weighing.

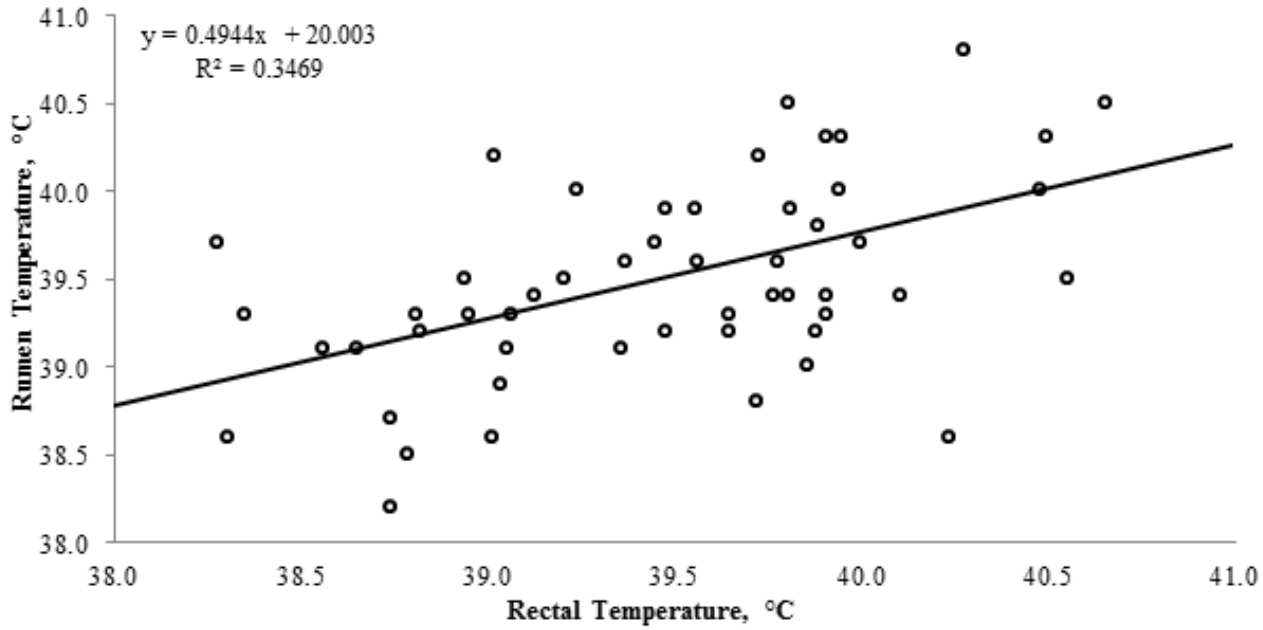


Figure 5.2.8: Linear trend between rectal temperature and rumen temperature

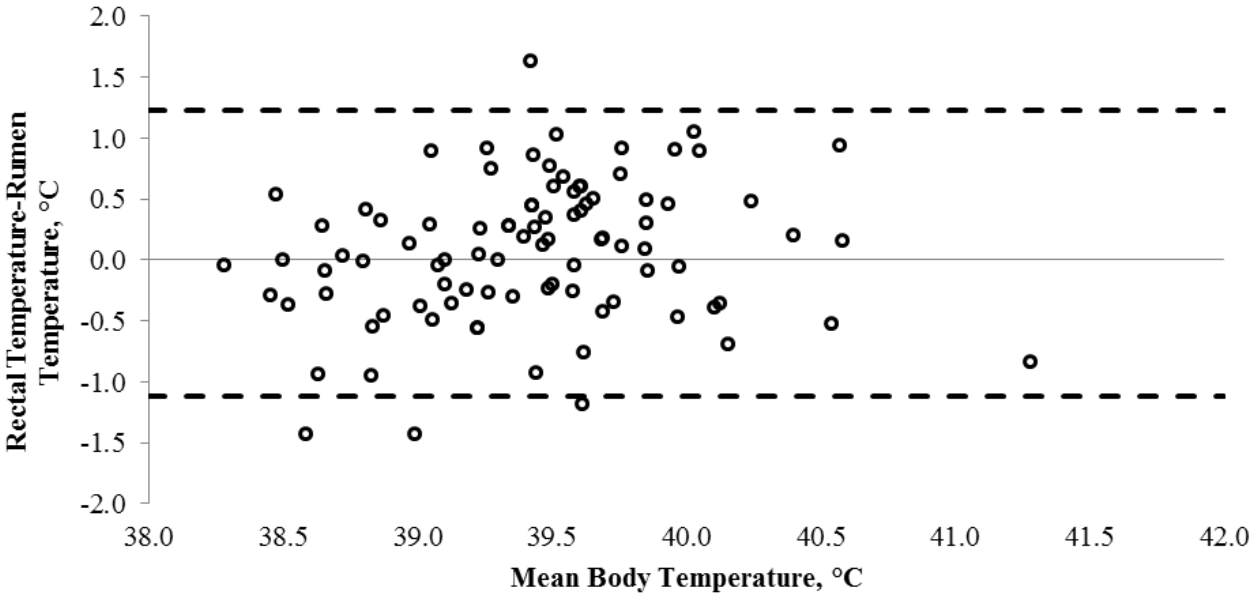


Figure 5.2.9: Bland Altman plot assessing the use rumen temperature as an assessment of core body temperature where the differences between rectal temperature and rumen temperature were evaluated against the combined mean of rectal temperature and rumen temperature, also showing the confidence intervals (95 % = mean ± 1.96 × SD; dashed line)

5.2.4 Discussion

Within the current experiment there was a moderate to strong relationship (r = 0.52) between mean hourly rumen temperature and rectal temperature, albeit from a small data set (n = 93). By using the Bland-Altman method of comparison, results from this study indicated that the mean difference between rumen temperature and rectal temperature was small, 0.06 ± 0.06 °C, indicating that there is an agreement between the two measures; thus rumen temperature can be identified as a functional estimate of core body temperuatre. Mean 130 day rumen temperature for breed × treatment groups (pooled) was 39.4 ± 0.04 °C. Maximum rumen temperature recorded throughout the experiment was 43.7 °C in an un-shaded Angus steer during a heat wave event, where HLI and accumulated heat load for un-shaded Angus (AHL_{86}) categories were classified as “very hot” and “extreme”. Given the small temperature difference between rumen temperature and rectal temperature observed within this experiment, the rumen temperature of 43.7 °C potentially could have been lethal, as rectal temperature greater than 42 °C has been associated with mortality in cattle (Findlay, 1958).

Data from this experiment has clearly shown that a diurnal temperature rhythm for rumen temperature existed. Furthermore the diurnal rhythm observed appears to trend with increasing and decreasing ambient conditions. However further investigation is required to determine the true nature of the relationship between ambient conditions and rumen temperature. The diurnal temperature rhythm for rumen temperature indicates that minimum and maximum temperatures occurred at approximately 0900 h and 1600 h. In addition, results from the current experiment

indicate that in all months there was a general trend of a secondary increase of rumen temperature at approximately 2000 h, which could be assumed to be associated with feed intake with feed offered once daily at approximately 1430 h. There was a trend for decreasing night time rumen temperature, where un-shaded Angus cattle tended to have lower rumen temperature than shaded Angus during night time hours. Similar findings, using other methods of measuring body temperature, have been reported by Gaughan et al. (2004b) via rectal temperature; Brown-Brandl et al. (2005a) and Gaughan et al. (2010a) using abdominal temperature via radiotelemetry; and Mader et al. (2010a) via tympanic temperature. It was suggested by Brown-Brandl et al. (2005a) that the greater decrease in un-shaded cattle's body temperature could be due to an increase in the thermal gradient for radiative exchange mechanisms, through increased exposure to the night time sky, providing an opportunity to dissipate greater thermal loads with little influence from radiative surfaces e.g. shade structures. Further to this Mader et al. (2010a) reported that cattle that had higher maximum tympanic temperatures during the day had a greater decrease resulting in lower tympanic temperatures during the night. Similarly Lefcourt and Adams (1996) reported peaks in core body temperuatre, through abdominal radiotelemetry, during the evening where peaks generally occurred after T_A began to decline with nightfall. However Lefcourt and Adams (1996) concluded that evening peaks in body temperature are likely to be representing a fundamental aspect of thermoregulation, whereby the increase in body temperature becomes a physiological reset mechanism.

These evening peaks in body temperature increase the temperature gradient between the animal and the environment thus increasing thermal exchange pathways, rather than a greater rate of heat dissipation, via radiative exchange mechanisms, due to night time exposure as suggested by Brown-Brandl et al. (2005a). In the current study small increases, ranging between 0.07°C and 0.14 °C, in rumen temperature were observed in Angus and Charolais between 1900 h and 2000 h. From 2000 h rumen temperature continued to decrease until approximately 0900 h. If these evening peaks in body temperature are a reset mechanism, the small increases to rumen temperature in the evenings and through the night could be a result of the animals' adjusting thermoregulatory mechanisms in order to increase heat dissipation. The increase in heat dissipation provides the animal with an opportunity to decrease body temperature to a physiologically acceptable baseline temperature prior to the commencement of the next day's heat challenge. However the physiological reasons for the greater decline in body temperature of un-shaded cattle are yet to be defined.

Whilst heat wave event 3 showed the greatest variability in rumen temperature, heat wave event 2 was still the most significant in terms of the dynamic responses of the cattle to hot weather

conditions. The main difference between heat wave event 2 and 3 was night time relief. During heat wave event 3, night time conditions were such that the un-shaded Angus had at least 5 hours of zero accumulated heat load. This indicates that the night time conditions were sufficient to incite heat dissipation allowing the cattle to decrease body temperature overnight, returning a thermal equilibrium (AHL_{86} = 0) prior to the commencement of the next day. Limited night time relief during heat wave event 2 resulted in greater increases of rumen temperature throughout the duration of the heat wave event, particularly in un-shaded Angus steers which expectantly were the most affected by the hot conditions. During heat wave event 2, un-shaded Angus steers had rumen temperatures that were ≥ 1.1 °C and ≥ 1.2 °C ($P < 0.0001$) compared with un-shaded and shaded Brahman between 0900 h and 1700 h. The amount of time animals are exposed to climatic conditions above the thermoneutral zone may provide an indication of time required below thresholds to return to thermal equilibrium. However it is not yet fully understood the amount of time animal required below thermal thresholds, i.e. HLI ≤ 86 for un-shaded Angus, for cattle to return to a thermal equilibrium and more studies into this area are warranted. However as ambient conditions increase, sensible and non-sensible heat exchange mechanisms between the animal and its surrounding environment become ineffective.

For an animal to maintain core body temperature, heat accumulated through heat energy exchange mechanisms and metabolic functions must equal the amount of heat dissipated from the body (Hahn, 1985). Therefore during hot conditions where heat is accumulated by the animal throughout day time conditions, night time conditions must allow for dissipation of accumulated heat load or the animal will enter the consecutive day, or days, with an elevated body temperature. Cooler night time conditions initiates heat dissipation, via convective and radiant exchanges, as the temperature gradient between the body surface and surrounding environment is reduced (Robertshaw, 1985). Thus cooler night time conditions provide an opportunity to decrease elevated body temperature (Scott et al., 1983), prior to the next day. This was emphasized during heat wave event 2 where climatic conditions remained abnormally high during night time hours where accumulated heat load for un-shaded Angus did not abate, i.e. $AHL_{86} \neq 0$ overnight. Whilst conditions remained abnormally high after sunset the effect of solar radiation (W/m^2) is removed, rapidly decreasing HLI. However during heat wave 2 accumulated heat load for un-shaded Angus (AHL_{86}) remained stable, categorized as extreme (≥ 50.1), on days 3, 4 and 5 for 3, 5, and 4 hours post sunset respectively, indicating that there was limited opportunity to dissipate accumulated heat load, particularly in un-shaded Angus, during early evening hours. This would suggest that these animals were predisposed to higher accumulated heat load during consecutive days. Moreover this may be indicating that the HLI may not be accurately describing the effect of night time conditions during

heat wave events due to the reliance on solar radiation within the model. Similar heat wave conditions were reported throughout the mid-central USA in July 1995 where cattle deaths were estimated at 4 000 head (Hahn and Mader, 1997). During the 1995 event, cattle were exposed to extended periods of continuous hot weather conditions with limited night time cooling (Hahn and Mader, 1997). Consequently, these events highlight the importance of a night time recovery period on the regulation of body temperature and maintenance of animal comfort and welfare during high heat load events.

During heat wave events it has been reported that daily means and ranges in body temperature are markedly increased (Hahn and Mader, 1997). This was evident during heat wave events 2, 3 and 4 particularly in Charolais and Angus steers. Greatest deviations from 130 day mean rumen temperatures were observed during heat wave event 3 noticeably in Angus and Charolais steers where mean rumen temperature variability was 0.64 ± 0.06 °C, 0.38 ± 0.04 °C, 0.33 ± 0.05 °C and 0.36 ± 0.03 °C for un-shaded and shaded Angus and Charolais respectively. Mechanisms of heat accumulation and dissipation often require a temperature gradient between the body and the surrounding environment. Therefore if the body temperature of an animal is lower than that of the surrounding air the animal becomes a heat "sink" where it will accumulate heat from the environment (Silanikove, 2000). However if the body temperature of the animal is similar to the surrounding environment, heat accumulation by the animal is reduced (Schmidt-Nielsen et al., 1957). Therefore greater daily increases in body temperature allows for greater heat accumulation and storage during the day, as long as it is within a physiologically acceptable rage, then dissipation via non-evaporative thermoregulatory mechanisms at night (Finch, 1986). Thus it could be concluded that the greater variability in rumen temperature of un-shaded Angus and Charolais steers is in fact a thermoregulatory mechanism to regulate heat accumulation and storage throughout the day. This may actually be a thermoregulatory adaptation whereby un-shaded cattle are acclimating to local conditions. Defining acclimation as a process where the animal is able to adapt and widen the dynamic range in body temperature (Horowitz, 2001), in order to survive; the variability in rumen temperature observed within the current study indicates that these animals were indeed acclimating to their thermal environment.

The current experiment used *Bos taurus* and *Bos indicus* breeds for the procurement of large individualized data sets. This has provided an opportunity to assess the magnitude of thermoregulatory capacity of these breeds, in terms of body temperature regulation. Within the current experiment no hour or treatment differences were observed between un-shaded and shaded Brahman steers. Furthermore monthly data also indicate that there was no difference in the rumen

temperature of shaded and un-shaded Brahman steers. However mean 130 day rumen temperatures of shaded Angus were lower ($P < 0.05$) than un-shaded Angus between 1200 and 1600 h. Rumen temperatures of un-shaded Angus were higher than shaded and un-shaded Brahman steers, whereby during heat wave event 2 the difference was ≥ 1 °C between 0900 h and 1700 h. These data provide further indication that *Bos indicus* breeds are better able to regulate body temperature compared with *Bos taurus* breeds. Furthermore these data indicate that body temperature, as measured by rumen temperature, is influenced by breed, treatment and hour. This was particularly evident in Angus where rumen temperatures of un-shaded animals were 0.55 °C and 0.53 °C higher ($P < 0.001$) higher than their shaded counterparts at 1400 h and 1500 h. Furthermore between mid to late afternoon hours (1200 h to 1600 h) un-shaded Angus had significantly higher ($P < 0.02$) rumen temperature compared with shaded Angus. These data suggest that shaded cattle were utilizing the shade to support body temperature regulation throughout mid to late afternoon. The importance of the availability of shade in the regulation of body temperature was reinforced throughout the 4 heat wave events. Shade has been reported to support the maintenance of body temperature during hot weather conditions (Mader et al., 1999a; Brown-Brandl et al., 2005a; Mader et al., 2010a). This was also emphasized during heat wave event 3 where un-shaded Angus steers had rumen temperatures that were ≥ 1 °C ($P < 0.0001$) higher than their shaded Angus counterparts between 1300 h and 1700 h. During heat wave event 1, mean hourly rumen temperature of shaded Charolais steers was lower ($P < 0.03$) than that of un-shaded Charolais at all hours excluding those between 2000 h and 2300 h.

It is widely accepted that *Bos indicus* heritage cattle have genetic adaptations for thermotolerance and consequently are less susceptible to the negative effects of hot climatic events (Hansen, 2004). In support of this, rumen temperature of Brahman steers did not show the same variability in rumen temperature as that exhibited by Charolais and Angus. Genes controlling the thermoregulatory abilities of *Bos indicus* cattle are extended to the cellular level (Hansen, 2004), where it has been suggested that water metabolism in *Bos indicus* cattle is similar to that of the camel (Finch, 1986). Summer body temperature of camels with once daily, *ad libitum*, water intake exhibited variations (± 2 °C) in body temperature similar to those identified during winter (Schmidt-Nielsen et al., 1957). Thus Brahman steers within this study could be utilising water consumption as a thermoregulatory mechanism to maintain rumen temperature throughout hot weather conditions. The rumen temperature of shaded and un-shaded Brahman steers did not differ throughout any of the 4 heat wave events.

The current HLI model accumulated heat load thresholds are HLI ≥ 96 for un-shaded Brahman and HLI ≥ 100 for shaded Brahman (Gaughan et al., 2008b), although all of the heat wave events resulted in conditions where HLI ≥ 95, where the duration of these conditions were not sufficient to warrant large variations in the body temperature of shaded and un-shaded Brahman steers. Furthermore Gaughan et al. (2008b) indicated that the upper HLI threshold of a purebred *Bos indicus* animal may be greater than 96, the authors acknowledged that during their experiment climatic conditions were not sufficient to result in large number of days where HLI ≥ 95. Therefore the authors were unable to identify a definitive HLI threshold for 100 % *Bos indicus* genotyped animals, indicating that further investigation in this area is required. However, it must be acknowledged that climatic conditions where HLI ≥ 95 are difficult to replicate and assess in natural and controlled environments.

The availability of modern data acquisition equipment has allowed for the collection of continuous measurement of real time body temperature. Remote sensing technology may potentially allow for the identification of animals susceptible to heat load and animals that are not coping with increasing thermal loads. Furthermore these data indicate that the availability of shade also influences rumen temperature, with shaded cattle generally having lower rumen temperature, particularly between 1200 h and 1600 h. Rumen bolus technology allows for the assessment of an animal's thermal status to be undertaken without physically moving or restraining the animal. Restraining an animal can incite the stress response that potentially masks the intended response (Hahn et al., 1990), i.e. an increase in body temperature. This is potentially important during heat wave events, where restraining or moving the animal leads to an increase in metabolic heat generation resulting in an increase in body temperature, contributing to the overall impact of heat load on the animal and ultimately increasing the animal's susceptibility to succumbing to heat load.

5.2.5 Conclusion

Results within this experiment indicate that the use of remote sensing technology to assess rumen temperature has the potential to provide real time body temperature information from animals without the stress of handling and restraint. Data obtained from the current experiment suggests that rumen temperature has the potential to become a functional predictor of rectal temperature, and that it is possible that rumen temperature can be used as a proxy of core body temperature. However more data is required to fully determine the use of rumen temperature as a determination of core body temperature. Results from this experiment indicate that breed, ambient conditions and availability of shade influence rumen temperature, indicating that rumen temperature can be used to assess an animal's thermal status.

5.3 Behavioural Responses of Feedlot Cattle to Heat Load

Experimental Hypothesis: Breed and shade availability will influence panting score, shade utilisation and the behavioural responses of feedlot cattle to heat load.

5.3.1 Introduction

Feedlot cattle are particularly susceptible to changes in climatic conditions, often exhibiting reduced performance and wellbeing during periods of hot climatic conditions (Hahn, 1999; Mader, 2003). How an animal responds to hot climatic conditions is dependent on a number of individual characteristics, therefore no two animals will respond to heat load in the same manner. In response to increasing thermal loads, cattle will initiate purposeful behavioural changes. Observations of cattle behaviour can be used to quantify animal responses to heat load (Mitlöhner et al., 2001a). These behavioural adaptations are the animal's first response to increasing thermal loads. Under conditions where behavioural responses are insufficient to support the regulation of core body temperature, cattle will adjust physiologically and haematologically to negate the adverse effects of hot conditions (Hahn, 1999; Gaughan et al., 2008b).

Quantifiable physiological measures such as increased sweating rate (Mader et al., 2010a); respiration rate (breaths per minute) (Gaughan et al., 2000); panting score (Mader et al., 2006); and body temperature (°C) (Robertshaw, 1985) are all useful indicators of thermal stress. Cattle also use adaptive behaviours to reduce heat load, primarily consisting of shade seeking, under shade structures or other animals, and the alignment of the body in accordance with solar radiation (**SR**; W/m^2) to reduce whole body exposure to direct sunlight (Nienaber et al., 2003). Other behavioural changes exhibited by cattle include alterations in posture, including increasing proportion of time standing; increased duration in shaded areas or increased shade seeking, including shade provided from other animals; and body splashing at water troughs (Young and Hall, 1993). An additional response to increasing thermal loads is to reduce dry matter intake (**DMI**) (Ray, 1989; Hahn et al., 1992). The reduction in DMI is associated with a decrease in heat production, via ruminal fermentation, and metabolism, thus aiding in maintaining the overall heat balance of the animal (Beede and Collier, 1986; Hahn, 1999). However a voluntary reduction in DMI is also associated with reduced gut motility and rumination (Beede and Collier, 1986).

Cattle have an ability to recognise and learn behaviours that support thermoregulation. These learnt behaviours are continually developed through exposure to hot environmental conditions where the experience provides the animal with strategies to cope with thermal loads (Castaneda et al., 2004).

As ambient heat load conditions increase, animal observations can provide some insight to the severity of heat load the animal is experiencing. Therefore the objectives of this section of the experiment were to;

i) *determine the differences in the behavioural responses of Bos taurus and Bos indicus breeds to heat load; and*

ii) *further determine the effect of shade on the behavioural responses of Bos taurus and Bos indicus to heat load*

5.3.2 Materials and Methods

5.3.2.1 Statistical Analysis

Behavioural observational data were converted to count for each breed × treatment group for each observation time point. Data collected included activity (feeding, drinking, or ruminating) and posture (standing or lying), i.e. number of un-shaded Angus steers standing at 0600 h. Shade utilisation was calculated by determining the count of steers standing or laying under shaded regions. Counts per breed were then converted to a proportion per breed × treatment group.

Panting score counts were used to calculate a mean panting score for each breed × treatment group for each observation. Mean panting score was calculated as described by Gaughan et al. (2008b) using the following equation;

$$Mean\ Panting\ Score = \frac{\sum_{i=0}^{4.5} N_i \times i}{\sum_{i=0}^{4.5} N_i}$$

Where N_i = the number of cattle observed at PS i

For this experiment mean panting score was divided into four stress categories as described by Gaughan et al. (2008b). The four categories are described as;

1. No Stress, mean panting score, $0 \leq 0.4$
2. Low Stress mean panting score, $0.4 \leq 0.8$
3. High Stress, mean panting score, $0.8 \leq 1.2$
4. Severe Stress, mean panting score, ≥ 1.2

Feeding, drinking, ruminating, posture, shade utilisation and mean panting score responses were analysed using an analysis of variance, Generalised Linear Model with a binomial structure (R, R Foundation for Statistical Computing, Vienna, Austria). For feeding, drinking, ruminating, posture and mean panting score the model analysed the effect of breed, treatment, hour (time of day; **h**), day

of experiment (**d**), HLI, breed × treatment, treatment × HLI, treatment × h, breed × d, h × d, breed × h, breed × HLI, breed × treatment × h, breed × treatment × HLI and breed × h × d. For shade utilisation the model analysed the effect of breed, h, d, HLI, breed × d, h × d, breed × h, breed × HLI and breed × h × d. Data were analysed for all data over the duration of the study (154 d), monthly (November 2012, d 2 to d 31; December 2012, d 32 to d 62; January 2013, d 63 to d 93; February 2013, d 94 to d 121; and March 2013, d 122 to d 152) and heat wave (event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147) data periods.

5.3.3 Results

5.3.3.1 Feeding Behaviour

During the study (154 d; Figure 5.3.1), feeding behaviours were affected by breed ($P < 0.0001$), treatment ($P = 0.04$), h ($P < 0.0001$), d ($P < 0.0001$), HLI ($P < 0.0001$; Figure 5.3.2), breed × HLI ($P = 0.002$), breed × treatment ($P = 0.25$), breed × h ($P = 0.10$), treatment × HLI ($P = 0.48$), treatment × h ($P = 0.0003$), breed × d ($P < 0.0001$), h × d ($P < 0.0001$). Feeding behaviours were not affected by breed × treatment × h ($P = 0.99$), breed × treatment × HLI ($P = 0.62$) and breed × d × h ($P = 0.40$). Interactions for feeding behaviours are presented in Appendix 1 and Appendix 2, for monthly and heat wave data periods respectively.

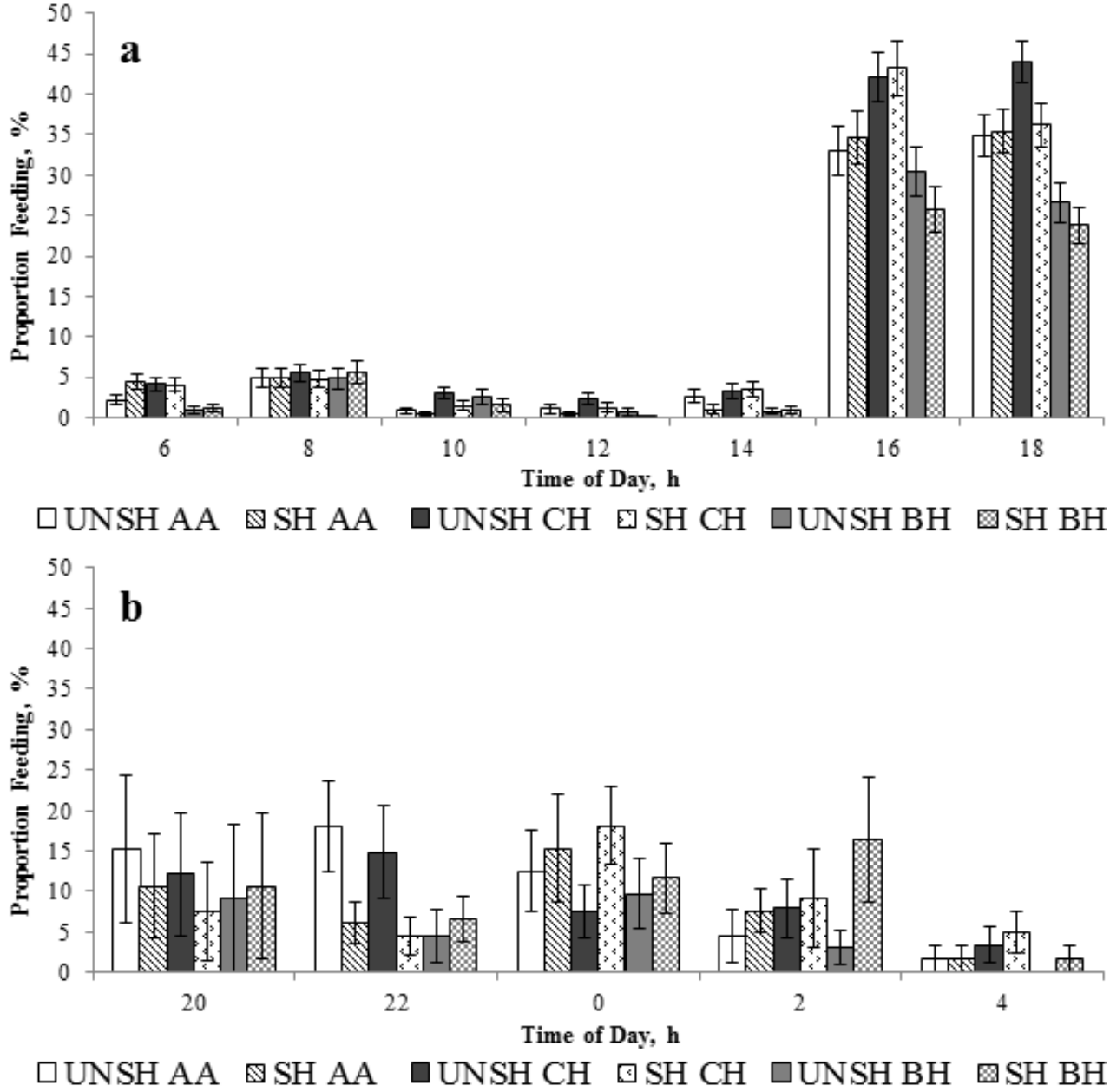


Figure 5.3.1: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers observed feeding during a) daytime (0600 h to 1800 h) and b) night time (2000 h to 0400 h) hours

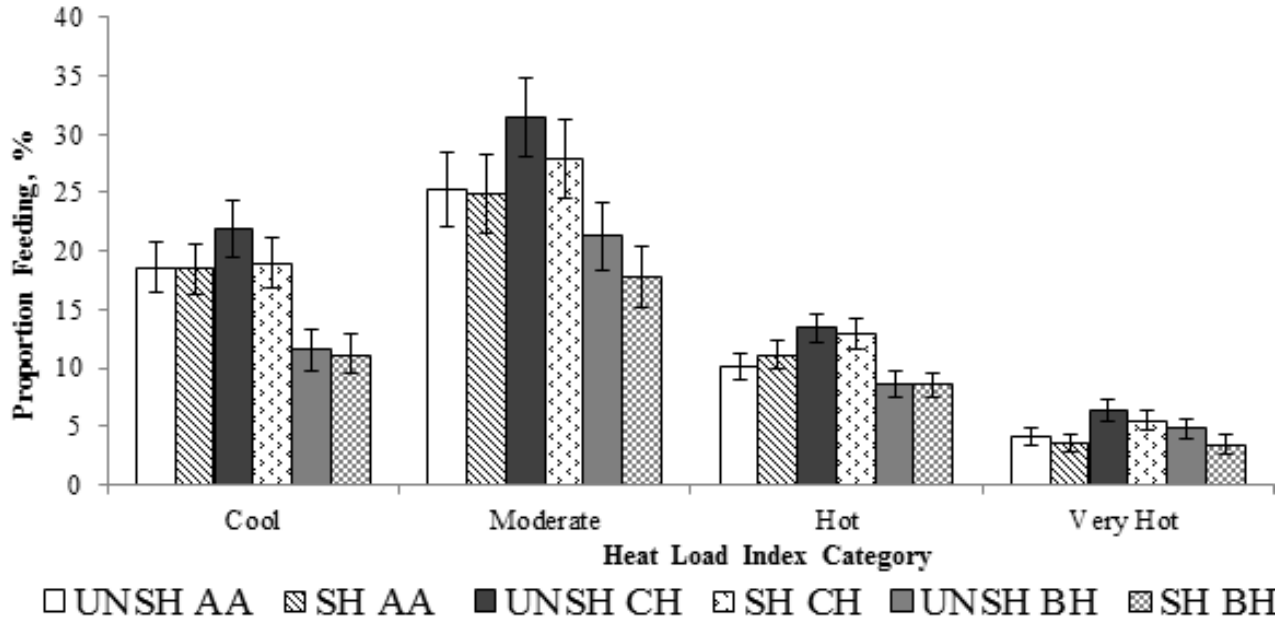


Figure 5.3.2: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers feeding within Heat Load Index (HLI) categories Cool (HLI ≤ 70), Moderate (HLI 70.1 ≤ 77), Hot (HLI 77.1 ≤ 86) and Very Hot (HLI ≥ 86)

5.3.3.2 Drinking Behaviour

During the study (154 d; Figure 5.3.3), drinking events observed were affected by breed ($P < 0.0001$), treatment ($P < 0.0001$), h ($P < 0.0001$), d ($P = 0.0005$), breed × treatment ($P = 0.03$), breed × h ($P < 0.0001$), breed × HLI ($P < 0.0001$), treatment × h ($P = 0.02$), breed × d ($P < 0.0001$). There were no effects of HLI ($P = 0.06$; Figure 5.3.4), treatment × HLI ($P = 0.11$), h × d ($P = 0.27$), breed × treatment × h ($P = 0.63$), breed × treatment × HLI ($P = 0.53$) and breed × h × d ($P = 0.45$). Interactions for drinking observations are presented in Appendix 3 and Appendix 4, for monthly and heat wave data periods respectively.

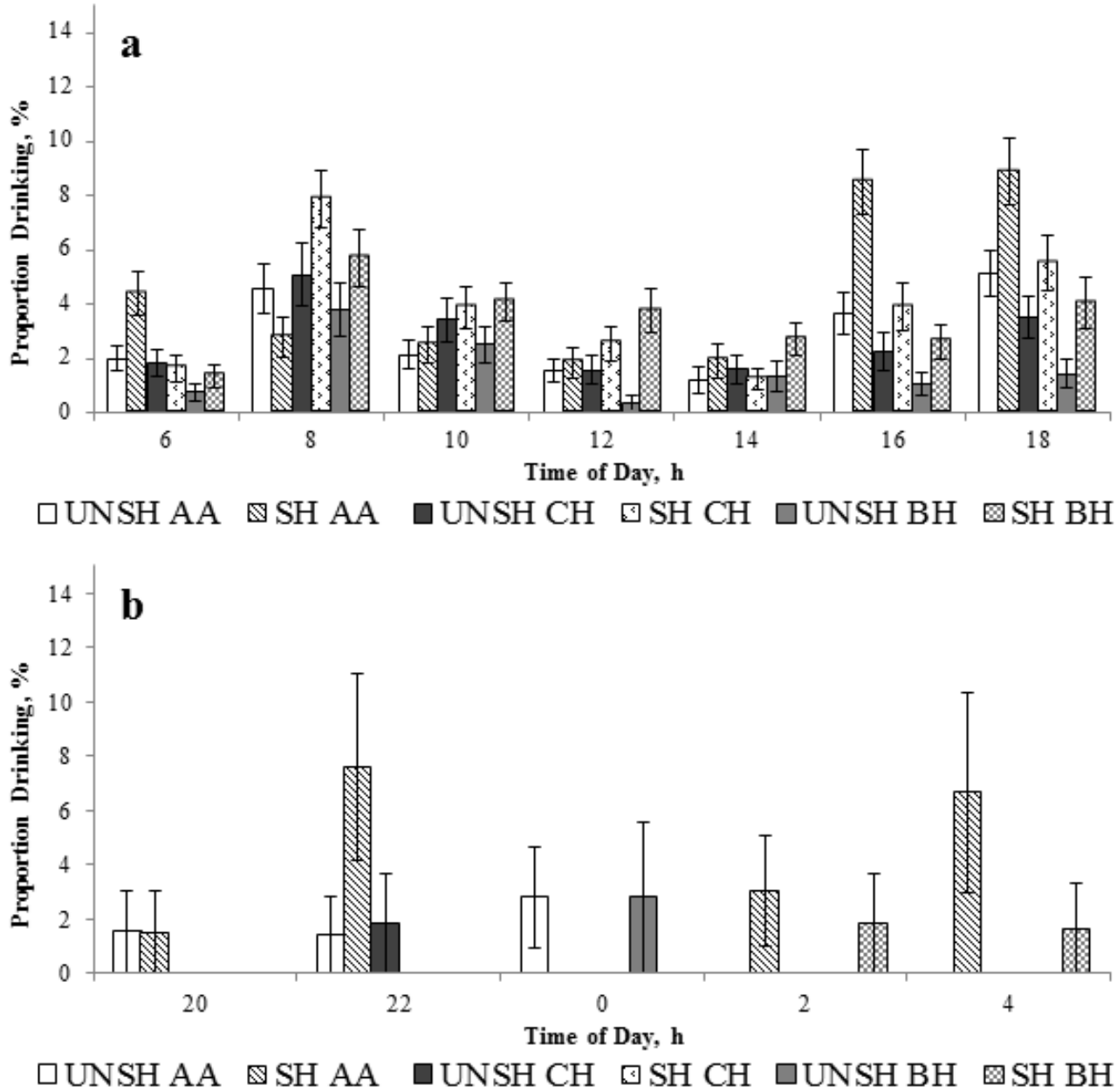


Figure 5.3.3: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers observed drinking during a) daytime (0600 h to 1800 h) and b) night time (2000 h to 0400 h) hours

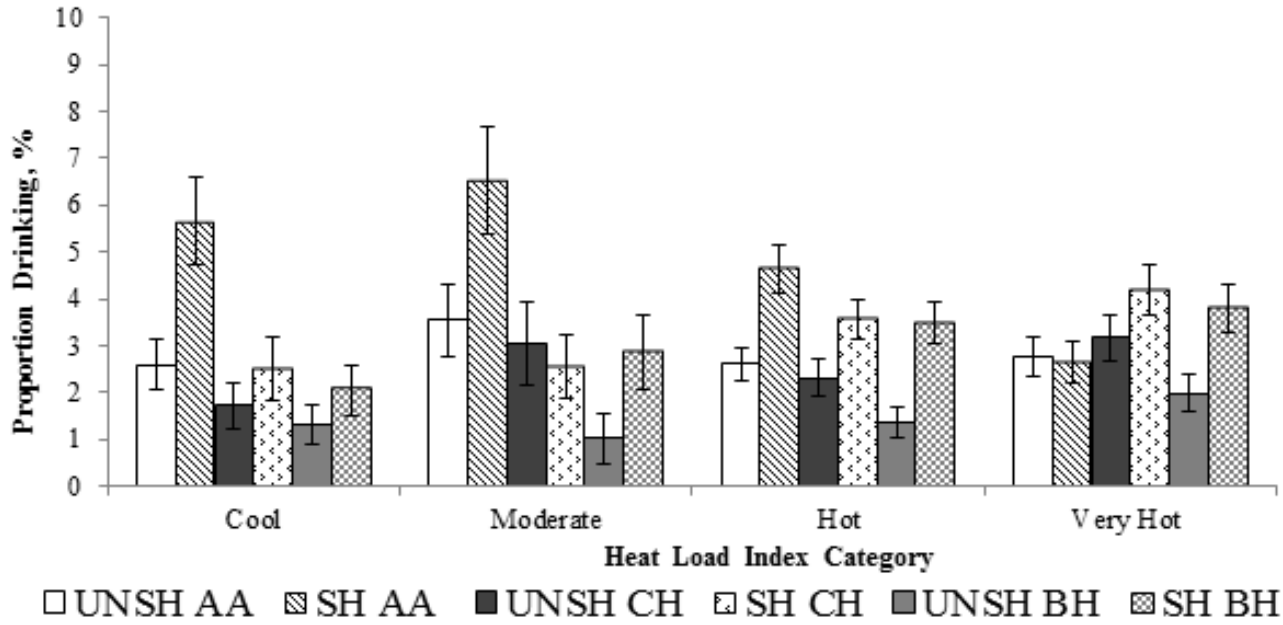


Figure 5.3.4: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers drinking within Heat Load Index (HLI) categories Cool (HLI ≤ 70), Moderate (HLI 70.1 ≤ 77), Hot (HLI 77.1 ≤ 86) and Very Hot (HLI ≥ 86)

5.3.3.3 Rumination Behaviour

During the study (154 d; Figure 5.3.5), rumination was affected by breed ($P < 0.0001$), h ($P < 0.0001$), d ($P < 0.0001$), breed × treatment ($P < 0.0001$), treatment × HLI ($P = 0.02$), breed × d ($P < 0.0001$), h × d ($P < 0.0001$), breed × treatment × HLI ($P = 0.01$). There were no effects of treatment ($P = 0.23$), HLI ($P = 0.97$; Figure 5.3.6), breed × h ($P = 0.12$), breed × HLI ($P = 0.30$), treatment × h ($P = 0.07$), breed × treatment × h ($P = 0.80$), and breed × h × d ($P = 0.48$). Interactions for rumination are presented in Appendix 5 and Appendix 6, for monthly and heat wave data periods respectively.

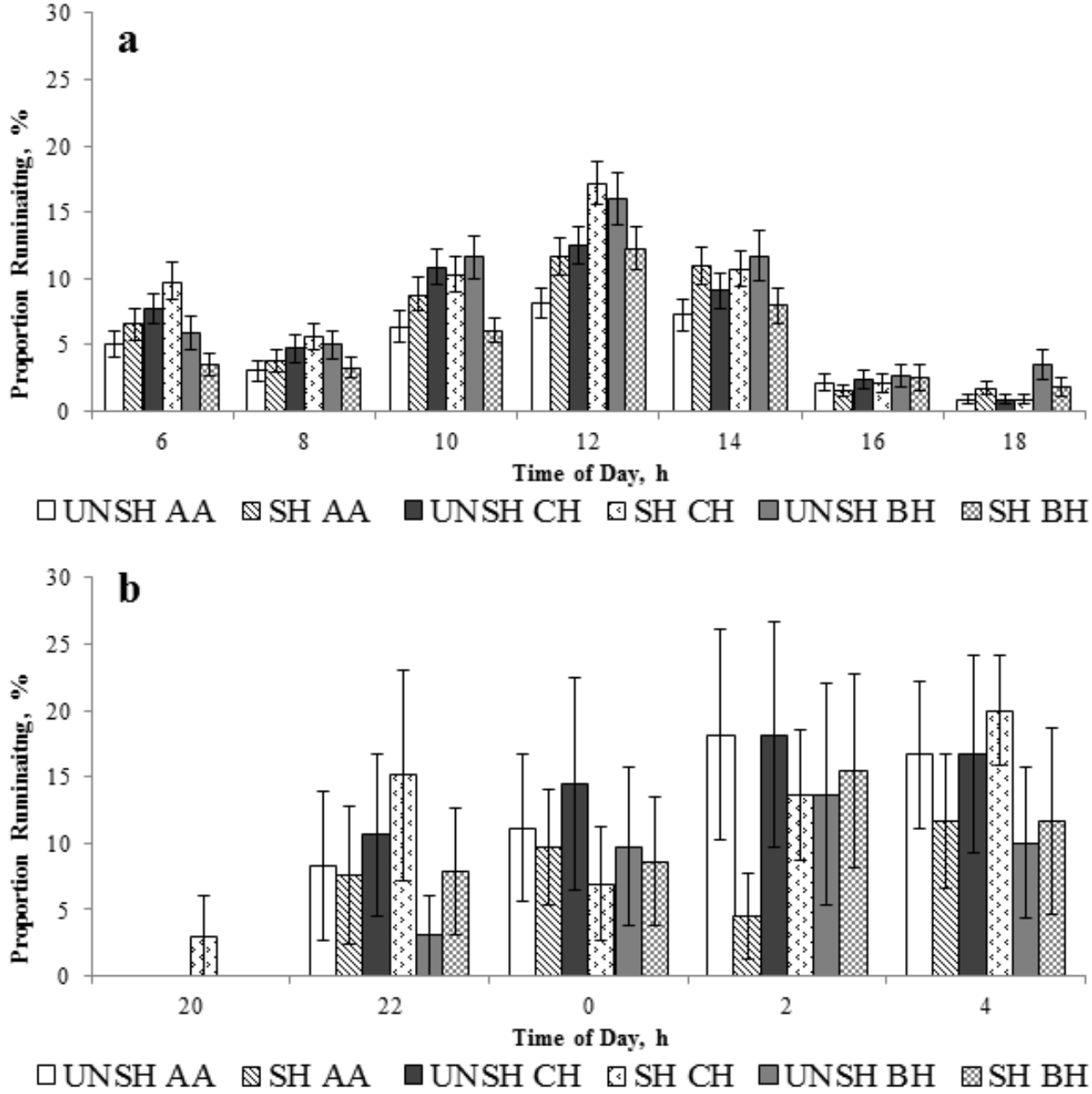


Figure 5.3.5: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers observed ruminating during a) daytime (0600 h to 1800 h) and b) night time (2000 h to 0400 h) hours

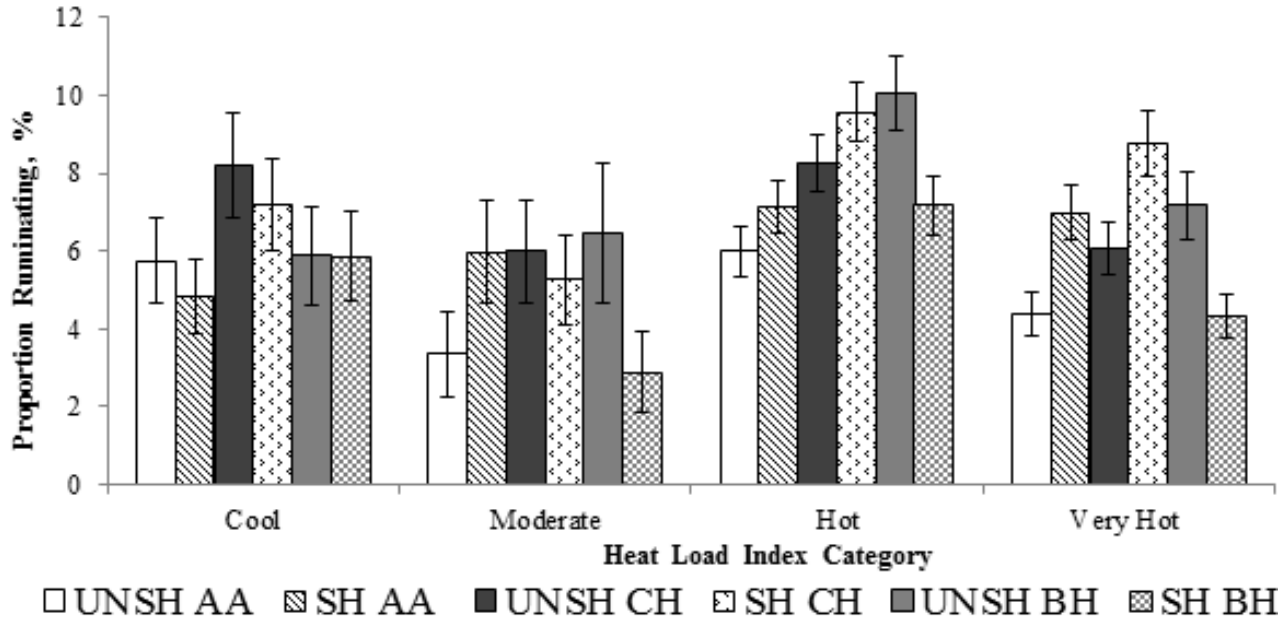


Figure 5.3.6: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers ruminating within Heat Load Index (HLI) categories Cool (HLI ≤ 70), Moderate (HLI 70.1 ≤ 77), Hot (HLI 77.1 ≤ 86) and Very Hot (HLI ≥ 86)

5.3.3.4 Postural Changes

5.3.3.4.1 Standing

During the study (154 d; Figure 5.3.7), proportion of steers standing was affected by breed ($P < 0.0001$), treatment ($P < 0.0001$), h ($P = 0.002$), d ($P < 0.0001$), HLI ($P < 0.0001$; Figure 5.3.8), breed × treatment ($P < 0.0001$), treatment × HLI ($P < 0.0001$), breed × d ($P < 0.0001$), h × d ($P < 0.0001$), breed × HLI ($P < 0.0001$). There were no effects of treatment × h ($P = 0.27$), breed × h ($P = 0.21$), breed × treatment × h ($P = 0.40$), breed × treatment × HLI ($P = 0.08$) or breed × h × d ($P = 0.18$). Interactions for number of steers standing are presented in Appendix 7 and Appendix 8, for monthly and heat wave data periods respectively.

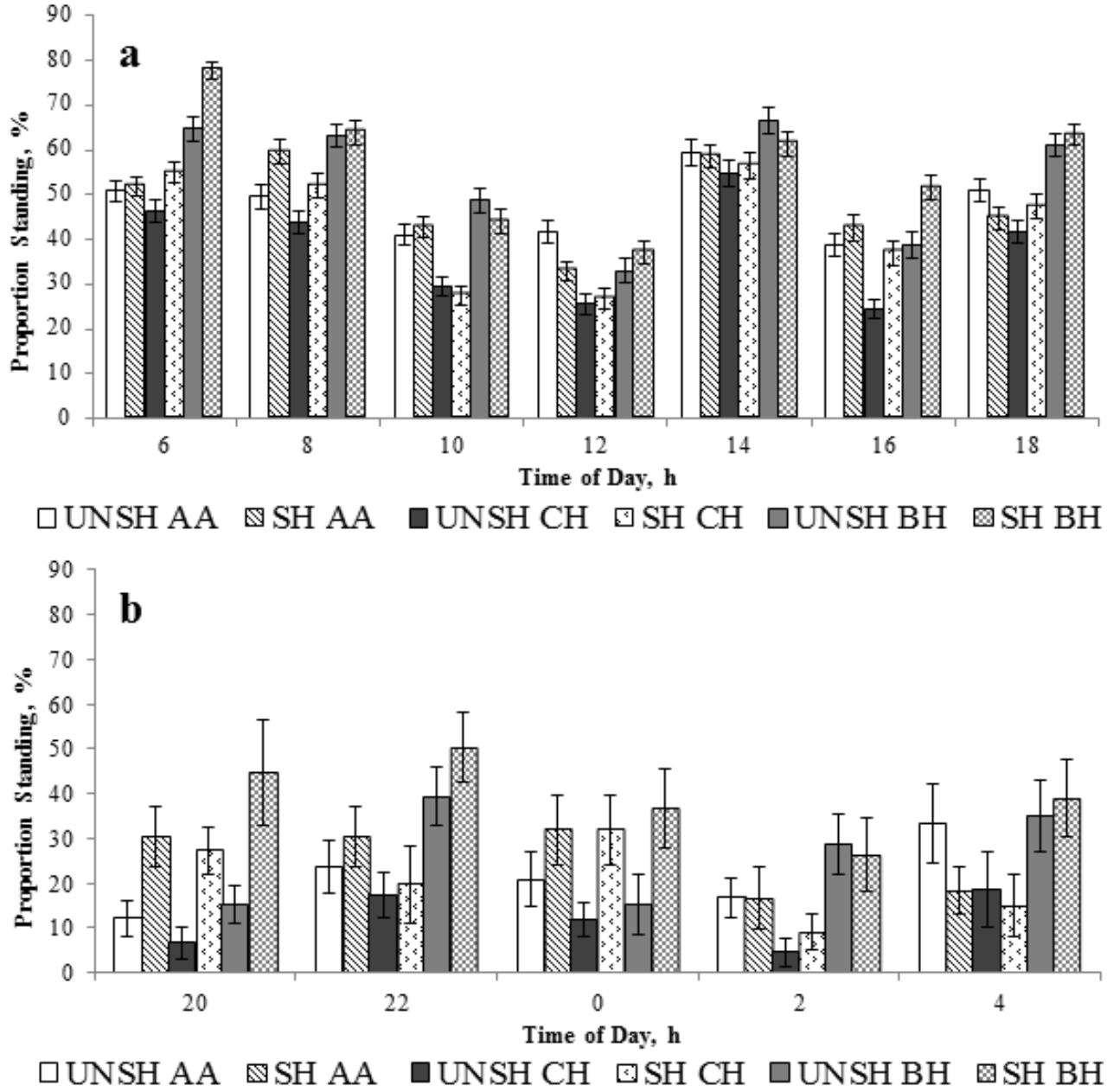


Figure 5.3.7: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers observed standing during a) daytime (0600 h to 1800 h) and b) night time (2000 h to 0400 h) hours

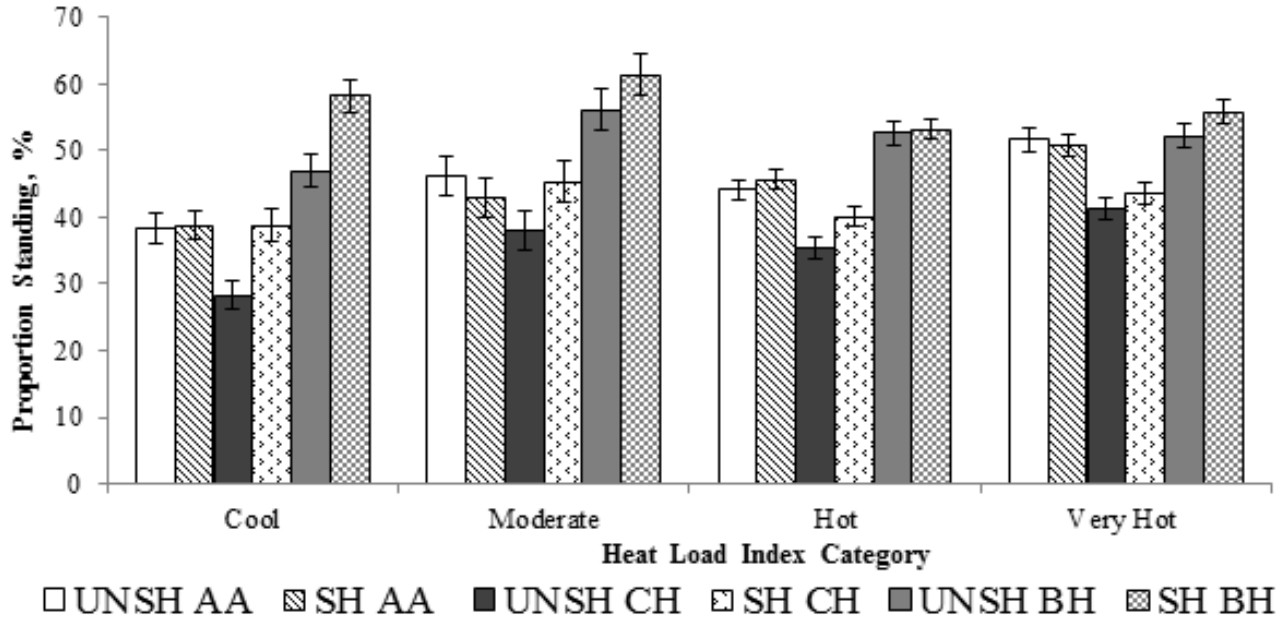


Figure 5.3.8: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers standing within Heat Load Index (HLI) categories Cool (HLI ≤ 70), Moderate (HLI 70.1 ≤ 77), Hot (HLI 77.1 ≤ 86) and Very Hot (HLI ≥ 86)

5.3.3.4.2 Lying

During the study (154 d; Figure 5.3.9), proportion of steers lying was affected by breed ($P < 0.0001$), treatment ($P < 0.0001$), h ($P < 0.0001$), day ($P < 0.0001$), HLI ($P < 0.0001$; Figure 5.3.10), breed × treatment ($P = 0.007$), treatment × HLI ($P < 0.0001$), breed × d ($P < 0.0001$), h × d ($P < 0.0001$), breed × h ($P < 0.0001$) and breed × h × d ($P = 0.007$). There were no effects of treatment × h ($P = 0.06$), breed × HLI ($P = 0.84$), breed × treatment × h ($P = 0.96$), breed × treatment × HLI ($P = 0.60$). Interactions for number of steers lying are presented in Appendix 9 and Appendix 10, for monthly and heat wave data periods respectively.

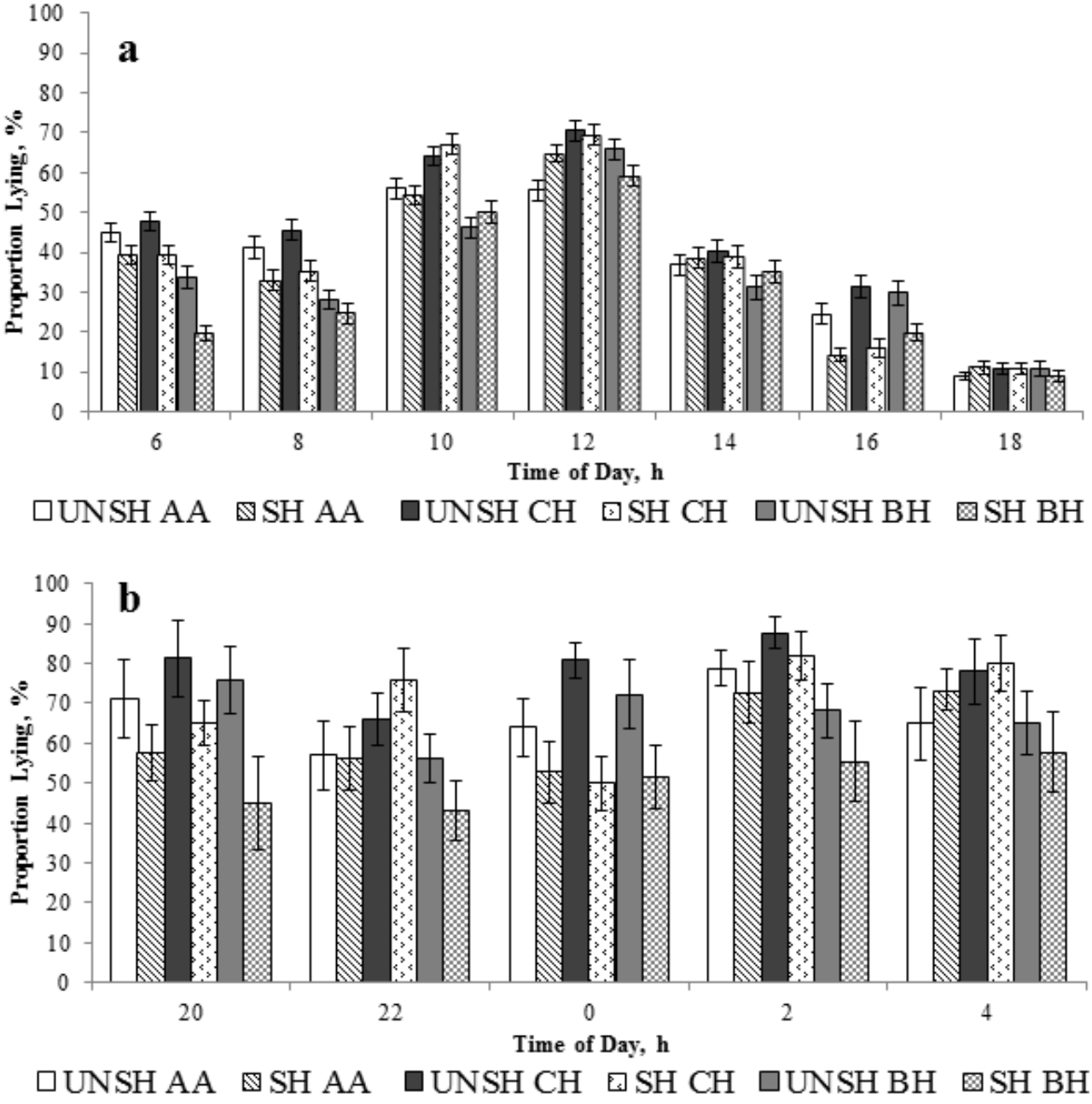


Figure 5.3.9: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers observed lying during a) daytime (0600 h to 1800 h) and b) night time (2000 h to 0400 h) hours

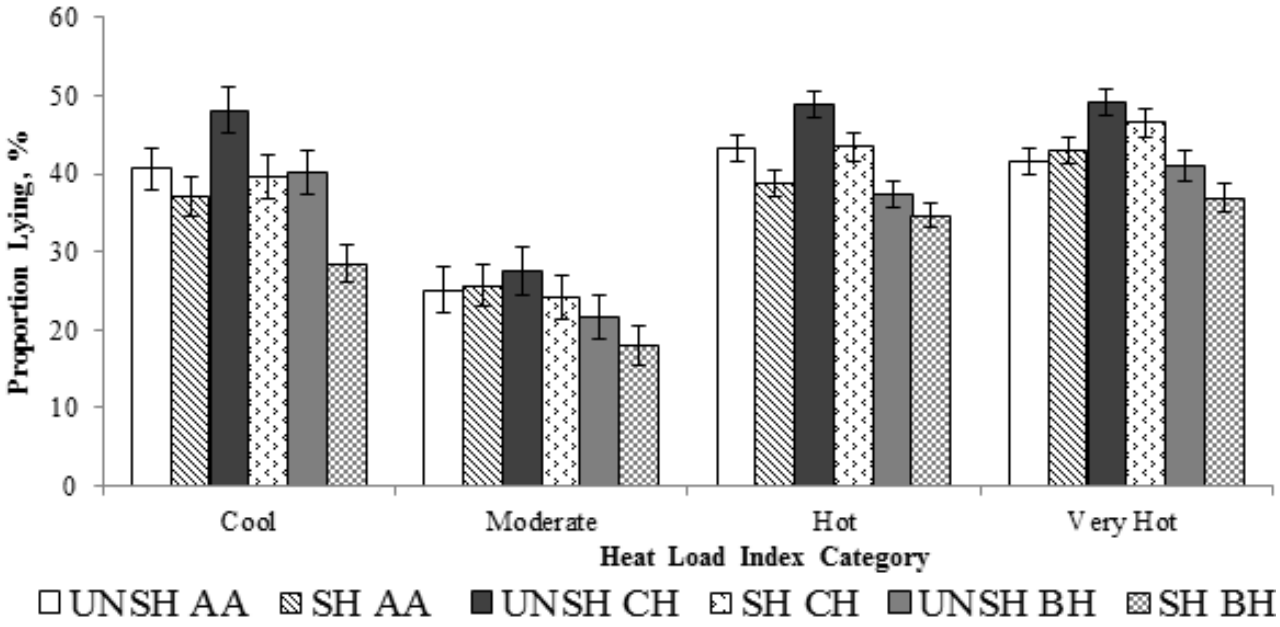


Figure 5.3.10: Proportion of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers lying within Heat Load Index (HLI) categories Cool (HLI ≤ 70), Moderate (HLI 70.1 ≤ 77), Hot (HLI 77.1 ≤ 86) and Very Hot (HLI ≥ 86)

5.3.3.5 Shade Utilisation

During the study (154 d; Figure 5.3.11), shade utilisation was influenced by breed ($P < 0.0001$), h ($P < 0.0001$), HLI ($P < 0.0001$; Figure 5.3.12), breed × h ($P < 0.0001$), breed × HLI ($P < 0.0001$), breed × d ($P < 0.0001$) and h × d ($P < 0.0001$). There were no effects of d ($P = 0.49$) or breed × h × d ($P = 0.83$). Interactions for shade utilisation are presented in Appendix 11 and Appendix 12, for monthly and heat wave data periods respectively.

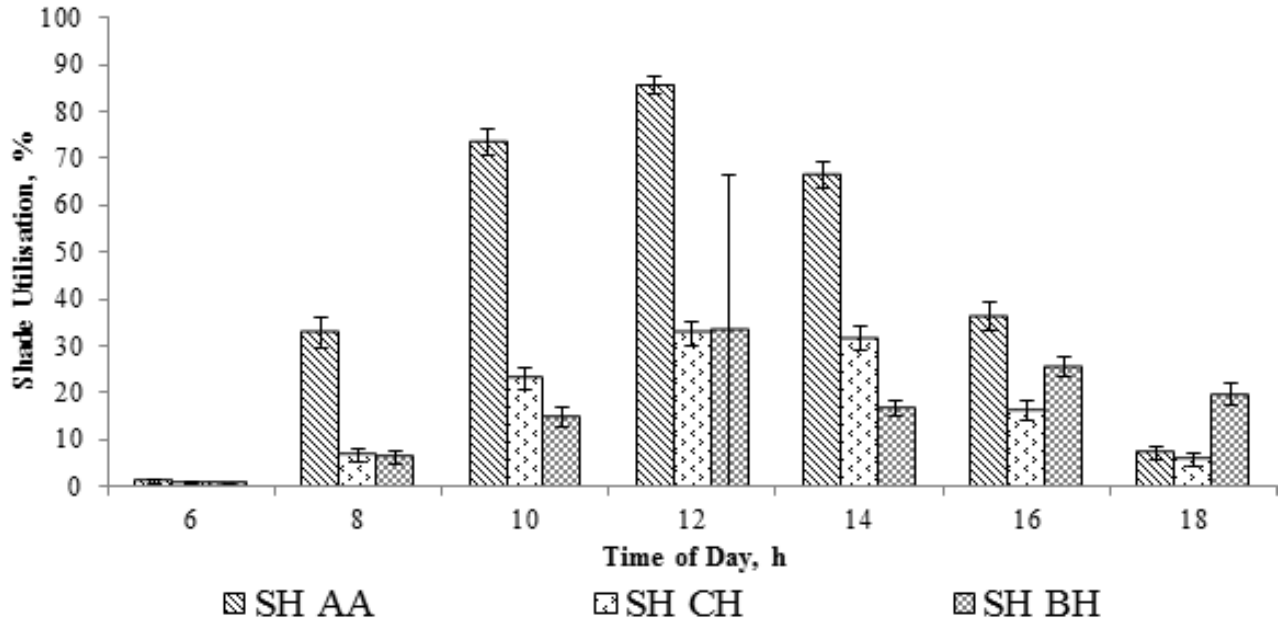


Figure 5.3.11: Shade utilisation of Angus (SH AA), Charolais (SH CH) and Brahman (SH BH) steers during day time (0600 h to 1800 h) hours

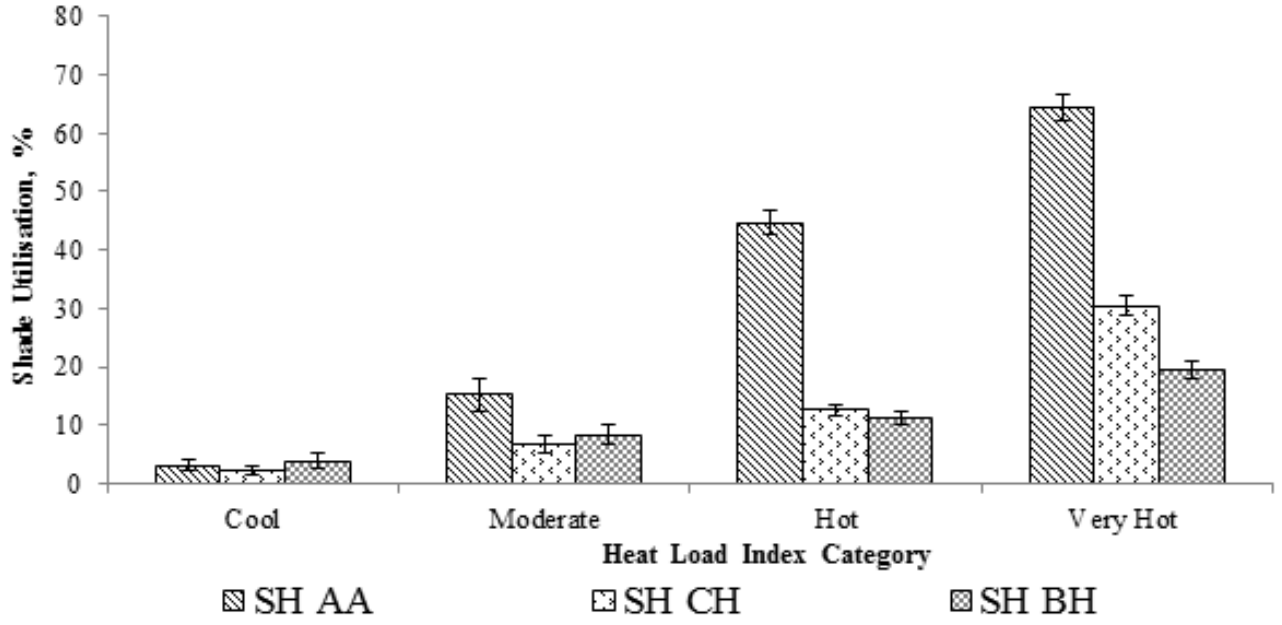


Figure 5.3.12: Proportion Angus (SH AA), Charolais (SH CH) and Brahman (SH BH) steers utilising shade within Heat Load Index (HLI) categories Cool (HLI ≤ 70), Moderate (HLI 70.1 ≤ 77), Hot (HLI 77.1 ≤ 86) and Very Hot (HLI ≥ 86)

Maximum shade utilisation was 85.5 %, 32.7 % and 33.3 % for Angus, Charolais and Brahman steers respectively at 1200 h. Interestingly Brahman steers had a 27.1 % increase in shade utilisation between 0800 h and 1200 h. Additionally the proportion of Brahman steers utilising shade increased from 4.0 % to 19.4 % respectively when HLI increased from cool (HLI ≤ 77) to very hot (HLI ≥ 86). Shade utilisation was similar between Brahman and Charolais steers when HLI was classified as cool (4.0 % versus 2.4 %), moderate (8.4 % versus 6.8 %) and hot (11.2 % versus 12.6 %).

However differences were observed between Brahman and Charolais steers when HLI was classified as very hot (19.4 % versus 30.5 %). Unsurprisingly Angus steers showed the greatest increase (61.3 %) in shade utilisation, however Charolais (28.1 %) and Brahman (15.4 %) steers also showed a significant increase in shade utilisation when HLI increased from cool (HLI ≤ 77) to very hot (HLI ≥ 86).

5.3.3.6 Panting Score

During the study (154 d; Figure 5.3.13), mean panting scores were affected by breed ($P < 0.0001$), treatment ($P < 0.0001$), day ($P < 0.0001$), HLI ($P < 0.0001$; Figure 5.3.14), breed × treatment ($P = 0.003$), breed × h ($P < 0.0001$), breed × HLI ($P < 0.0001$), breed × d ($P < 0.0001$), h × d ($P = 0.02$). There was no effect of h ($P = 0.06$), treatment × HLI ($P = 0.33$), treatment × h ($P = 0.17$), breed × treatment × h ($P = 0.21$), breed × treatment × HLI ($P = 0.37$) and breed × h × d ($P = 0.30$) on mean panting score. Interactions for mean panting score are presented in Appendix 13 and Appendix 14, for monthly and heat wave data periods respectively.

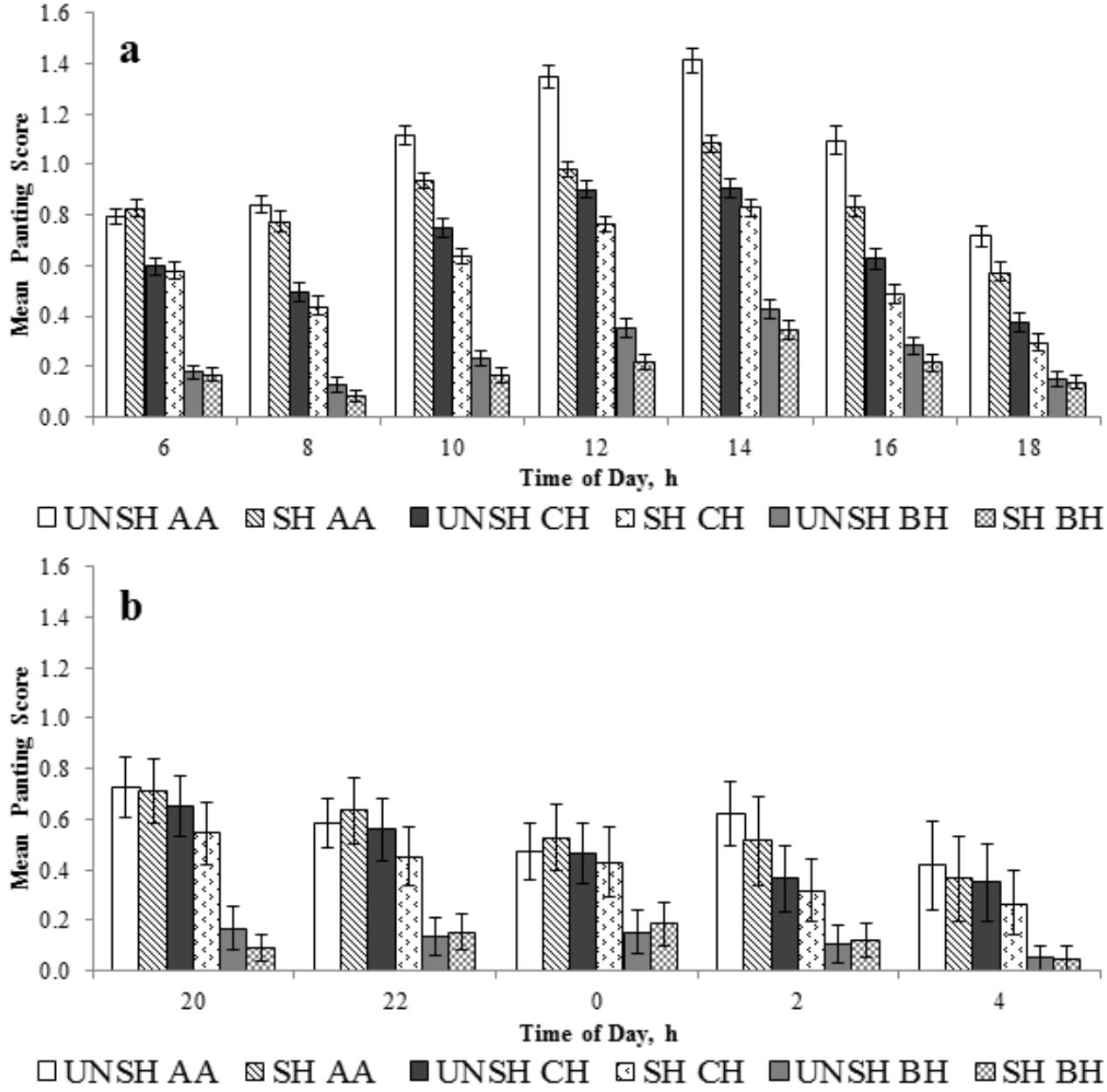


Figure 5.3.13: Mean panting score of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers observed during a) daytime (0600 h to 1800 h) and b) night time (2000 h to 0400 h) hours

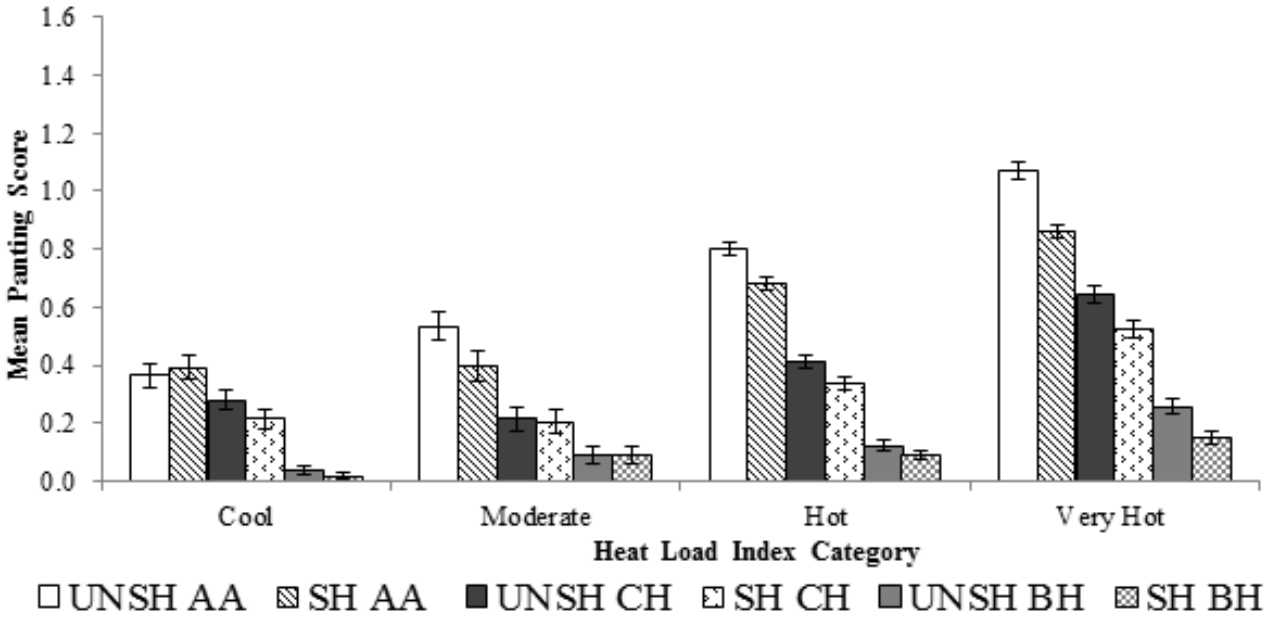


Figure 5.3.14: Mean panting score of shaded (SH) and un-shaded (UNSH) Angus (AA), Charolais (CH) and Brahman (BH) steers within Heat Load Index (HLI) categories Cool (HLI ≤ 70), Moderate (HLI 70.1 ≤ 77), Hot (HLI 77.1 ≤ 86) and Very Hot (HLI ≥ 86)

5.3.4 Discussion

Feedlot cattle are particularly susceptible to heat load, partially due to the nature of the diets they are fed (Blackshaw and Blackshaw, 1994), i.e. high energy concentrate feeds. However the response of cattle to hot climatic conditions is dependent on a number of individual characteristics. There are a number of factors that influence how an animal will respond to heat load conditions, including genotype, coat characteristics, health status, prior disease exposure, and days on feed. Vulnerable animals have been described as those with black or dark coats (and skin); compromised immune systems; greater fat cover (greater body condition score); and animals that are excitable in temperament (Brown-Brandl et al., 2006a). Therefore no two animals will respond to hot climatic conditions in the same manner.

When an animal encounters challenging climatic conditions, i.e. those which are outside the animal's thermoneutral zone, the immediate systemic response is directed towards self-preservation (DeShazer et al., 2009). In response to increasing thermal loads, cattle will initiate purposeful behavioural change to ensure survival. Any imbalance in heat accumulation and dissipation results in a change in core body temperature (Brown-Brandl et al., 2005b), which consequently influences other physiological functions. In response to ambient heat load, quantifiable measures such as sweating rate (Mader et al., 2010a); respiration rate (breaths per minute) (Gaughan et al., 2000); panting score (Mader et al., 2006); voluntary decrease in DMI; decrease the amount of time spent lying; and increased water consumption (Brown-Brandl et al., 2006b), can be used to described the

effect of heat load. As ambient heat load increases, behavioural observations can be used to provide insight regarding the severity of heat load that cattle are experiencing.

5.3.4.1 Feeding Behaviour

Heat load conditions are associated with a reduction in DMI (Beede and Collier, 1986; Ray, 1989; Hahn et al., 1992; Hahn, 1999; Brown-Brandl et al., 2005a). Reducing DMI appears to have a lag effect where animals are adjusting their feed intake based on numerous factors, including ambient conditions and previous feed intake (Brown-Brandl et al., 2005a). However feed intake and feeding behaviours are not considered as a suitable measure of thermal status as these behaviours are intermittent (Brown-Brandl et al., 2005a). As feed intake was not measured individually it was not possible to separate out breed differences for DMI. However Hicks et al. (1989) suggested that feeding pattern in cattle may be highly repeatable, therefore the trends in observed feeding behaviours were investigated.

Within the current experiment the greatest proportions of animals observed feeding were at 1600 h and 1800 h. Although all breed × treatment groups had the highest proportion of animals feeding at 1600 h and 1800 h, results from this experiment suggest that the proportion of Charolais steers feeding at 1600 h and 1800 h was greater when compared to Angus and Brahman steers. Results within this experiment also suggest that all breed × treatment groups continued feeding throughout the night time hours. Ray and Roubicek (1971) reported that there was a peak in feeding activity where a majority of feed consumption occurred in the late afternoon and early evening. Brown-Brandl et al. (2005a) also reported that un-shaded cattle appear to be adapting feed consumption times by shifting feed intake to the cooler hours of the day. Results within the current experiment may be suggestive of these animals consuming small portions of feed at regular intervals. Brown-Brandl et al. (2005a) indicated that cattle compensate for hotter conditions by consuming smaller meals more frequently. The consumption of small frequent meals may be an adaptation to regulate body heat content by regulating metabolic heat production. The reduction in DMI has been associated with a decrease in metabolic heat production, via ruminal fermentation, thus aiding in maintaining the overall heat balance of the animal (Beede and Collier, 1986; Hahn, 1999).

Feeding behaviours were affected by hour ($P < 0.0001$) and HLI ($P < 0.0001$), although it is important to acknowledge that these results are somewhat confounded as cattle were offered feed once daily at approximately 1430 h. Afternoon feeding was deliberately implemented within this experiment, as time of feeding has been identified to effect heat production and heat balance (Brosh et al., 1998). Additionally the feed offered within this experiment was regulated to i) reduce

excessive feed intake during high heat load periods, i.e. reducing metabolic heat load and ii) ensure that cattle were growing to comply with Australian domestic market specifications. Therefore it becomes difficult to define the response of feeding behaviours to hot climatic conditions within the current experiment.

5.3.4.2 Drinking Behaviour

There are numerous factors that influence daily water intake including ambient conditions; diet type; and breed (genotype) (Arias and Mader, 2011). McDowell and Weldy (1967) indicated that daily water intake appears to be primarily driven by DMI, where the level of intake (kg/d) and type of ration, i.e. concentrates versus roughage diets, influences the amount of water consumed. Results from this experiment indicate that observed drinking events were highly variable for all breed × treatment groups, whereby observed drinking behaviours occurred at all hours. However greater proportions ($P < 0.0001$) of shaded Angus were observed drinking at 1600 h and 1800 h. These results also suggest that there appears to be a trend of increased observed drinking events at 0800 h and 1800 h in all breed × treatment groups. Increases in observed drinking events at 1800 h are potentially associated with feeding events. Observed drinking events appeared to be more regular in shaded Angus steers during day time and night time observations. Additionally there may be a reluctance to drink during the hottest hours of the day as the water troughs were located within an un-shaded region of the pens, potentially increasing water temperature. However within the shaded pens the shade footprint would cover the water troughs from approximately 1600 h, potentially decreasing water temperature.

5.3.4.3 Rumination

Rumination appears to be highly variable, although the results from this experiment indicate that cattle spent more time ruminating during night time hours. During day time hours there were greater observations of ruminating at 1000 h, 1200 h and 1400 h in all breed × treatment groups. Generally proportion of steers ruminating were not affected by HLI ($P = 0.97$), however there were month and heat wave periods where ruminating was affected by HLI ($P < 0.01$). These results suggest that rumination was largely dependent on time of day rather than HLI, however where HLI conditions were classified as very hot, HLI ≥ 86, observed rumination in shaded Angus and Charolais steers was greater ($P < 0.05$). Additionally under these conditions un-shaded Brahman steers were more likely to be ruminating ($P < 0.05$). However these results are likely to be confounded by feed intake, i.e. voluntary feed reductions, by each breed × treatment group, and as feed intake was not measured individually it is difficult to draw substantial conclusions from these results. Young and Hall (1993) refer to stage 8 in the diagnosis of the degree of heat load an animal is exposed to as a

reduction, or a complete termination, of rumination. However it is important to consider that rumination is a necessary component of digestion in ruminants. Therefore a complete termination of rumination seems unlikely, unless illness/excessive heat load is likely to become fatal. Beede and Collier (1986) indicated that a confounding factor in voluntary DMI reductions was reduced gut motility and rumination. Therefore a reduction in rumination during periods of hot weather is potentially directly related to the amount of feed consumed, rather than high heat load.

5.3.4.4 Postural Changes

Feedlot and dairy cattle will increase their proportion of time spent standing during periods of hot weather (Shultz, 1984; Igono et al., 1987; Frazzi et al., 2000; Brown-Brandl et al., 2006a; Gaughan et al., 2008a). An increase in the proportion of time spent standing can be considered as a sign of discomfort, in both dairy and feedlot cattle, during hot weather conditions (Young and Hall, 1993; Dikmen et al., 2012). Standing and lying postures were highly variable for each breed × treatment group. However all breed × treatment groups were more likely to be observed standing during day time hours, and lying during night time hours ($P < 0.05$). The proportion of un-shaded and shaded Angus steers observed standing increased 13.4 % and 12 % respectively where HLI was classified as cool (HLI $\leq$ 77) versus very hot (HLI $\geq$ 86). Similarly an increase in the proportion of un-shaded and shaded Charolais steers was observed, whereby the proportion standing increased 12.9 % and 4.8 % respectively. Unexpectedly there was a 5.3 % increase in the proportion of un-shaded Brahman steers standing. An increase in the proportion of time spent standing during heat load conditions may be representative of the animals' inability to dissipate body heat via evaporation. When pen surface temperature exceeds that of skin temperature, the animal will accumulate heat from the ground, thus increasing the overall accumulated heat load that the animal must dissipate (Mader et al., 2002). By standing, the animal is exposing greater body surface area in an attempt to increase the proportion of heat dissipation through i) evaporative exchanges via the coat surface; and ii) convective mechanisms via air movement around the body.

5.3.4.5 Shade Utilisation

During hot conditions cattle will seek shade where available (Blackshaw and Blackshaw, 1994). Shade seeking behaviour can be considered as a thermoregulatory mechanism whereby the shaded areas provide a change in microclimate, therefore assisting in the regulation of core body temperature (Bennett et al., 1985). Whilst shade reduces the impact of solar load, it does not completely remove exposure to heat load conditions. However the provision of shade structures for feedlot cattle remains beneficial as the animals are able to utilise shaded regions voluntarily. As

expected the proportion of Angus steers utilising shaded areas was greater ($P < 0.05$) than Charolais and Brahman steers between 0800 h and 1600 h.

Although shade utilisation was only calculated on animals within the shaded treatment, steers within the un-shaded pens were observed expressing shade seeking behaviours from the shade footprint of other animals and from structures around the pen, i.e. fence lines, feed bunks and water troughs. This is supported by the findings Mitlöhner et al. (2001b) and Castaneda et al. (2004) indicated that un-shaded cattle seek shade by placing their heads in the shade footprint of feed bunks during the hot hours of the day. Additionally Gaughan and Mader (2014) recorded observations of un-shaded cattle utilising the shade footprint of other animals, water troughs and fence posts. These findings suggest that it is impossible to completely remove access from shade footprints in feedlot pens. These findings highlight the strong expression of shade seeking behaviours, further indicating the importance of providing shade structures to feedlot cattle.

5.3.4.6 Panting Score

As ambient conditions change, changes to panting score provide a good indication of the changing thermal status of the animal (Mader et al., 2006). Panting score can be used to determine an individual's heat load status (Gaughan et al., 2010b), or a group of animals by using a mean panting score for the group of animals (Brown-Brandl et al., 2006b; Gaughan et al., 2008b).

Mean panting score showed a marked increase in all breed × treatment groups during day time hours, i.e. between 0600h to 1400 h, then declined overnight between 2000 h to 0400 h. Maximum increase in mean panting score was observed in un-shaded Angus steers from 0.80 ± 0.03 (low stress) at 0600 h to 1.41 ± 0.05 (severe stress) at 1400 h. Highest mean panting score at 0600 h was observed in shaded Angus (0.83 ± 0.03; moderate stress). Unsurprisingly the lowest mean panting score at 0600 h was observed in the shaded Brahman steers (0.17 ± 0.03; no stress). Maximum mean panting score of 3.5 (severe stress) was observed in un-shaded Angus at 1200 h on d 74 during heat wave event 2. At this time excessive heat load responses, i.e. panting scores 4 and 4.5 combined with head wetting behaviours (Figure 5.1.3), were observed in the un-shaded Angus steers. These symptoms of excessive heat load resulted in the un-shaded treatment pens being relocated to shaded pens until heat wave conditions abated (HLI ≤ 60; $AHL_{86} = 0$). Interestingly un-shaded and shaded Brahman steers had a lower ($P > 0.05$) mean panting score at 0800 h (0.13 ± 0.03; 0.08 ± 0.03) than at 0600 h (0.18 ± 0.03; 0.17 ± 0.03).

Mean panting score also increased when HLI increased from cool (HLI ≤ 77) to very hot (HLI ≥ 86). Where HLI conditions were very hot (HLI ≥ 86) the mean panting score of all breed × treatment groups differed ($P < 0.05$), whereby shaded cattle had lower mean panting scores compared with their un-shaded counterparts. An increase in mean panting score in conjunction with HLI category, irrespective of shade availability or breed, has been reported (Gaughan et al., 2010b; Sullivan et al., 2011). Interestingly where HLI conditions were very hot (HLI ≥ 86) the mean panting score of shaded (0.15 ± 0.02) and un-shaded (0.26 ± 0.03) Brahman steers differed ($P < 0.05$). Although the mean panting scores indicate that these animals were under no stress, these findings highlight that Brahman cattle will utilise shade to support thermoregulation. Gaughan et al. (2010b) reported that 100 % *Bos indicus* cattle also showed a high within breed variability in panting score response to very hot HLI (≥ 86.1) conditions. The findings from Gaughan et al. (2010b) and the current study suggest that there are also within breed variations in heat tolerance of 100 % *Bos indicus* breed types.

5.3.5 Conclusion

As ambient heat load conditions increase, animal observations can provide some insight into the severity of heat load the animal is experiencing. Observations of cattle behaviour can be used to quantify animal responses to heat load (Mitlöhner et al., 2001a). Results from this experiment suggest that HLI category is a useful predictor of the heat load status of different breed × treatment groups. The advantage of the HLI model is that it is able to be modified to reflect the different management style of feedlots. As HLI category increased from cool (HLI ≤ 77) to very hot (HLI ≥ 86) there was an increase in shade utilisation and mean panting score, indicating that HLI category can be used as a predictor of thermal comfort. Angus steers showed the greatest increase in shade utilisation and mean panting score. Interestingly Brahman steers exhibited a notable increase in mean panting score and shade utilisation as HLI category increased, suggesting that *Bos indicus* cattle will use shade to support comfort during hot conditions. However, the impact that shade utilisation has on thermoregulation in Brahman cattle still remains unclear.

5.4 Haematological Responses of Feedlot Cattle to Heat Load

Experimental Hypothesis: Breed and shade availability will influence haematological responses to heat load.

5.4.1 Introduction

An animal's body is designed to ensure survival, particularly when exposed to stressors. The impact of hot climatic conditions, as a stressor, can be difficult to quantify. However is has been well reported the impact of hot climatic conditions have on the welfare and performance of feedlot cattle (Bushby and Loy, 1997; Hahn and Mader, 1997; Hahn, 1999; Entwistle et al., 2000; Brown-Brandl et al., 2006a; Brown-Brandl et al., 2006b; Gaughan et al., 2008b; Gaughan et al., 2010a). Cattle are able to adjust behaviourally, physiologically and immunologically during hot climatic conditions to minimise the adverse effects of thermal stress (Hahn, 1999; Gaughan et al., 2008b). During hot conditions cattle will decrease dry matter intake (**DMI**); decrease the amount of time spent lying, therefore increase the amount of time standing, and increase water consumption (Brown-Brandl et al., 2006b).

Periods of heat load are also associated with an increase in core body temperature, potentially compromising cellular function and resulting in physiological changes (Hansen, 2004). The relationship between the body's response to stressors and immune function is exceptionally complex and dynamic (Carroll and Burdick Sanchez, 2014). However it is not uncommon for feedlot cattle to be exposed to a number of non-climatic stressors concurrently with climatic stressors, including variations in nutrition and housing management, which over a period of time potentially lead to chronic stress (Gaughan et al., 2013). Therefore defining the impact of a singular stressor on feedlot cattle becomes difficult.

Prolonged exposure to heat load has the potential to result in a reduction in the immune cell reactivity, potentially increasing the animal's susceptibility to disease (Lacetera et al., 2006), therefore influencing animal welfare and performance. Developing an understanding of the influence that heat load has on haematological responses of feedlot cattle is critical in the development of alleviation strategies to improve cattle performance and overall wellbeing during the summer months. Therefore haematological markers in the blood can be indicative of the degree of stress an animal is experiencing (Collier et al., 2008). Additionally it is well known that *Bos indicus* and *Bos taurus* cattle respond differently to heat load (Hansen, 2004); therefore the objective of this section of the experiment was *to determine if differences exist in the*

haematological responses of Bos indicus and Bos taurus breeds with and without access to shade during the summer months.

5.4.2 Materials and Methods

5.4.2.1 Creatine Kinase

Creatine Kinase (IU/L) concentrations were determined using an enzyme-linked immunosorbent assay (**ELISA;** Bioo Scientific Corp., USA). Plasma samples and reagent solution were bought to room temperature (24 °C) and vortexed prior to use. The standard curve was prepared using a serial dilution with creatine kinase concentration ranging from 25 IU/L to 800 IU/L. A 5 µl sample of standard solutions and plasma samples were added, in duplicate, into a 96 well blank microplate. Following this 250 µl of reagent was added to each microwell. The microplate was then incubated for 5 minutes, in a dark area. Absorbance measures were read at the end of the incubation period at a wavelength of 340 nm (Teacan, Sunrise, microplate reader, Tecan Group Ltd, Mannedorf, Switzerland).

5.4.2.2 Cytokine Interleukin-6

Cytokine interleukin 6 (pg/mL) concentrations were determined using a bovine cytokine interleukin 6 ELISA (Thermo Scientific™, Thermo Fisher Scientific, USA). Anti-bovine cytokine interleukin 6 coating antibody was diluted 1:100 with a carbonate-bicarbonate buffer (0.2M sodium-bicarbonate buffer, pH 9.4). A 96 well, blank microplate was coated with 100 µl of 1:100 diluted coating antibody and incubated for 16 hours on a microplate shaker at 300 rpm at room temperature (24 °C). All samples and solutions were brought to room temperature prior to use. Plasma samples were diluted in reagent diluent (4 % bovine serum albumin (**BSA**), 5 % sucrose in Dulbecco's phosphate buffer (**D-PBS**; Thermo Scientific™, Thermo Fisher Scientific, USA), pH 7.4) at 1:8 or 1:10. After the 16 hour incubation period, the coating antibody was aspirated from the microplate and 300 µl of blocking buffer (4 % BSA, 5 % sucrose in D-PBS), was added to each well and incubated for 1 hour on a microplate shaker at 300 rpm at room temperature. After the incubation period aspirate the blocking buffer and a 100 µl sample of standard solutions and plasma and standard samples, in duplicate, were added and incubated for 90 minutes on a microplate shaker at 750 rpm. Standard solutions were prepared using a serial dilution with cytokine interleukin 6 concentration ranging between 39 pg/mL to 5000 pg/mL. Prior to the completion of the incubation period a detection antibody solution was prepared, diluted 1:100 with reagent diluent. At the completion of the incubation period samples were aspirated and washed with 900 µl of wash buffer (0.05 % tween™20 in D-PBS, pH 7.4). 100 µl of diluted detection antibody was added to each well and incubated for 1 hour on a microplate shaker at 750 rpm. At the completion of the incubation period

the detection antibody was aspirated and the microplate was washed with 900 µl of wash buffer. Then 100 µl of 1:400 diluted Steptavidin-HRP:reagent diluent was added into each well and incubated for 30 minutes on a microplate shaker at 750 rpm. At the end of the incubation period the Steptavidin-HRP solution was aspirated and the wells were washed with 900 µl of wash buffer. 100 µl of substrate solution was added to each well and incubated, in the dark, for 30 minutes. At the completion of the incubation period 100 µl of stop solution (0.16M sulphuric acid) was added to each well. Absorbance measures were read at the end of the incubation period at a wavelength of 450 nm minus 550 nm (Teacan, Sunrise, microplate reader, Tecan Group Ltd, Switzerland).

5.4.2.3 Electrolytes, Lipids and Glucose

Bicarbonate (mmol/L); chloride (mmol/L); potassium (mmol/L); sodium (mmol/L); cholesterol (mmol/L); triglyceride (mmol/L); and glucose (mmol/L) concentrations were determined using an Olympus analyser (Olympus™ AU400® Clinical Chemistry Auto-Analyser, Olympus Life and Material Science Europa, Clare, Ireland). All substrates and reagents were brought to room temperature (24 °C) prior to use. All analyses and concentrations were determined according to the manufacturer's specifications.

5.4.2.4 Haptoglobin

Haptoglobin (mg/mL) concentrations were determined using an ELISA (Tridelta Development Ltd, Ireland). Plasma, reagent (reagent 1, stabilised haemoglobin; reagent 2, chromogen reagent) and diluent (phosphate buffered saline) were brought to room temperature (24 °C) and vortexed prior to use. The standard curve was prepared using a serial dilution with haptoglobin concentration ranging from 0.156 mg/mL to 2.5 mg/mL. A 7.5 µl sample of standard solutions and plasma samples were added, in duplicate, into a 96 well blank microplate. Following this 100 µl of reagent 1 was added to each microwell, then placed on a microplate shaker for 1 minute at 650 rpm. After the incubation period, 140 µl of reagent 2 was added to each microwell. The microplate was then incubated for 5 minutes at room temperature. Absorbance measures were read at the end of the incubation period at a wavelength of 630 nm (Teacan, Sunrise, microplate reader, Tecan Group Ltd, Switzerland).

5.4.2.5 Heat Shock Protein 70

Heat Shock Protein 70 (**HSP_{70}**) optical densities were determined using a pre-coated with an antibody specific HSP_{70} ELISA (EIAab Science Co. Ltd.; Optics Valley, Wuhan, China). All substrates and reagents were brought to room temperature (24 °C) prior to use. Standard solutions were prepared using a serial dilution with insulin concentrations ranging between 10 ng/mL and 0.156 ng/mL. Plasma samples were diluted 1:4 sample diluent. A 100 µl sample of standard

solutions and diluted plasma samples were pipetted, in duplicate, into the pre-coated 96 well microplate, and incubated for 2 hours at 37 °C. After the incubation period standard 100 µl of detection reagent A was added to each well, and incubated for 1 hour at 37 °C. At the completion of the incubation period the detection reagent A was aspirated and the microplate was washed with 1200 µl of wash buffer. After the microplate had been washed, 100 µl of detection reagent B was pipetted into each well and incubated at 37 °C for 1 hour. At the completion of the incubation period detection reagent B was aspirated and the microplate was washed with 1200 µl of wash buffer. 90 µl of substrate solution was added to each microwell and the microplate was incubated in the dark at 37 °C for 30 minutes. A 50 µl sample of stop solution was added into each well and the optical density was measured at a wavelength of 450 nm.

5.4.2.6 Insulin

Insulin (µIU/mL) concentrations were determined using a solid phase radioimmunoassay (**RIA**; Coat-A-Count® RIA Kit., Siemens, Dublin, Ireland). All substrates were brought to room temperature (24 °C) prior to use. Standard solutions were prepared using a serial dilution with insulin concentrations ranging between 25 µIU/mL and 800 µIU/mL. A 200 µl sample of standard solutions and plasma samples were added, in duplicate, to antibody coated tubes, and then 1 mL radiolabeled insulin (^{125}I) was added to each tube and all tubes vortex. Samples were then incubated at room temperature for 18 hours. Samples were then aspirated until thoroughly dried. Concentrations were determined by counting the bound radiolabeled insulin in a gammer counter (2470 WIZARD^2 automatic gamma-counter, PerkinElmer, Massachusetts, USA).

5.4.2.7 Statistical Analysis

Individual haematoliogcal parameters from samples collected on d 8, d 9, d 36, d 37, d 64, d 65, d 99, d 100, d 127 and d 128 were pooled by breed × treatment to determine a mean concentration for each sample period, i.e. concentrations from d 8 and 9, period 1; 36 and 37, period 2; 64 and 65, period 3; 99 and 100, period 4; and 127 and 128, period 5; were pooled. Plasma haematoliogcal concentrations were also pooled by breed × treatment for each of the heat wave events 1 (d 29, d 32 and d 36), 2 (d 71, d 74 and d 78), 3 (d 120, d 123 and d 127). Insulin and IL-6 were transformed into Log_{10}, to account for the large variability in plasma concentrations, whereby the data did not meet the assumptions of the analysis of variance proceedures.

Period (1 to 5) and heat wave events (1 to 3) haematoliogcal parameters were analysed using a repeated measures model (PROC MIXED; SAS® Inst. Inc. Cary, NC, version 9.3). The model included fixed effects breed, treatment, period, treatment × breed, treatment × period, breed ×

period and treatment × breed × period. Pen nested within treatment and treatment × breed × animal ID nested within pen as random effects within the model.

Pearson's correlaiton coefficients were determined for each haematological parameter and their association with i) HLI and accumulated heat load at 12, 24, 36 and 48 hours prior to blood sample collection; ii) minimum, mean and maximum HLI for the day prior to blood sample collection; iii) minimum, mean and maximum T_A for the day prior to blood sample collection; iv) minimum, mean and maximum BGT for the day prior to blood sample collection; and v) rumen temperature at the time of blood sample collection; for pooled, breed and breed × treatment groups by period and heat waves. Additonally Pearson's correlaiton coefficient was determined for rectal temperature for pooled, breed and breed × treatment groups by period. Although there were some significant Pearson's correlaiton coefficients, they tended to be weak to moderate. Additionally there were no consistent trends in significant Pearson's correlaiton coefficients; therefore these data are not presented here.

5.4.3 Results

5.4.3.1 Creatine Kinase

Plasma creatine kinase concentrations were not affected by breed ($P = 0.49$), treatment ($P = 0.11$), treatment × breed ($P = 0.76$), treatment × period ($P = 0.83$), breed × period ($P = 0.88$) or treatment × breed × period ($P = 0.74$; Figure 5.4.1); however there was an effect of period ($P < 0.0001$; Figure 5.4.2). Additionally the shaded treatment had a higher pooled plasma creatine kinase concentration compared with the un-shaded treatment groups (194.1 ± 14.0 IU/L *v* 234.7 ± 14.0 IU/L) during period 1.

During the heat wave events, plasma creatine kinase concentrations were not affected by treatment ($P = 0.95$), breed ($P = 0.16$), treatment × breed ($P = 0.48$), treatment × heat wave ($P = 0.68$), breed × heat wave ($P = 0.76$) or treatment × breed × heat wave ($P = 0.46$); however there were effects of heat wave ($P = 0.001$). Heat wave 3 had a pooled mean plasma creatine kinase concentration greater (153.6 ± 10.0 IU/L) than heat waves 1 (112.1 ± 10.0 IU/L) and 2 (115.8 ± 10.0 IU/L). There were also effects of sample within heat wave ($P = 0.0005$), where pooled mean plasma creatine kinase concentration were greater in samples collected on d 3 during heat waves 1 (d 29; 158.6 ± 14.6 IU/L) and 3 (d 120; 177.8 ± 14.6 IU/L; Figure 5.4.2). There were some individual samples where plasma concentrations were above the reference range for bovines, particularly in period 1 where 5 steers had creatine kinase concentrations between 293.5 IU/L (shaded Angus) and 394.0 IU/L (shaded Brahman). No trends were observed in these individuals for an increased creatine kinase

concentration during period or heat wave sampling time points. The inter and intra assay coefficients of variation were 24.6 % and 6.3 % respectively.

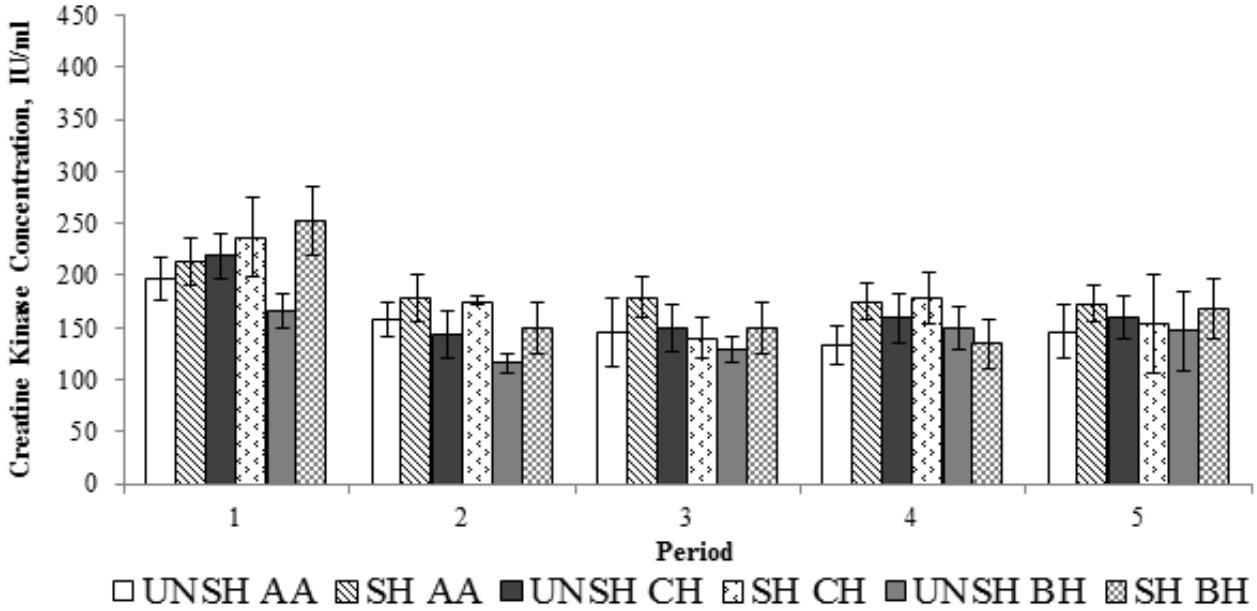
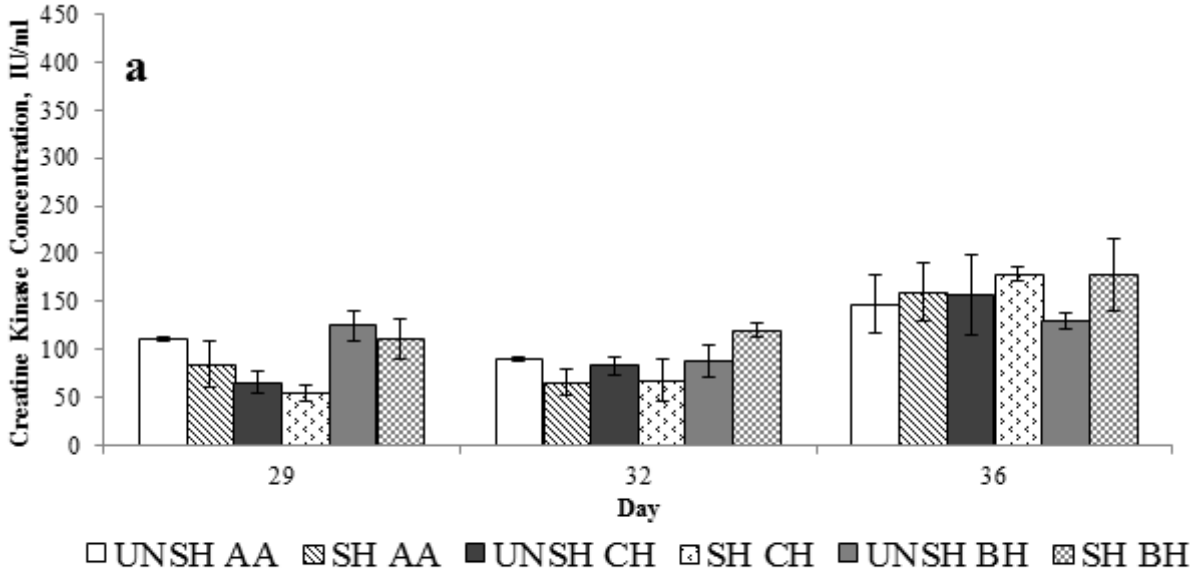
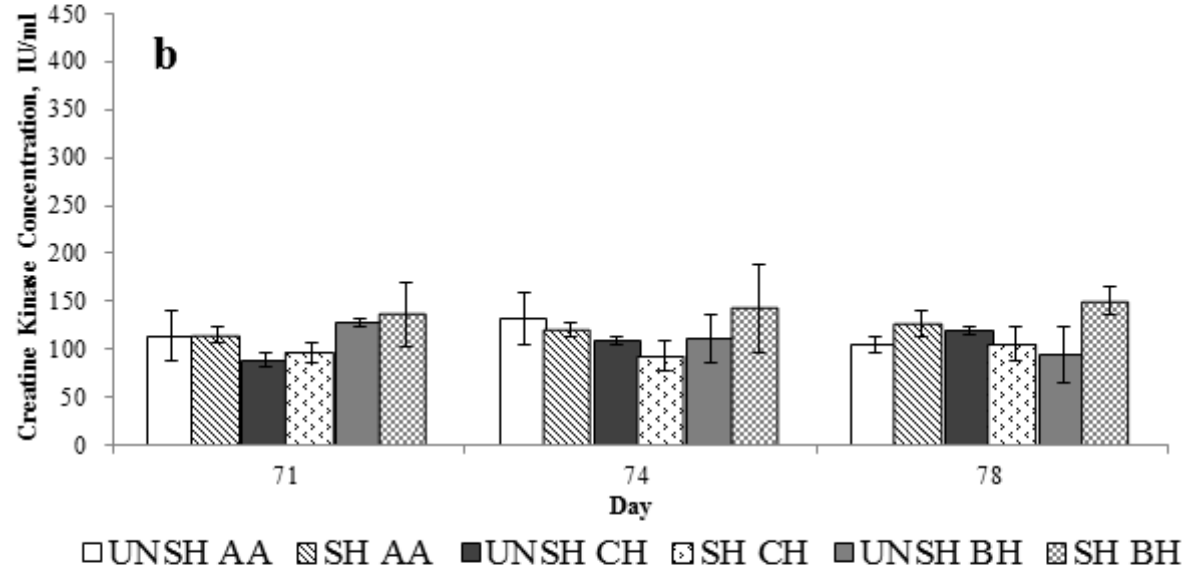


Figure 5.4.1: Plasma creatine kinase (IU/L) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers





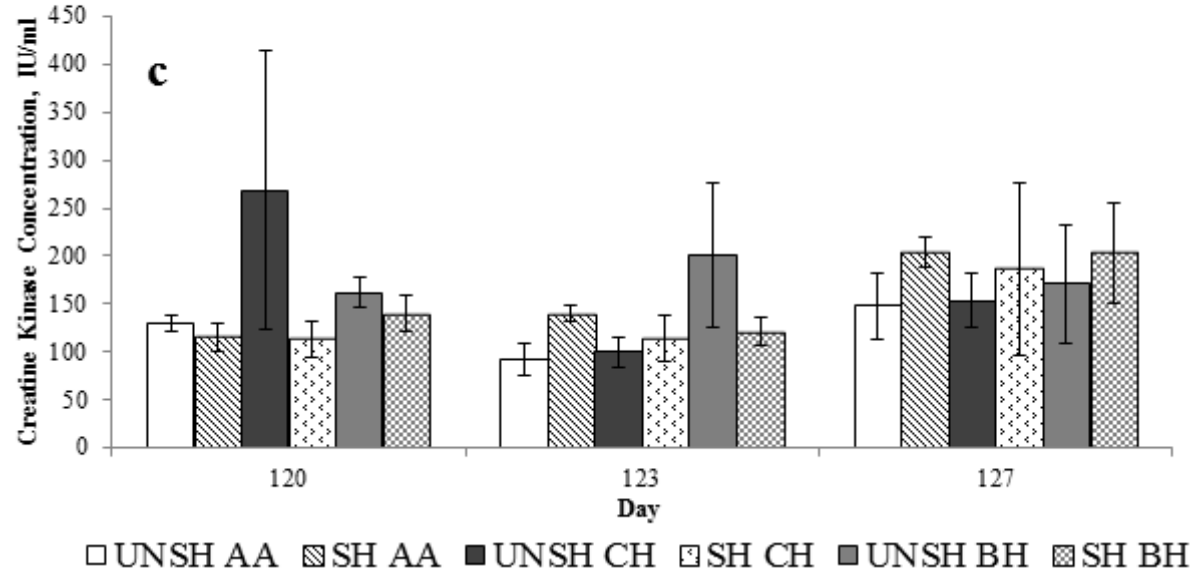


Figure 5.4.2: Plasma creatine kinase (IU/L) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.2 Cytokine Interleukin-6

Plasma cytokine interleukin 6 concentrations were highly variable across breed × treatment groups during periods 1 to 5 (Appendix 15) and throughout each heat wave event (Appendix 16). The data did not meet the assumptions of the analysis of variance proceedures; therefore concentrations were converted transformed into Log_{10} for analysis. The inter and intra assay coefficients of variation were 23.2 % and 4.0 % respectively.

Log_{10} cytokine interleukin 6 concentrations were not affected by breed (P = 0.45), treatment (P = 0.85), treatment × breed (P = 0.83), treatment × period (P = 0.46), or breed × period (P = 0.36). However there were effects of period (P = 0.05) whereby period 2 had a pooled Log_{10} cytokine interleukin 6 values that was higher (3.5 ± 0.1) compared to the other periods. There was a treatment × breed × period (P = 0.03) effect, although it appears to be a false significance (Figure 5.4.3). During the heat wave events Log_{10} cytokine interleukin 6 concentration was not influenced by heat wave (P = 0.25), sample within heat wave (P = 0.70), treatment (P = 0.22), breed (P = 0.94), treatment × breed (P = 0.19), treatment × heat wave (P = 0.94), breed × heat wave (P = 0.93) or treatment × breed × heat wave (P =0.42; Figure 5.4.4).

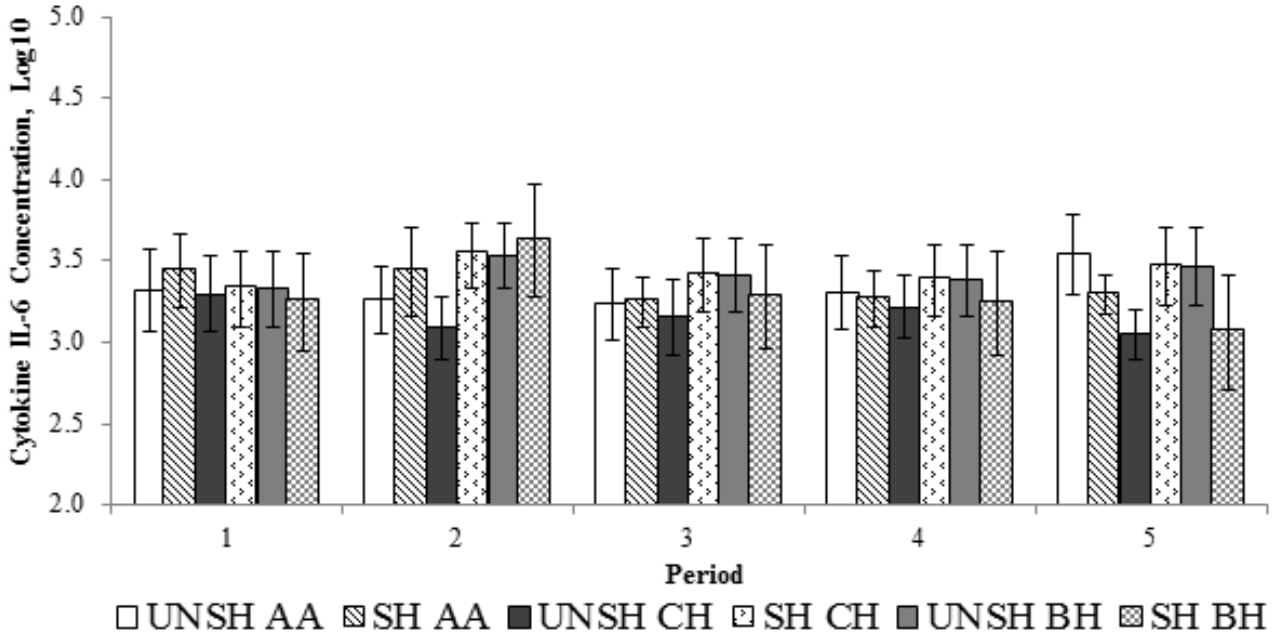
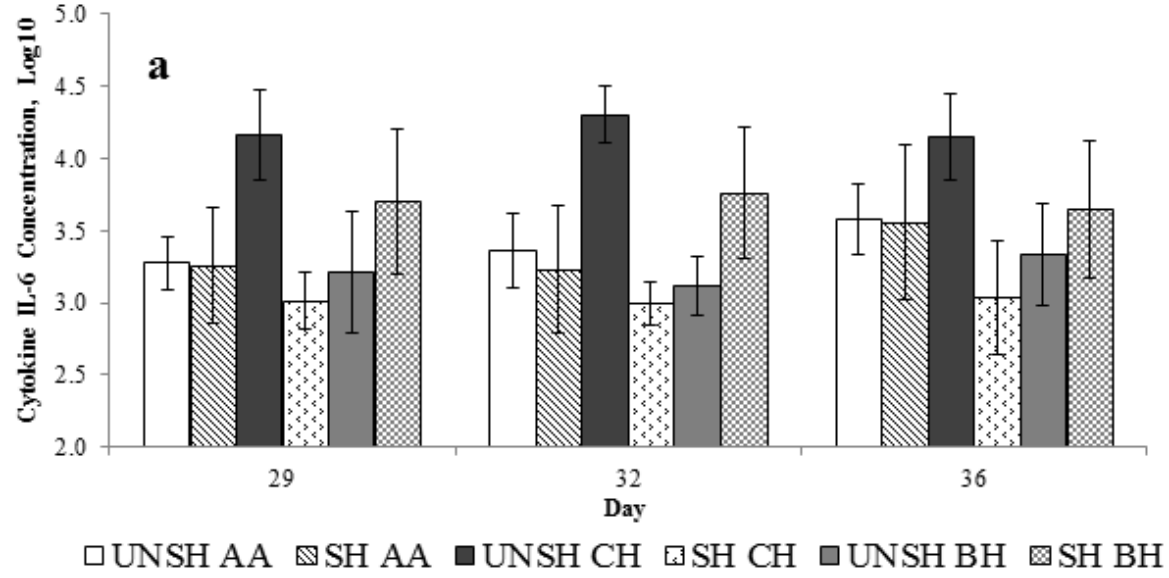
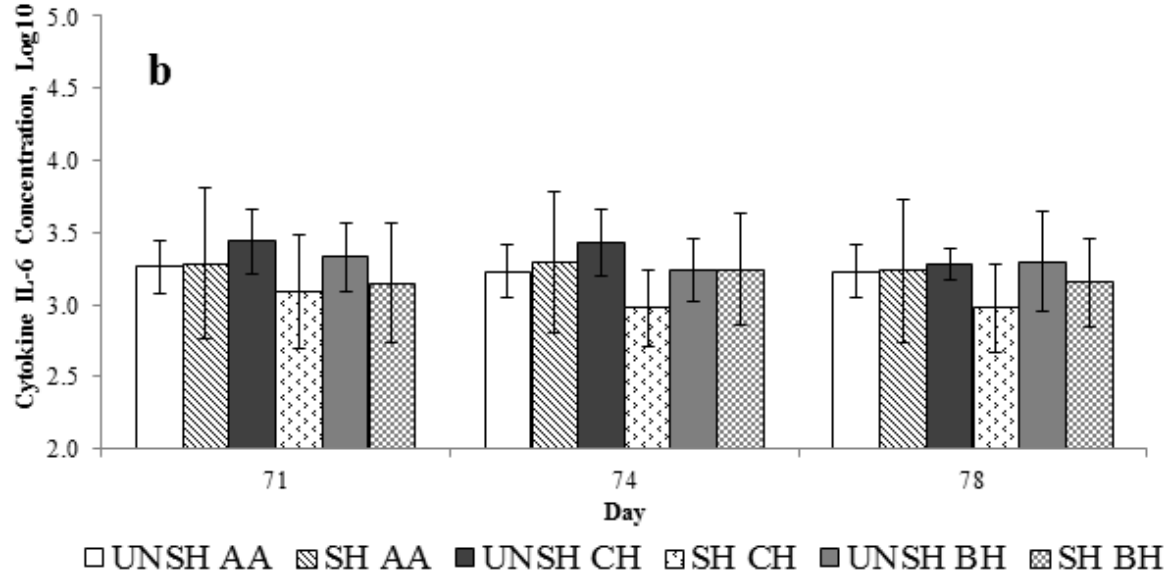


Figure 5.4.3: Log_{10} Plasma cytokine interleukin-6 (IL-6) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers





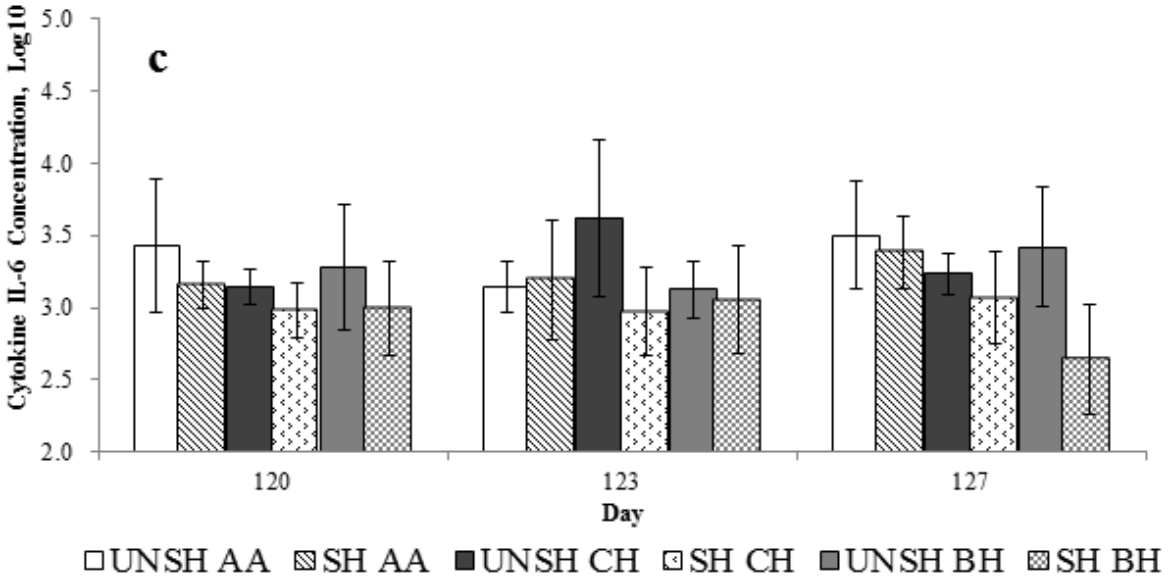


Figure 5.4.4: Log$_{10}$ plasma cytokine interleukin-6 (IL-6) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.3 Electrolytes

5.4.3.3.1 Bicarbonate

Plasma bicarbonate concentrations were not affected by breed (P = 0.32), treatment (P = 0.94), treatment × breed (P = 0.09), treatment × period (P = 0.19), breed × period (P = 0.98) or treatment × breed × period (P = 0.33); however there were period (P < 0.0001) effects where pooled bicarbonate concentration increased over time (Figure 5.4.5). During the heat wave events, plasma bicarbonate concentrations were not affected by treatment (P = 0.40), breed (P = 0.52), treatment × breed (P = 0.21), treatment × heat wave (P = 0.59), breed × heat wave (P = 0.85) or treatment × breed × heat wave (P =0.39). However there were effects of heat wave (P < 0.0001), where pooled bicarbonate concentration was greater during heat wave 3 (21.58 ± 0.4 mmol/L). There was a sample within heat wave (P < 0.0001) effect, where samples collected on d 3 within heat waves 1 (d 29; 21.0 ± 0.48 mmol/L), 2 (d 71; 19.0 ± 0.48 mmol/L), and 3 (d 120; 23.7 ± 0.48 mmol/L) were greater than samples collected on d 1 and 2 (Figure 5.4.6).

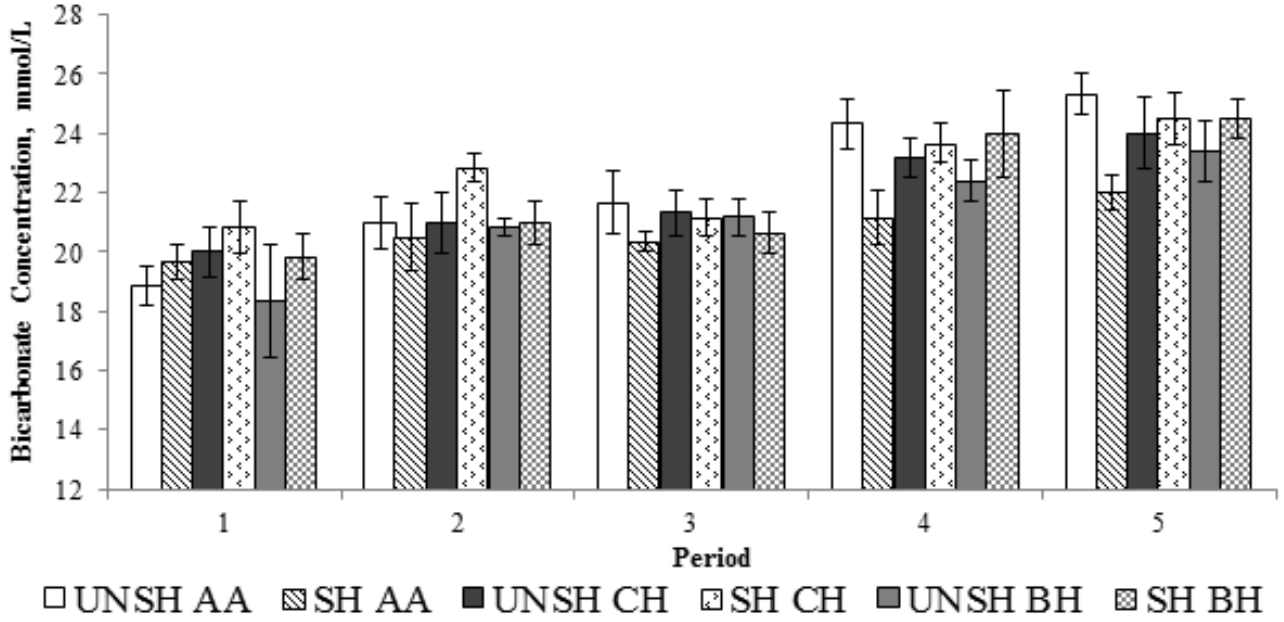
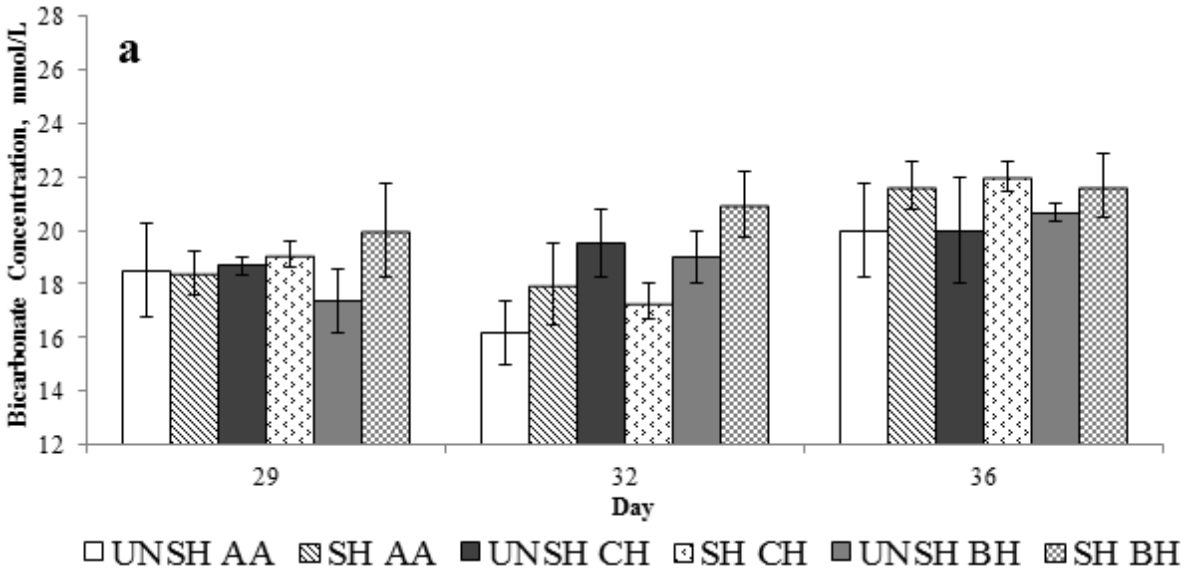
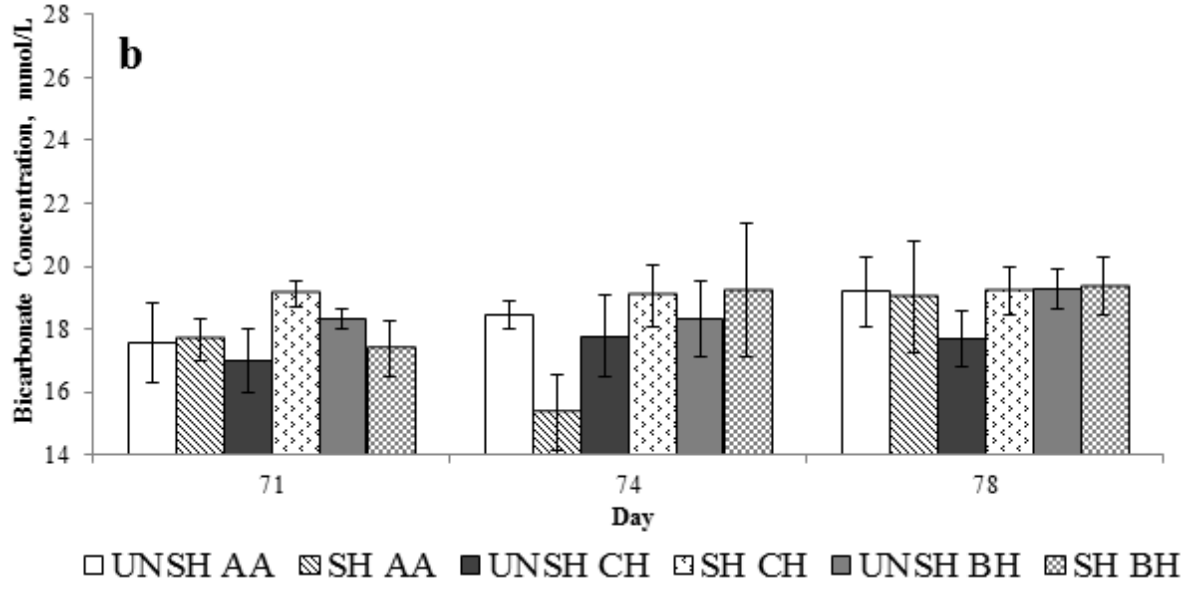


Figure 5.4.5: Plasma bicarbonate (mmol/L) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers





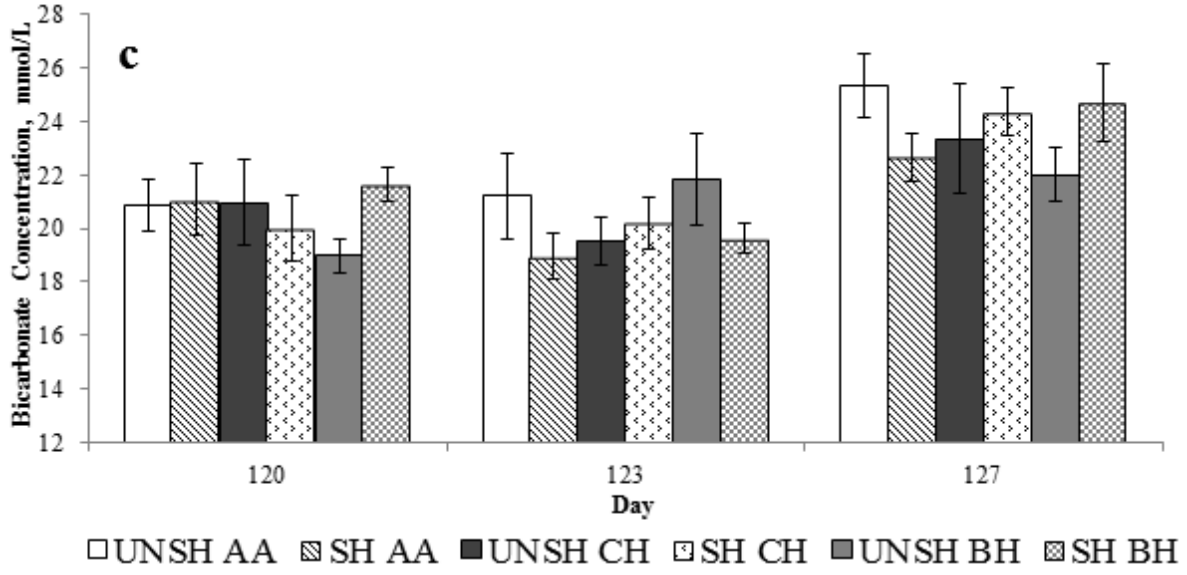


Figure 5.4.6: Plasma bicarbonate (mmol/L) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.3.2 Chloride

Plasma chloride concentrations were not affected by breed (P = 0.63), treatment (P = 0.10), treatment × breed (P = 0.72), treatment × period (P = 0.31), breed × period (P = 0.58) or treatment × breed × period (P = 0.80); however there was an effect of period (P = 0.003; Figure 5.4.7) whereby period 4 had pooled plasma chloride concentrations (99.3 ± 0.4 mmol/L) that were lower than other periods. During the heat wave events plasma chloride concentrations were not affected by treatment (P = 0.64), breed (P = 0.85), treatment × breed (P = 0.75), treatment × heat wave (P = 0.95), or breed × heat wave (P = 0.29); however there were effects of heat wave (P < 0.0001), where pooled chloride concentrations differed during heat wave 1 (96.4 ± 0.7 mmol/L), 2 (91.5 ± 0.7 mmol/L) and 3 (99.4 ± 0.7 mmol/L); and sample within heat wave (P < 0.0001), where plasma chloride concentrations were highly variable (Figure 5.4.8). There were also treatment × breed × heat wave (P = 0.05), whereby during heat wave event 1, samples collected on d 3 (d 36; Figure 5.4.8). There were differences in the plasma chloride concentration of un-shaded (95.5 ± 2.8 mmol/L) and shaded (101.3 ± 2.2 mmol/L) Angus and within samples collected on d 2 (d 74; Figure 5.4.8). Additionally there were differences between un-shaded (95.1 ± 1.6 mmol/L) and shaded (83.3 ± 4.4 mmol/L) Charolais steers. Similarly during heat wave event 2, there were differences in the plasma chloride concentrations of shaded (78.7 ± 3.5 mmol/L) and un-shaded (95.5 ± 2.8 mmol/L) Angus within samples collected on d 3 (d 78; Figure 5.4.8) and Charolais steers within samples collected on d 1 (d 71; Figure 5.4.8).

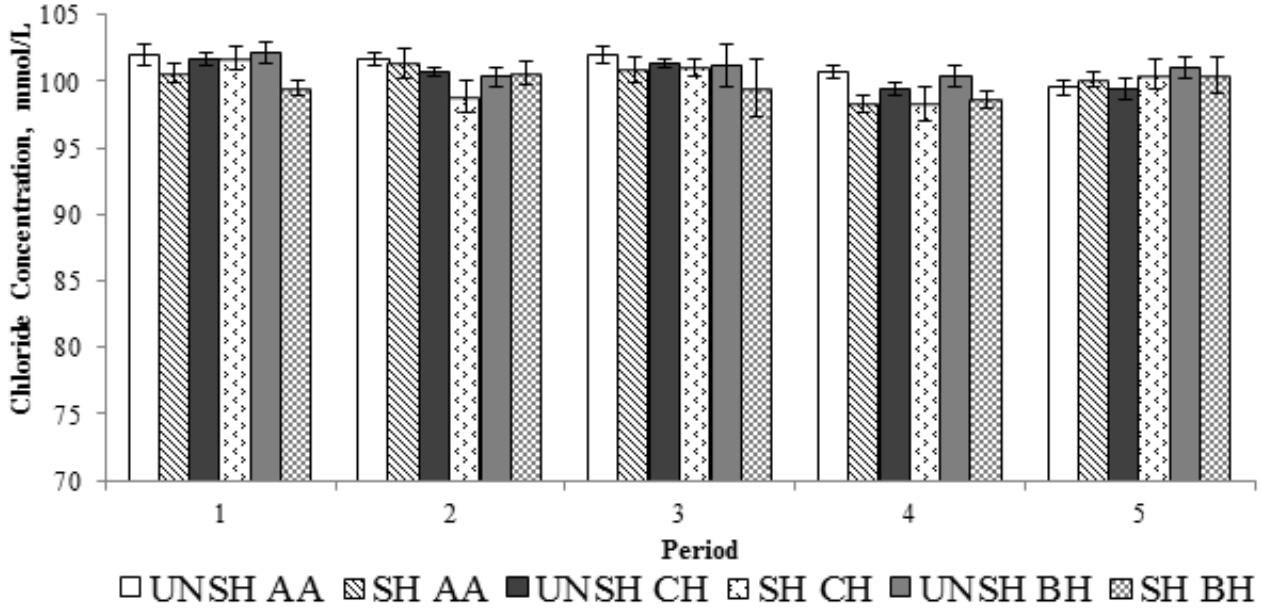
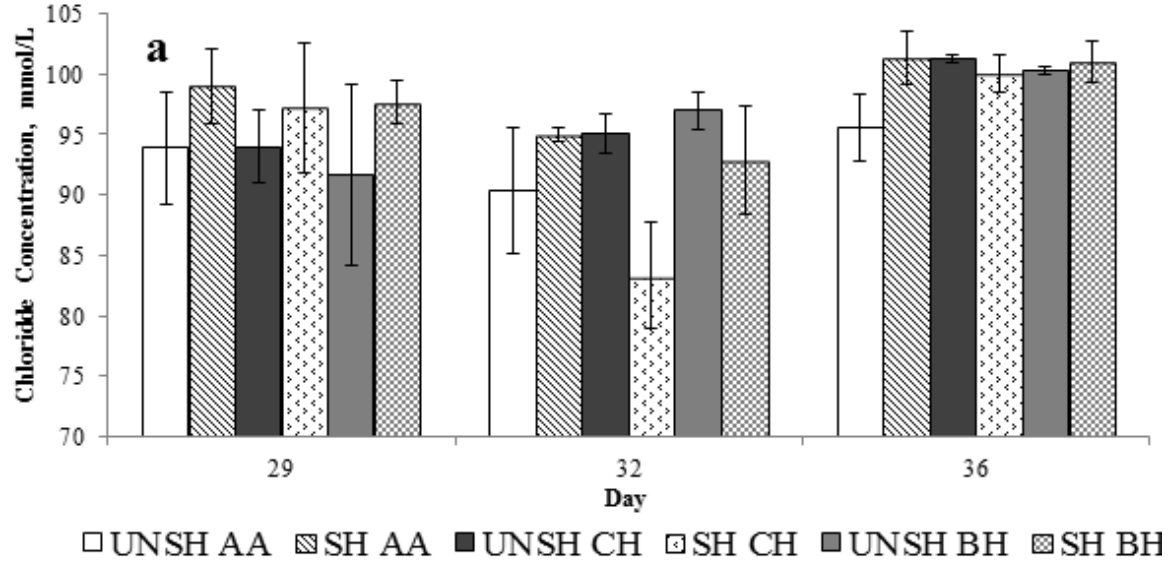
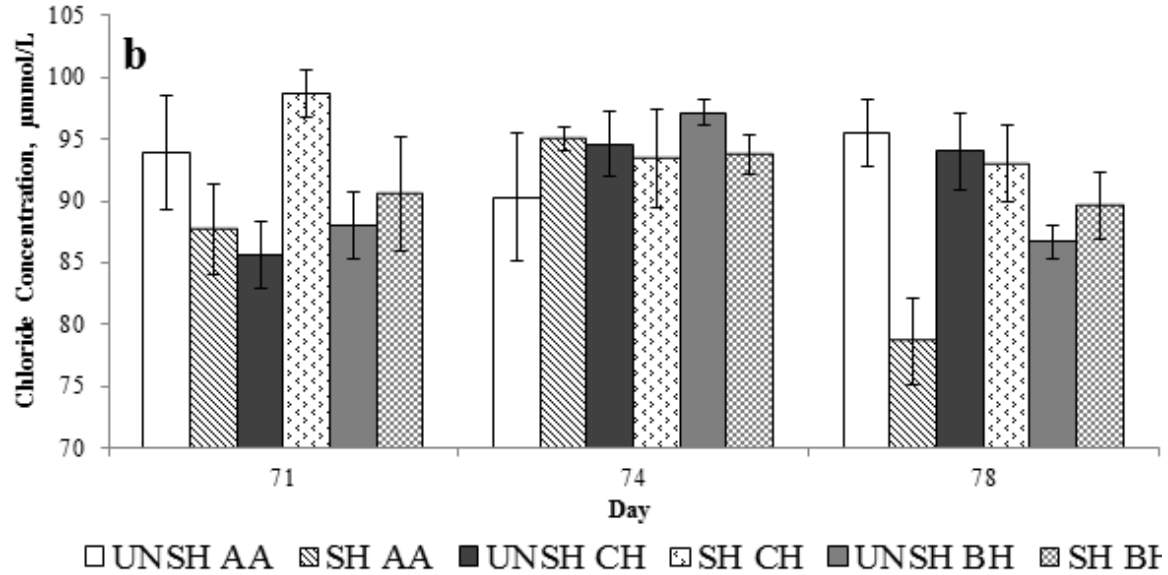


Figure 5.4.7: Plasma chloride (mmol/L) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers





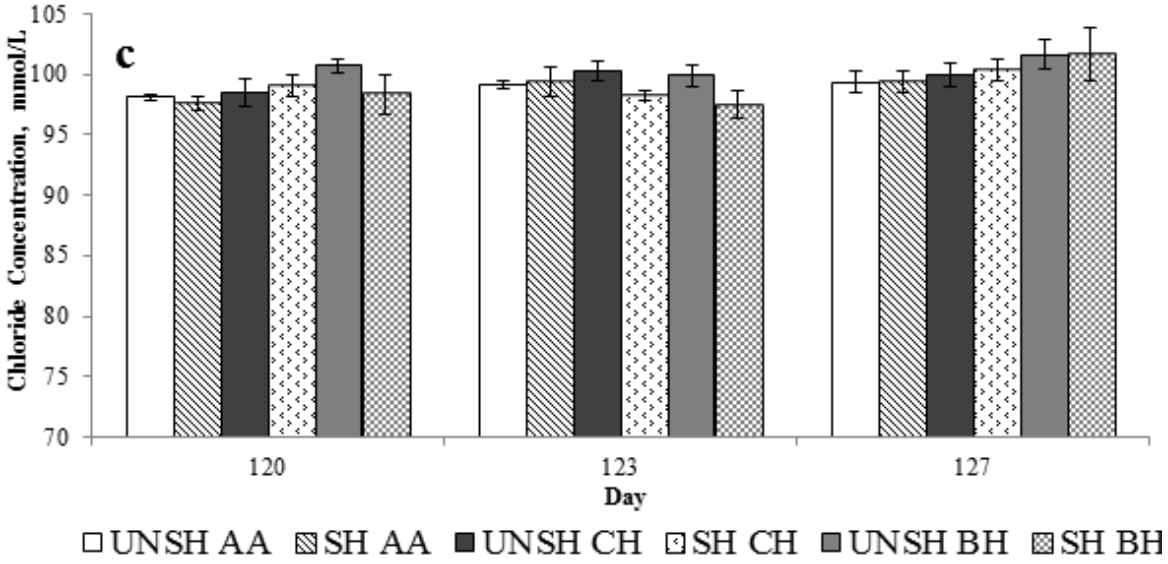


Figure 5.4.8: Plasma chloride (mmol/L) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.3.3 Potassium

Plasma potassium concentrations were not affected by breed (P = 0.23), treatment (P = 0.35), treatment × breed (P = 0.89), treatment × period (P = 0.81), breed × period (P = 0.32) or treatment × breed × period (P = 0.91); however there was an effect of period (P < 0.0001), where pooled plasma potassium concentration was greater in period 1 (Figure 5.4.9). During the heat wave events plasma potassium concentrations were not affected by treatment (P = 0.18), breed (P = 0.18), treatment × heat wave (P = 0.87), breed × heat wave (P = 0.17), or treatment × breed × heat wave (P = 0.75); however there were effects of treatment × breed (P = 0.04; Figure 5.4.10). Heat wave had an effect on potassium concentration (P = 0.006), where pooled potassium concentrations during heat wave 1 (4.4 ± 0.06 mmol/L), 2 (4.3 ± 0.06 mmol/L) and 3 (4.6 ± 0.06 mmol/L) differed. There were also sample within heat wave effects (P < 0.0001), where during heat wave 1 samples collected on d 2 (d 32; 4.3 ± 0.09 mmol/L) had a lower pooled potassium concentration than samples collected on d 1 (d 29; 4.6 ± 0.09 mmol/L) and 3 (d 29; 4.4 ± 0.09 mmol/L). During heat wave 2, samples collected on d 3 (d 78; 4.5 ± 0.09 mmol/L) had a higher pooled potassium concentration than samples collected on d 1 (d 71; 4.3 ± 0.09 mmol/L) and samples collected on d 2 (d 74; 4.1 ± 0.09 mmol/L). Within heat wave 3, samples collected on d 3 (d 127; 4.3 ± 0.09 mmol/L) had a lower pooled potassium concentration than samples collected on d 1 (d 120; 4.8 ± 0.09 mmol/L) and samples collected on d 2 (d 123; 4.7 ± 0.09 mmol/L).

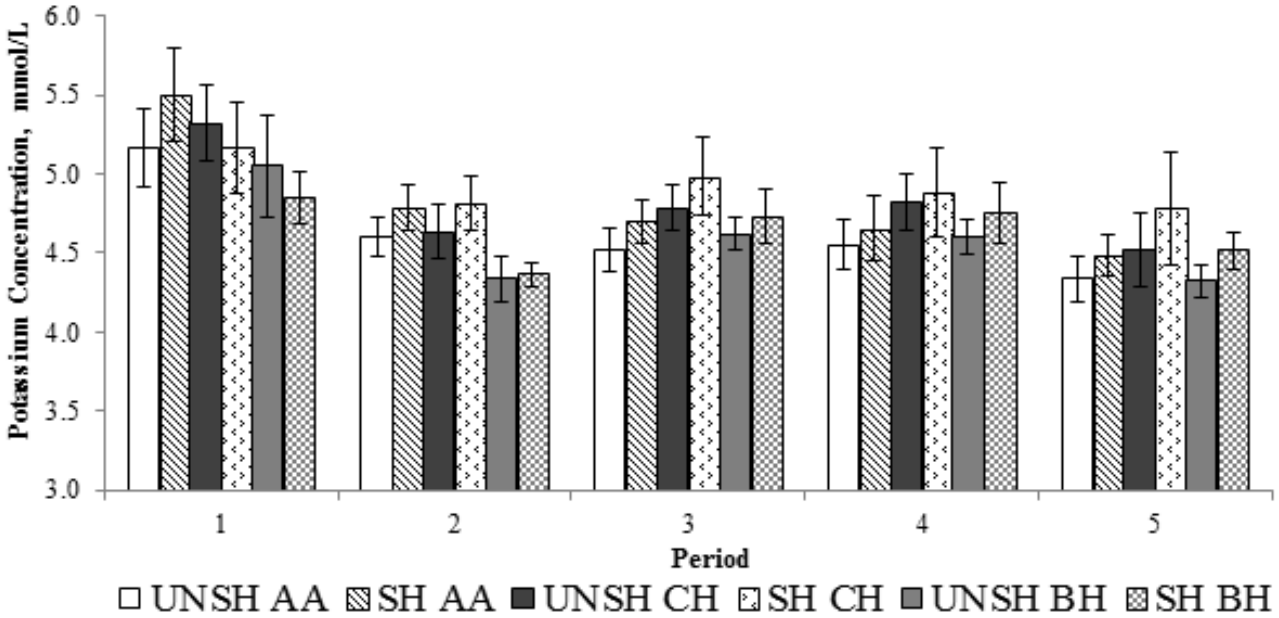


Figure 5.4.9: Plasma potassium (mmol/L) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

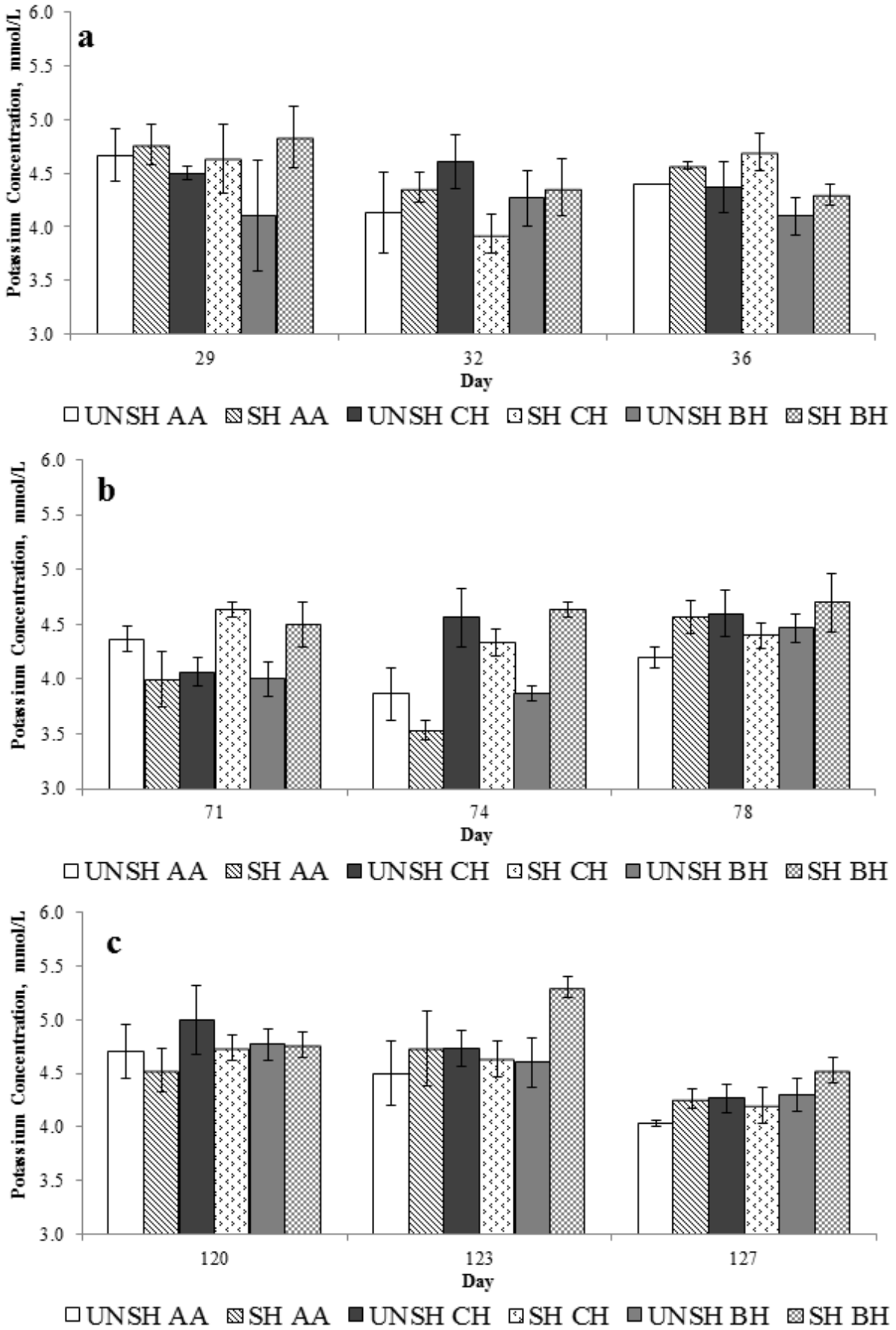


Figure 5.4.10: Plasma potassium (mmol/L) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.3.4 Sodium

Plasma sodium concentrations were not affected by breed (P = 0.45), treatment (P = 0.12), treatment × breed (P = 0.71), breed × period (P = 0.55) or treatment × breed × period (P = 0.75); however there were treatment × period (P = 0.01) and period (P = 0.0008), effects (Figure 5.4.11). Plasma sodium concentration was lower (137.5 ± 0.4 mmol/L) in period 4 due to a treatment effect (P = 0.0004), whereby pooled sodium concentrations were lower in shaded cattle (135.9 ± 0.6 mmol/L v 139.2 ± 0.6 mmol/L). During the heat wave events plasma sodium concentrations were not affected by treatment (P = 0.88), breed (P = 0.85), treatment × breed (P = 0.31), treatment × heat wave (P = 0.94) or breed × heat wave (P = 0.38). However plasma sodium concentrations were effected by treatment × breed × heat wave (P = 0.04), where there was large variability in the plasma sodium concentration of treatment × breed groups during heat wave 1 and 2; heat wave (P < 0.0001), whereby pooled plasma sodium concentration was greatest during heat wave 3 (139.2 ± 1.1 mmol/L) and lowest during heat wave 2 (125.9 ± 1.0 mmol/L). There were also sample within heat wave (P = 0.0003) effects, where pooled sodium concentrations tended to be lower in samples collected on d 2 during all heat wave events (Figure 5.4.12).

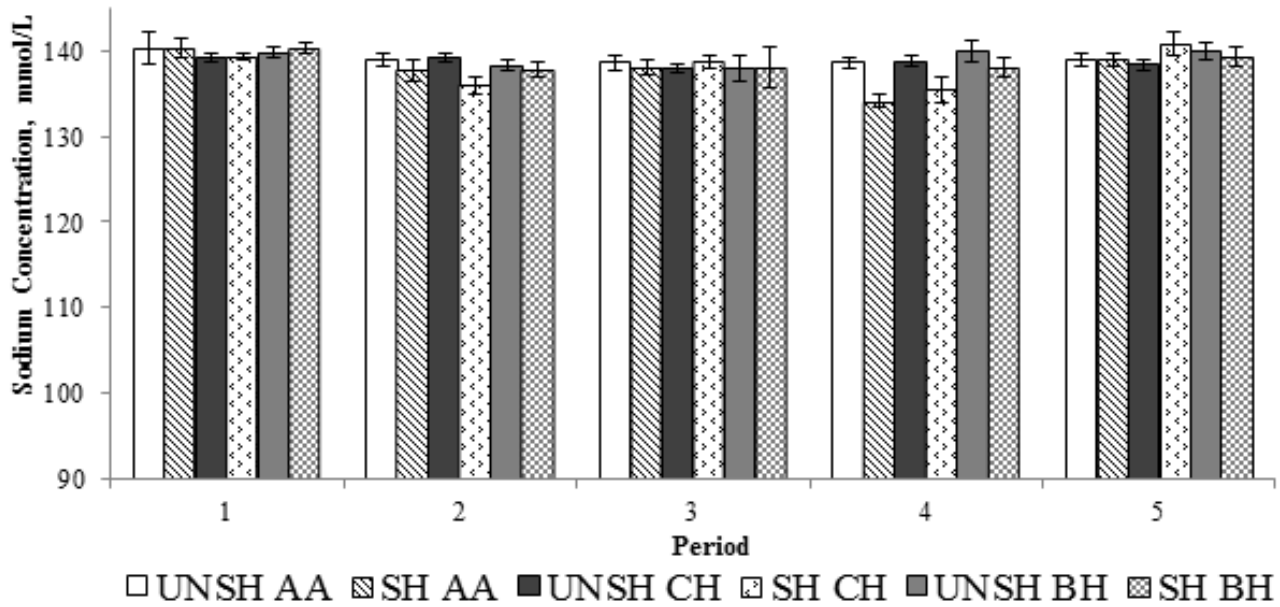
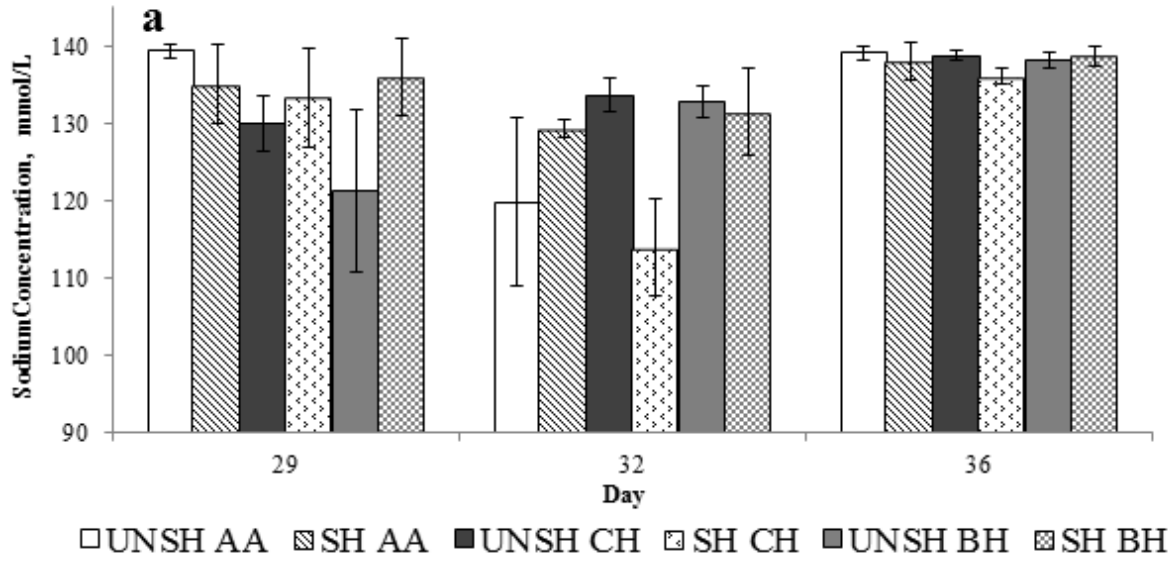
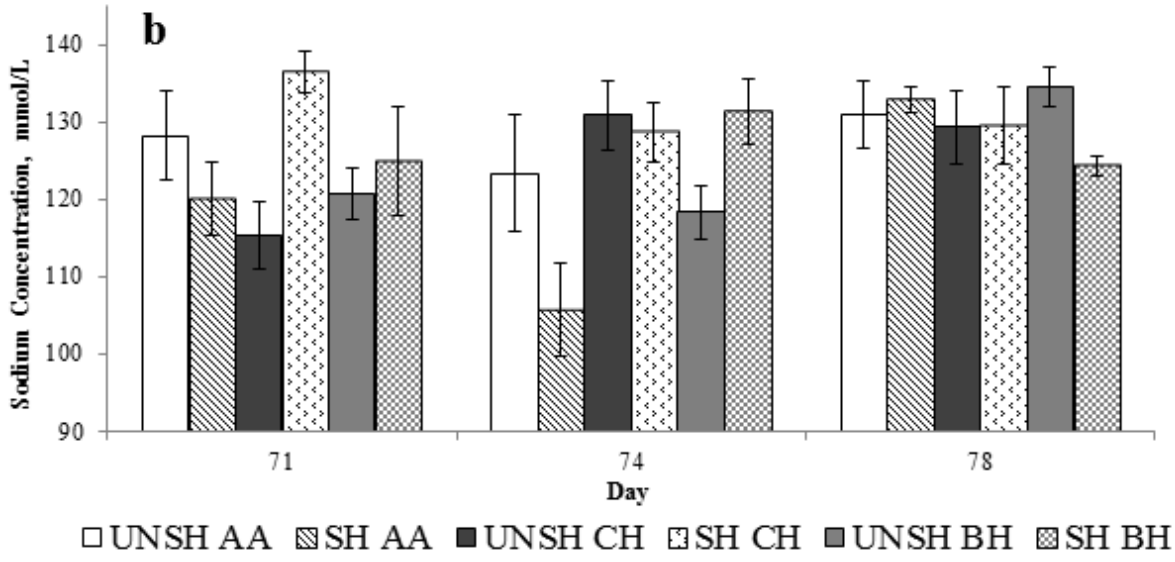


Figure 5.4.11: Plasma sodium (mmol/L) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers





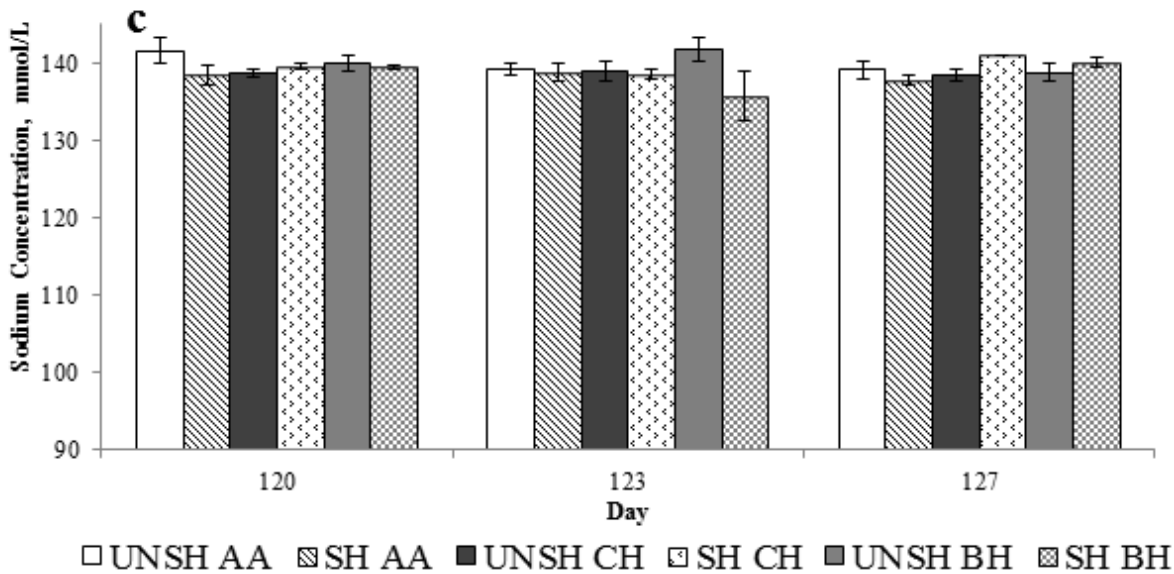


Figure 5.4.12: Plasma sodium (mmol/L) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.4 Glucose

Plasma glucose concentrations were not affected by treatment (P = 0.97), treatment × breed (P = 0.39), treatment × breed × period (P = 0.48) or treatment × period (P = 0.09); however there were breed (P = 0.03), period (P = 0.0003) and breed × period (P < 0.0001) effects (Figure 5.4.13). Brahman steers tended to have higher plasma glucose concentrations than Angus steers particularly during periods 1 (P < 0.0001) and 2 (P = 0.003). During the heat wave events, circulating glucose concentrations were not affected by treatment (P = 0.33), breed (P = 0.14), treatment × breed (P = 0.56), heat wave (P = 0.13), treatment × heat wave (P = 0.54), treatment × breed × heat wave (P = 0.72), or sample within heat wave (P = 0.74; Figure 5.4.14). There were effects of breed × heat wave (P = 0.04); whereby during heat wave 1 Brahman (5.1 ± 0.3 mmol/L) steers had greater circulating glucose concentrations compared to Angus (4.5 ± 0.3 mmol/L) and Charolais (4.5 ± 0.3 mmol/L; P = 0.008) steers.

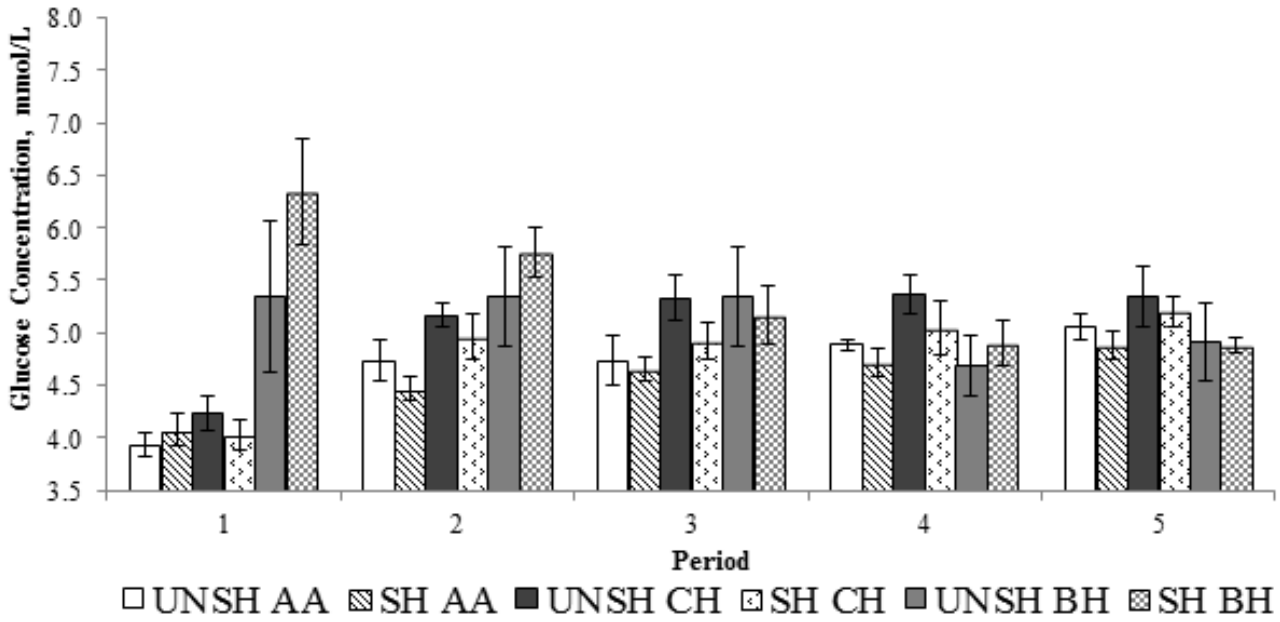


Figure 5.4.13: Plasma glucose (mmol/L) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

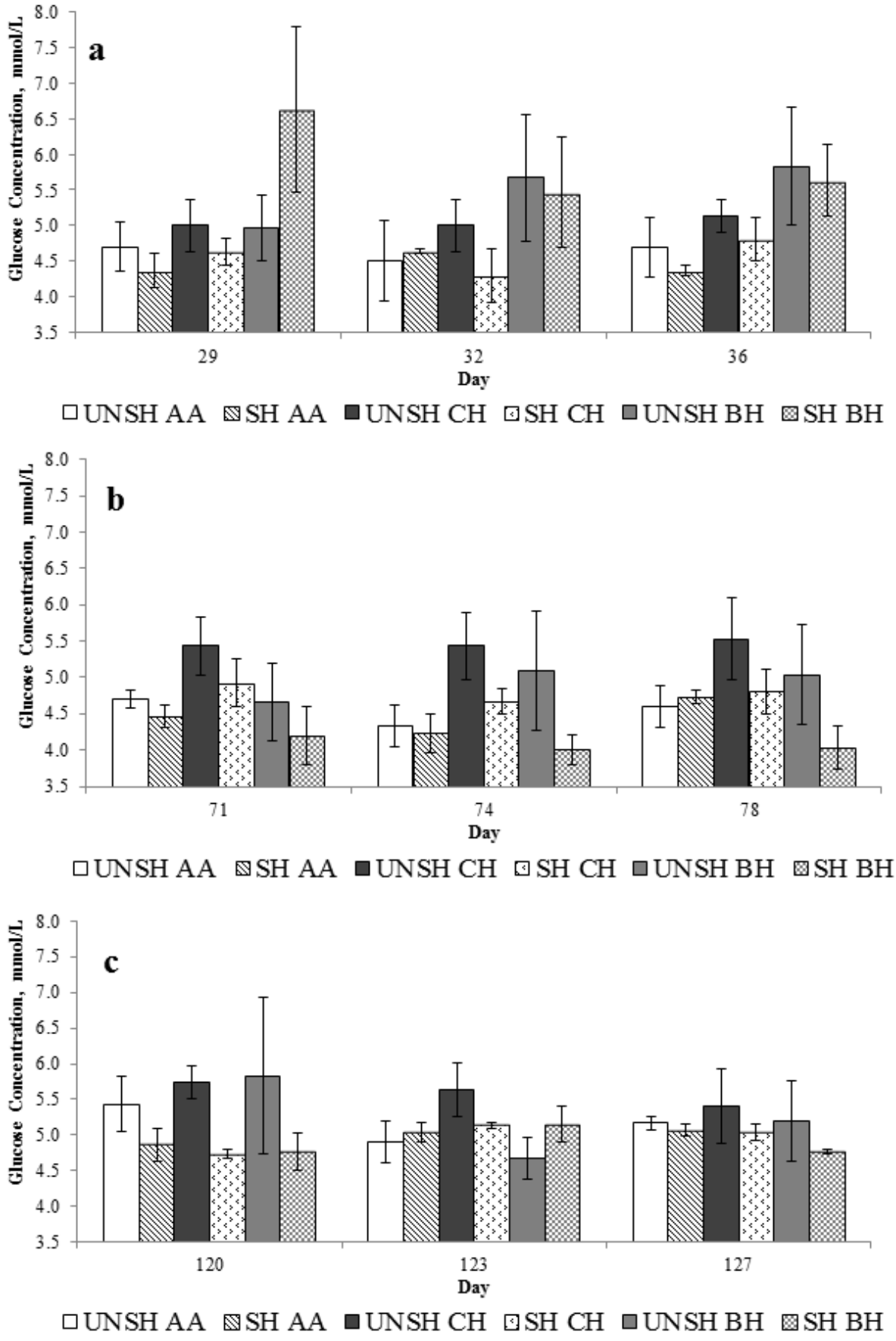


Figure 5.4.14: Plasma glucose (mmol/L) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.5 Haptoglobin

Plasma haptoglobin concentrations (mg/mL) were not affected by treatment (P = 0.65), treatment × breed (P = 0.99), treatment × period (P = 0.62), breed × period (P = 0.58) or treatment × breed × period (P = 0.45); however there were breed (P = 0.05) and period (P = 0.003) effects (Figure 5.4.15), whereby plasma haptoglobin concentrations were lower in Angus steers during period 1 (P = 0.01). Circulating haptoglobin concentrations during heat wave events were not affected by treatment (P = 0.14), breed (P = 0.85), treatment × heat wave (P = 0.26), breed × heat wave (P = 0.25), treatment × breed × heat wave (P = 0.61) or sample within heat wave (P = 0.18). There were effects of treatment × breed (P = 0.05), whereby pooled plasma haptoglobin concentration was greater in un-shaded (0.34 ± 0.02 mg/mL) steers than their shaded (0.27 ± 0.02 mg/mL) counterparts during heat wave 3 (P = 0.04). Additionally there were also effects of heat wave (P = 0.0008) where pooled plasma haptoglobin concentration increased with each heat wave (event 1, 0.23 ± 0.02 mg/mL; event 2, 0.30 ± 0.02 mg/mL, event 3, 0.31 ± 0.02 mg/mL; Figure 5.4.16). The inter and intra assay coefficients of variation were 19.7 % and 3.6 % respectively.

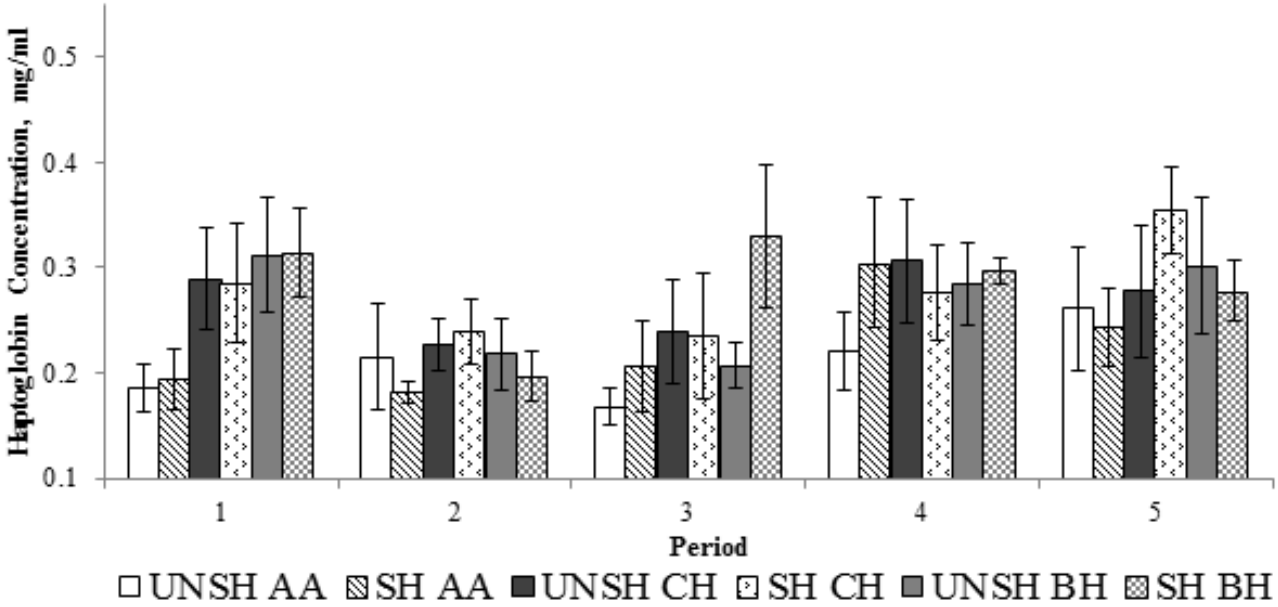


Figure 5.4.15: Plasma haptoglobin (mg/mL) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

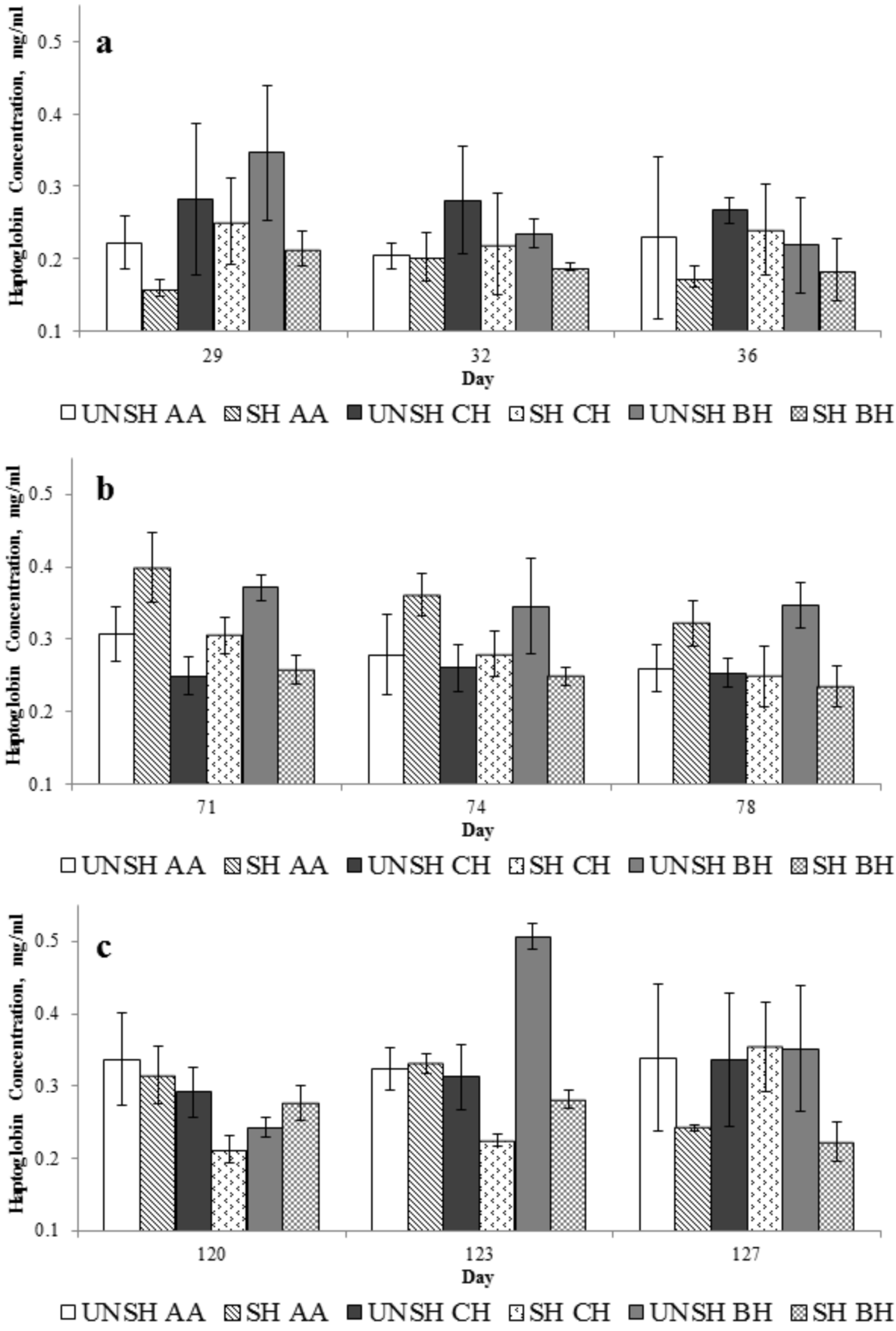


Figure 5.4.16: Plasma haptoglobin (mg/mL) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.6 Heat Shock Protein 70

Change in optical density (nm) for HSP_{70} was calculated by subtracting the optical density for each duplicate from the blank (B_0) from each assay (Figure 5.4.17). These calculations indicate that in many circumstances there was no response in circulating HSP_{70} concentrations. However there was considerable within breed × treatment variability in HSP_{70} optical densities (Appendix 17), particularly within the shaded Angus (animal ID 2081 and 2075), un-shaded (animal ID 2094 and 2096) and shaded (animal ID 2093 and 2106) Brahman steers. Given this, HSP_{70} optical density was not affected by breed (P = 0.12), treatment (P = 0.31), treatment × breed (P = 0.73), breed × period (P = 0.14) or treatment × breed × period (P = 0.27). There were period (P = 0.002) and treatment × period (P = 0.05).

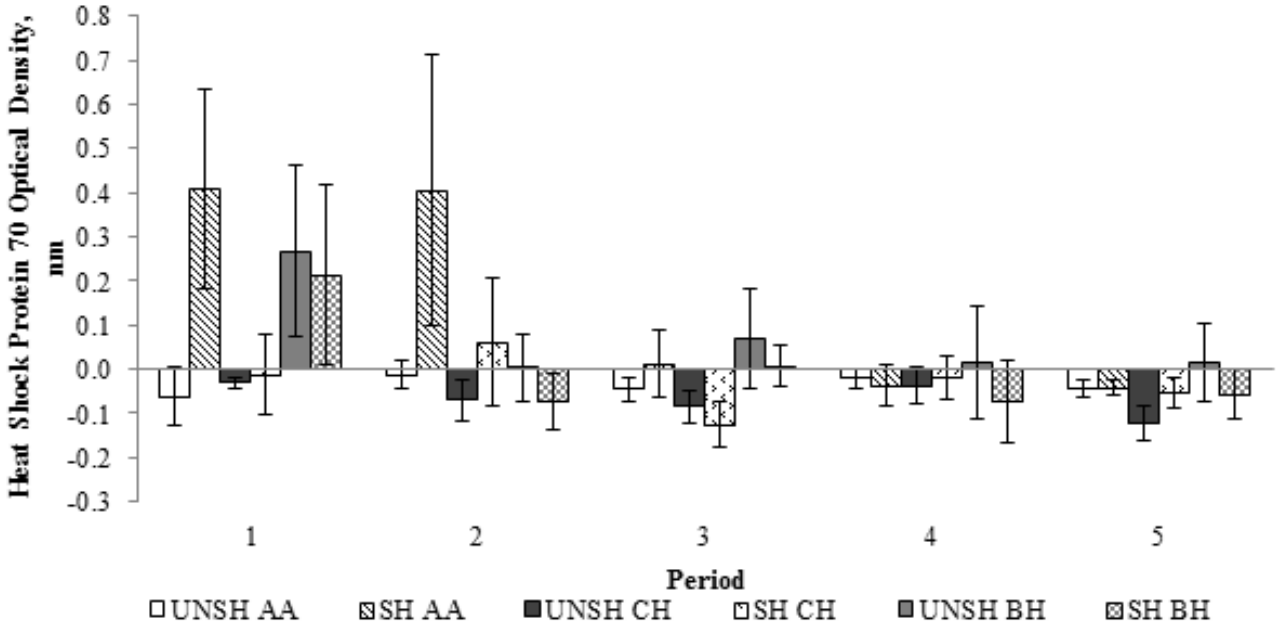


Figure 5.4.17: Change in optical density (nm) of heat shock protein 70 (HSP70) during period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.7 Insulin

Plasma insulin concentrations were highly variable across breed × treatment groups during periods (Appendix 18) and throughout each heat wave event (Appendix 19); therefore concentrations were transformed into Log_{10} for analysis. Log_{10} insulin concentrations were not affected by treatment (P = 0.81), treatment × breed (P = 0.38), or treatment × breed × period (P = 0.50); however there was a tendency for a treatment × period (P = 0.06), and there were breed (P = 0.03), period (P < 0.0001) and breed × period (P = 0.03) effects (Figure 5.4.18). During the heat wave events Log_{10} insulin concentrations were not affected by treatment (P = 0.95), treatment × breed (P = 0.57), treatment ×

heat wave (P = 0.92), treatment × breed × heat wave (P = 0.08) or sample within heat wave (P = 0.66); however there were effects of breed (P = 0.006), heat wave (P < 0.0001) and breed × heat wave (P = 0.01; Figure 5.4.19). Angus steers had lower pooled Log_{10} insulin concentrations during heat wave event 1 (P = 0.29); however during events 2 and 3 Charolais steers had lower pooled Log_{10} insulin concentrations. Insulin concentration increased over time (Figure 5.4.18) whereby each successive heat wave had a greater insulin concentration than the previous event (Figure 5.4.19). The inter and intra assay coefficients of variation were 20.4 % and 7.9 % respectively.

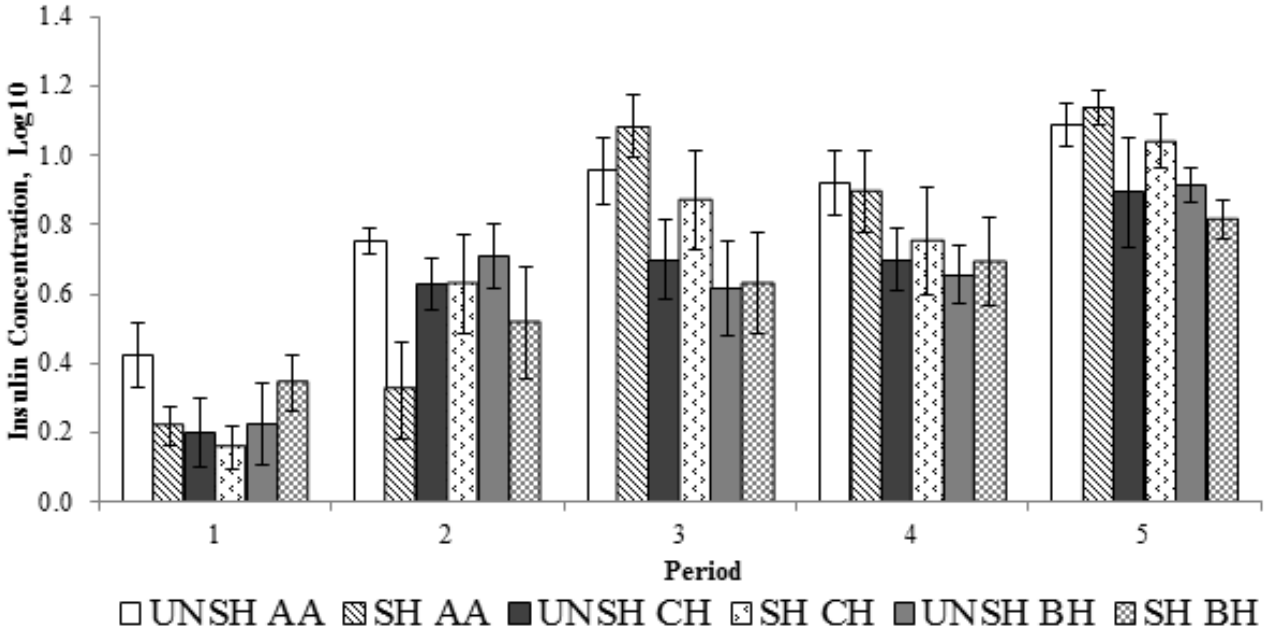


Figure 5.4.18: Log_{10} plasma insulin concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

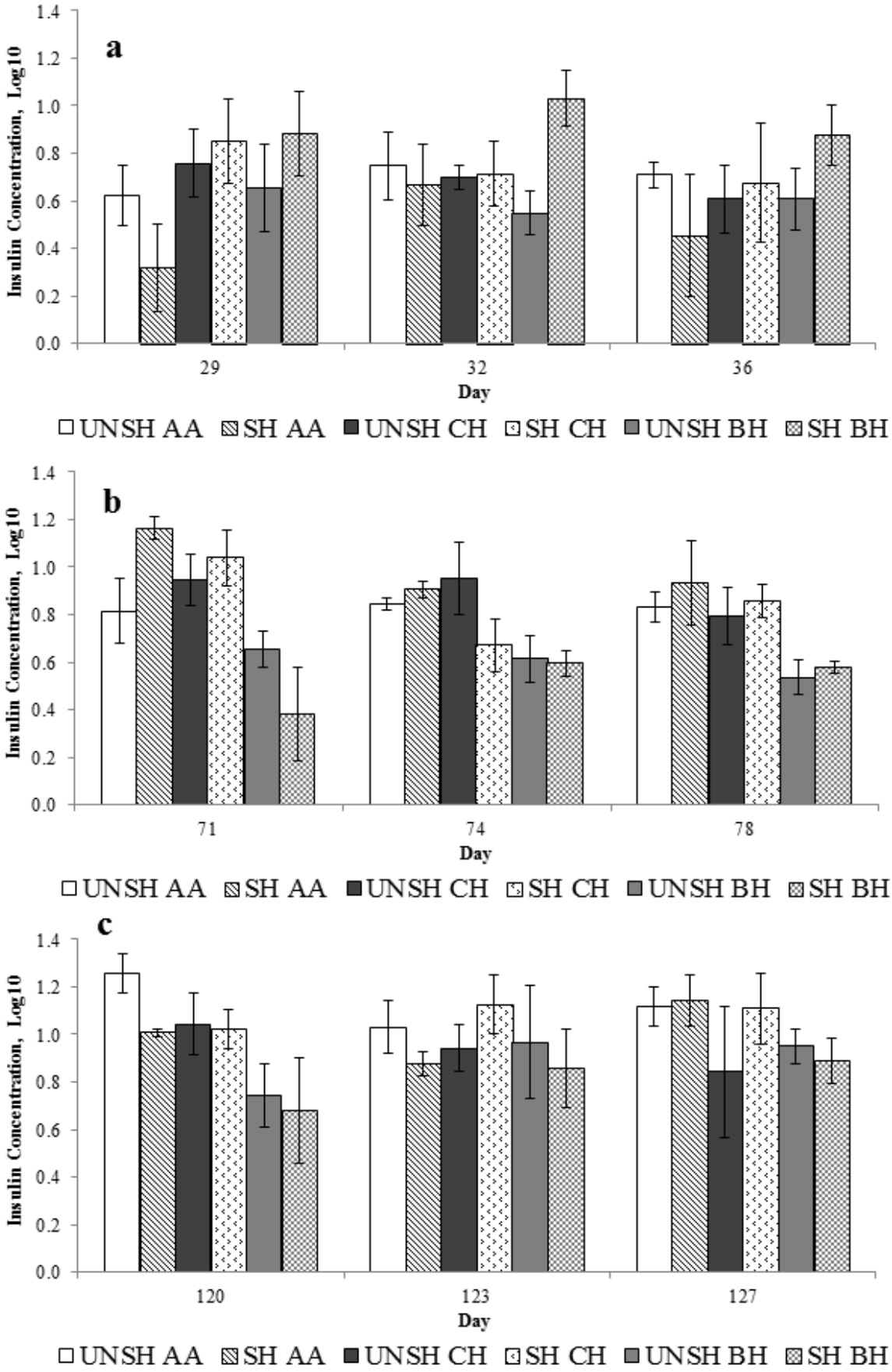


Figure 5.4.19: Log_{10} plasma insulin concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.7.1 Glucose Insulin Ratio

Glucose to insulin ratio were not affected by treatment (P = 0.75), treatment × period (P = 0.78), breed × period (P = 0.18) or treatment × breed × period (P = 0.24); however there were treatment × breed (P = 0.05), breed (P = 0.05) and period (P < 0.0001) effects (Figure 5.4.20). During the heat wave events, there were no effects of treatment (P = 0.98), treatment × breed (P = 0.28), treatment × heat wave (P = 0.87), breed × heat wave (P = 0.13), treatment × breed × heat wave (P = 0.14) or sample within heat wave (P = 0.68); however there were effects of breed (P = 0.02) and heat wave (P = 0.0002). Glucose to insulin ratio decreased over time (heat wave 1, 1.3 ± 0.1; heat wave 2, 0.9 ± 0.1; heat wave 3, 0.6 ± 0.1). Brahman steers also tended to have a greater glucose to insulin ratio, particularly evident during heat wave 2 (P = 0.0097) where pooled ratio for Brahman steers was 1.3 ± 0.2, compared with 0.6 ± 0.2 and 0.8 ± 0.2 for Angus and Charolais steers respectively (Figure 5.4.21).

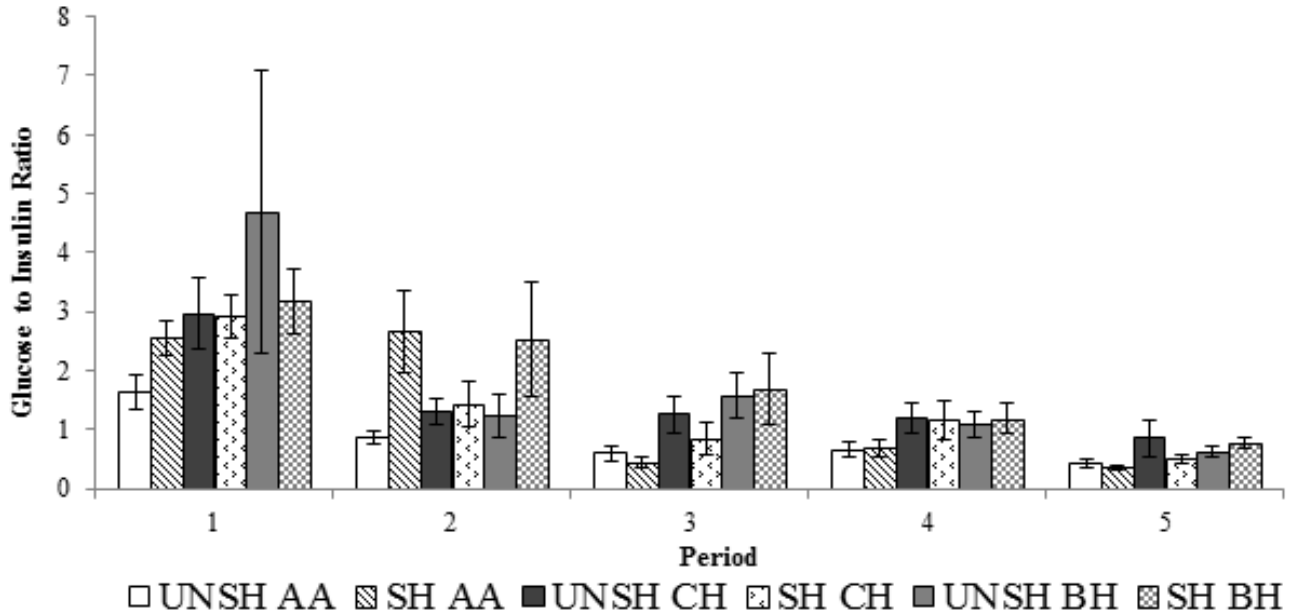


Figure 5.4.20: Plasma insulin glucose (glucose:insulin) ratio for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

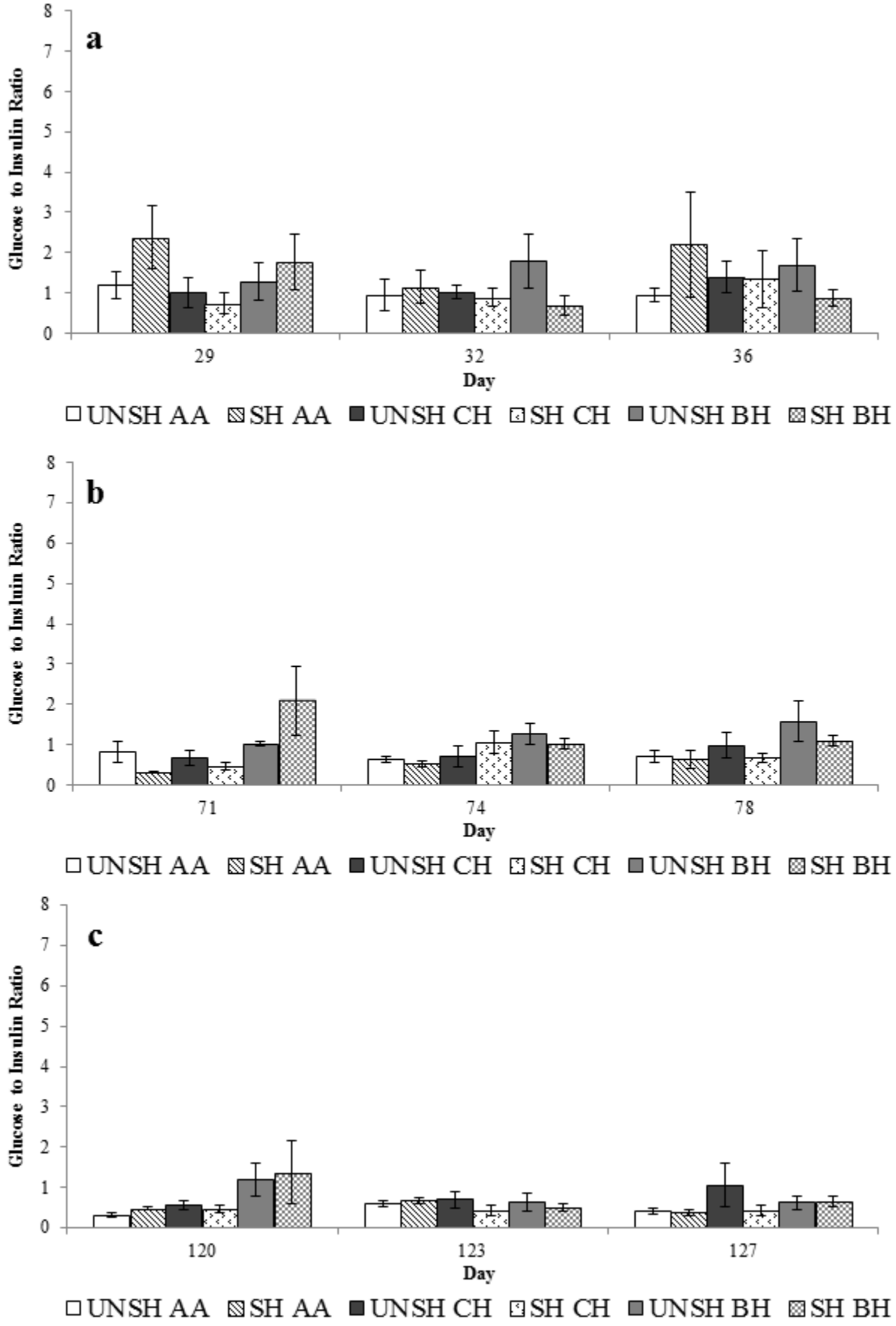


Figure 5.4.21: Plasma insulin glucose (glucose:insulin) ratio for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.8 Lipids

5.4.3.8.1 Cholesterol

Plasma cholesterol concentration was not effected by treatment (P =0.92), treatment × breed (P = 0.52), breed × period (P = 0.28), treatment × breed × period (P = 0.90) or treatment × period (P = 0.08); however there were breed (P = 0.0001; Figure 5.4.22) and period (P < 0.0001) effects, whereby Brahman steers had plasma cholesterol concentrations that were greater than Angus and Charolais steers. During the heat wave events, plasma concentrations were not affected by treatment (P = 0.33), treatment × breed (P = 0.31), treatment × heat wave (P = 0.21), breed × heat wave (P = 0.60) or treatment × breed × heat wave (P = 0.73); however there were effects of breed (P < 0.0001), heat wave (P < 0.0001) and sample within heat wave (P = 0.05; Figure 5.4.23). There was a trend for increasing plasma cholesterol concentration over time for all breed groups (Figure 5.4.22).

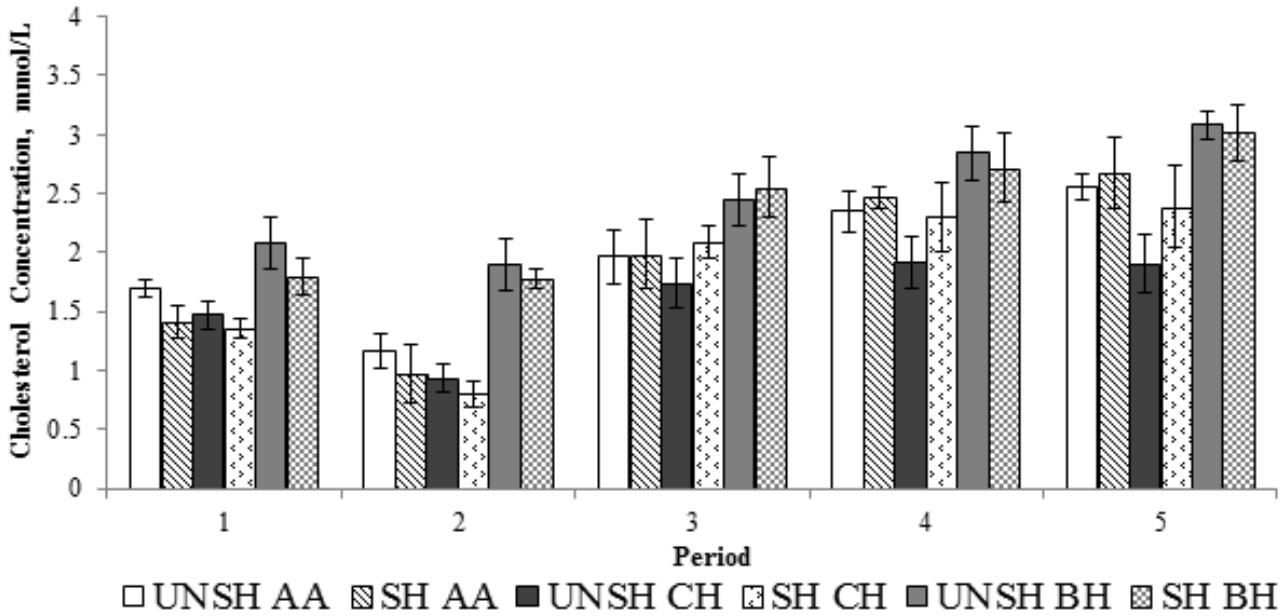


Figure 5.4.22: Plasma cholesterol concentration (mmol/L) for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

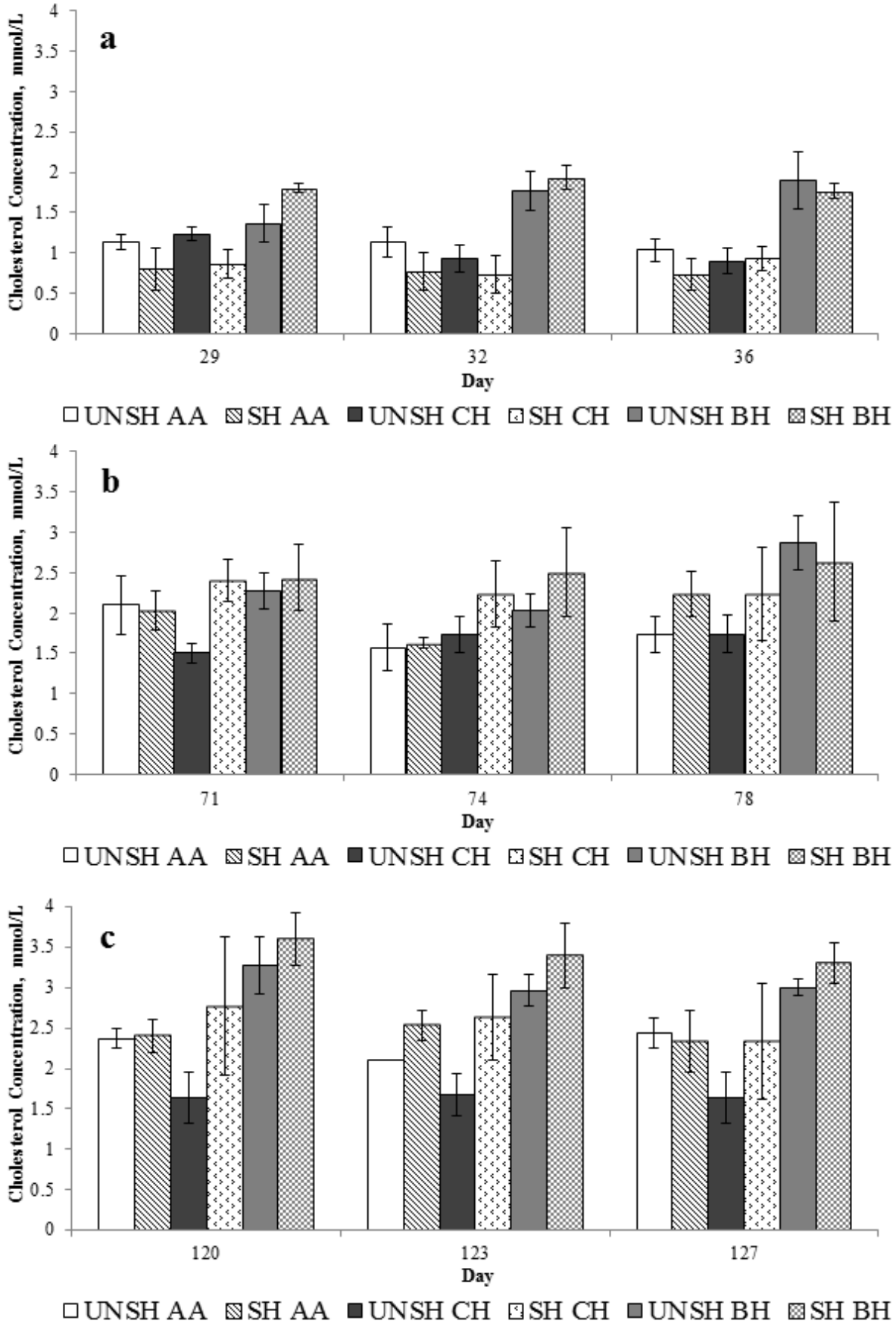


Figure 5.4.23: Plasma cholesterol concentration (mmol/L) for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.3.8.2 Triglycerides

Plasma triglyceride concentration was not effected by treatment (P = 0.67), treatment × breed (P = 0.50), treatment × period (P = 0.82), or treatment × breed × period (P = 0.13); however there were breed (P = 0.002), period (P = 0.004) and breed × period (P =0.0004) effects, where pooled plasma triglyceride concentrations were higher in Angus steers during period 1 and Brahman during periods 2 and 4 (Figure 5.4.24). During the heat wave events, plasma concentrations were not affected by treatment (P = 0.34), treatment × breed (P = 0.43), treatment × heat wave (P = 0.45), breed × heat wave (P = 0.48), or treatment × breed × heat wave (P = 0.29); however there were effects of breed (P < 0.0001), heat wave (P = 0.0018) and sample within heat wave (P < 0.0002; Figure 5.4.25). Pooled plasma triglyceride concentration increased with each heat wave event (heat wave 1, 0.18 ± 0.01 mmol/L; heat wave 2, 0.21 ± 0.01 mmol/L; heat wave 3, 0.24 ± 0.01 mmol/L).

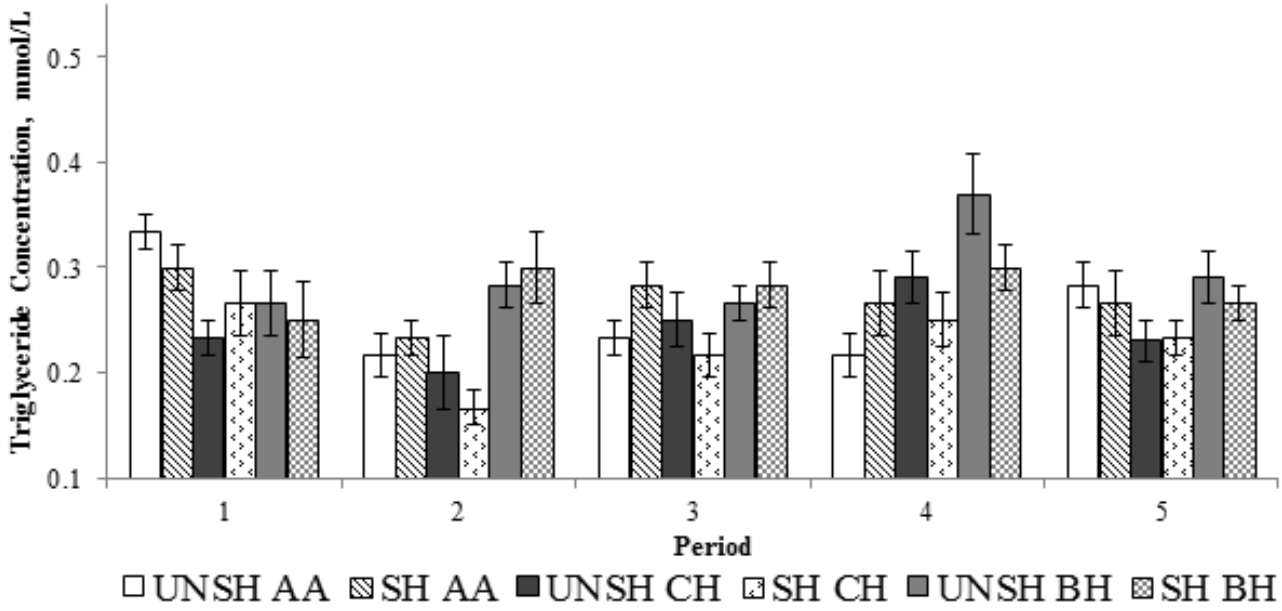


Figure 5.4.24: Plasma triglyceride concentration (mmol/L) for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

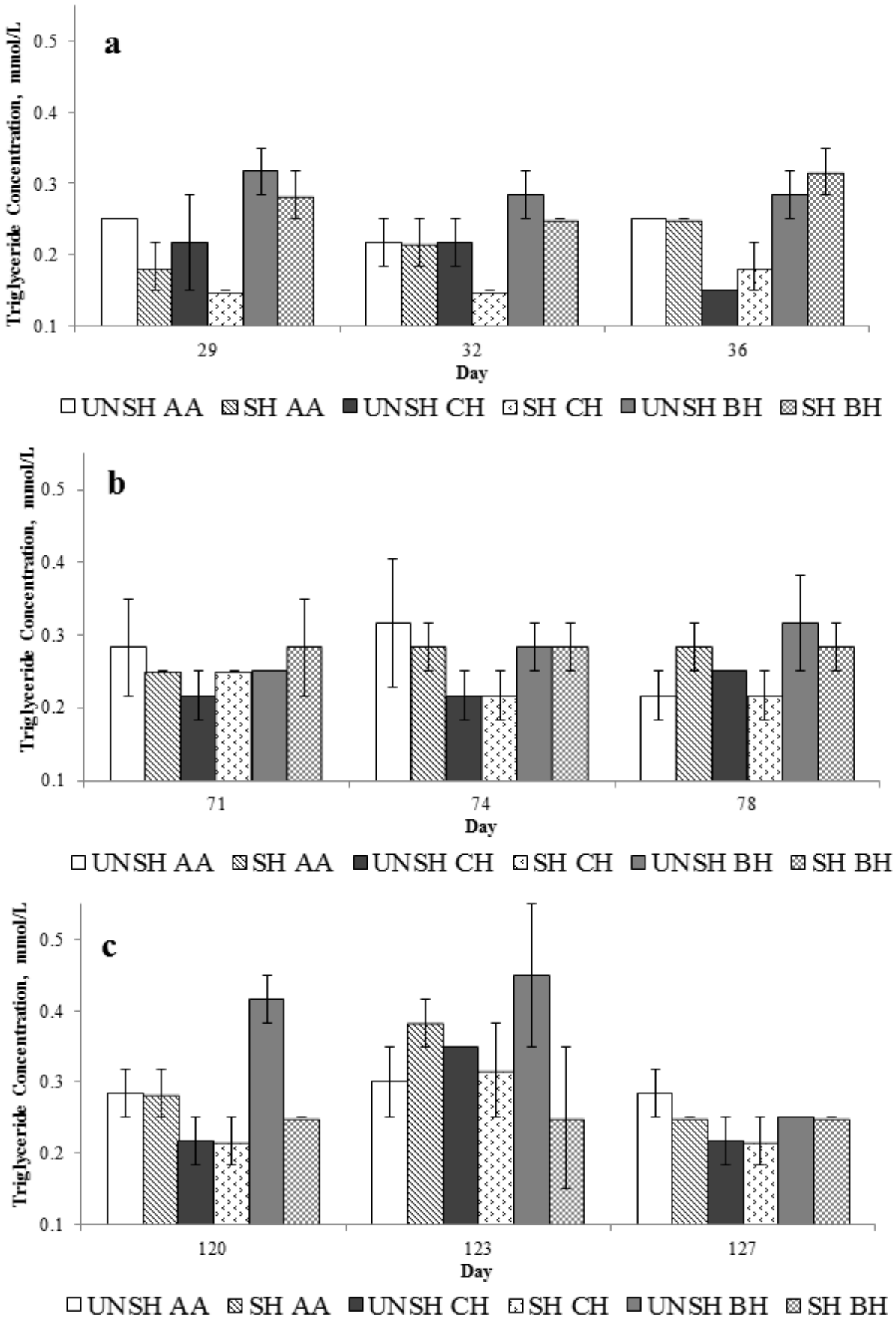


Figure 5.4.25: Plasma triglyceride concentration (mmol/L) for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

5.4.4 Discussion

Circulating haematological parameters, including enzymes and hormones, can be measured to determine the overall impact of heat load on an animal. These biological expressions to heat load arise due to an inability to maintain homeostasis (Carroll et al., 2012). Animal production and wellbeing is compromised during heat load as metabolism and the hierarchy of nutrient utilisation is altered (Johnson et al., 2015). Hyperthermia as a result of heat load potentially promotes oxidative stress (Lacetera et al., 2006), whereby the thermal challenge alters the antioxidant status (Bernabucci et al., 2002), compromising physiological and metabolic functions (Bernabucci et al., 2005) of the animal.

During periods of high heat load, absorbable nutrients are diverted from growth and development and directed to maintaining body temperature (Baumgard and Rhoads, 2012b), within a physiologically acceptable range. In addition to this there is a reduction in DMI and blood flow to the gastrointestinal tract. Therefore the concentration of absorbable nutrients per unit of blood volume must increase if the animal is to satisfy daily requirements (Beede and Collier, 1986) and maintain normal functions. Chang (1993) reported that common denominators in lethal cases of hyperthermia are due to the interactions between high metabolic heat loads and hot environmental conditions, particularly where environmental conditions are contributing to the accumulation of heat, or failure of heat dissipation. This may lead to a failure of thermoregulatory mechanisms (Chang, 1993), resulting in the accumulation of heat load greater than what is physiologically acceptable.

5.4.4.1 Creatine Kinase

In cattle creatine kinase is identified as three isoenzymes specific to i) skeletal muscle; ii) cardiac muscle; and iii) brain derived (Sattler and Fürll, 2004). In healthy cattle circulating concentrations are predominantly derived from skeletal muscle (Sattler and Fürll, 2004). In the current experiment creatine kinase concentrations, pooled by breed × treatment, were within the reference range for bovines. For bovines creatine kinase has a normal plasma concentration between 21 and 280 U/L (Radostits and Done, 2007). Kanelov et al. (2008) reported that the basal serum creatine kinase concentrations are highly variable, between 2 U/L and 39 U/L, between individuals. In the current experiment, plasma creatine kinase concentrations were also highly variable across all period and heat wave sampling time points.

Rhabdomyolysis, degradation of striated muscle, is a common feature of hyperthermia as a result of physical exertion (Chang, 1993). As creatine kinase is a measure of muscular degeneration it would

be expected that during heat load periods, cattle would have greater circulating levels due to changes in respiratory dynamics associated with heat load, i.e. an increase in respiration rate and panting score particularly un-shaded Angus steers. However this was not observed within the current experiment. Shaded cattle had a higher pooled creatine kinase concentration when compared with their un-shaded counterparts (194.1 ± 14.0 IU/L *v* 234.7 ± 14.0 IU/L) during period 1, although pooled concentrations were within the reference range for bovines. The results from this experiment support those of Scharf et al. (2010). The authors reported that there were no breed (P = 0.80) or heat exposure (P = 0.53) or breed × temperature (P = 0.98) effects on serum creatine kinase (U/L) concentrations in Angus (thermoneutral, 126.44 ± 20.90 U/L; heat 114.44 ± 20.90 U/L) or Romosinuano (thermoneutral, 131.00 ± 20.90 U/L; heat 118.00 ± 420.90 U/L) steers (Scharf et al., 2010).

During period 1 there was a tendency (P > 0.05) for creatine kinase concentrations to be greater. The elevated creatine kinase concentration in these individuals could be a reflection of the i) cattle being unaccustomed to handling and consistent exposure to people and/or ii) animals that are nervous or have flighty/excitable temperaments. Additionally climatic conditions may not have been sufficient to incite an elevation in plasma creatine kinase, associated with muscular degradation, prior to sample collection. Creatine kinase has a half-life of 2 to 4 hours; however persistently elevated creatine kinase concentrations are indicative of continuous muscular degeneration, or muscle damage has occurred within the previous 48 hours (Radostits and Done, 2007). Given that muscular damage can be detected within a 48 hour period, a creatine kinase response during heat wave event 2 would have been expected; however creatine kinase concentrations were within the reference range (34.76 IU/L to 233.15 IU/L). Therefore it is unclear whether sample collection time points were appropriate to assess creatine kinase concentration within the current experiment.

5.4.4.2 Cytokine Interleukin-6

Pro-inflammatory cytokines, specifically tumour necrosis factor-alpha, interleukin 1β and interleukin 6, are indicators of acute inflammation in cattle (Carroll and Burdick Sanchez, 2014). Nakajima et al. (1997) reported that the serum cytokine interleukin 6 concentration of clinically healthy dairy cows was < 20 pg/mL. In the current experiment minimum plasma cytokine interleukin 6 concentration determined was 100.8 pg/mL and the maximum was above maximum standard (50 000 pg/mL). These results indicate chronic inflammation in these cattle for the duration of the experiment. However it remains unclear as to the cause of this inflammation, as it is difficult to separate exposure to environmental, nutritional and social stressors. Additionally the

interrelationship of these stressors combined with the dynamic nature of the stress response makes it difficult to define a singular stressor resulting in the elevation in cytokine interleukin 6 concentration.

Within the current experiment there was no association between rumen or rectal temperatures and cytokine interleukin 6 concentrations. However Carroll et al. (2009) reported moderate association ($r = 0.21$; $P < 0.05$) between rumen temperature and cytokine interleukin 6. Additionally studies in mice have reported an increase in cytokine interleukin 6 concentration association with maximum core body temperature, where cytokine interleukin 6 concentration remained elevated 24 hours post maximum core body temperature (45.0 ± 17.4 pg/mL) (Leon et al., 2006). In human patients Leon et al. (2006) suggested that the variability in cytokine response may be indicative of the different roles pro-inflammatory cytokines have in heatstroke. A study by Hershko et al. (2003) indicated that human intestinal epithelial cells (Caco-2) treated with cytokine interleukin 6 (20 ng/mL) resulted in cellular thermotolerance when exposed to 48 °C for 2 hours. This suggests that cytokine interleukin 6 may have cell protecting properties. However the role of cytokine interleukin 6 in thermotolerance remains unclear (Hershko et al., 2003).

5.4.4.3 Electrolytes

From the literature it is unclear what influence heat load has on circulating electrolyte concentrations; however it is important to acknowledge that electrolyte balance is essential for maintaining a normal acid-base balance. Electrolyte losses occur through drooling, salivation, sweating and urination (Mader et al., 2010a). Given that heat load is associated with an increase in respiration rate/panting score (Brown-Brandl et al., 2006a; Mader et al., 2006; Gaughan and Mader, 2014), sweating rate (Carvalho et al., 1995; Collier et al., 2008; Mader et al., 2010a) and increased urination (Mader et al., 2010a), electrolyte losses are not unexpected during hot weather. Changes in electrolyte balance, and plasma pH, reduce the buffering capacity of the blood, therefore impairing normal cellular function (Sparke et al., 2001). The maintenance of the electrolyte balance is a function of glomerular filtration and tubular re-absorption within the kidney (Wilcox, 1983).

5.4.4.3.1 Bicarbonate

During heat load conditions bicarbonate may be influenced by an increase in respiration rate. In order for the blood to remain an effective pH buffering system, the body needs to maintain a bicarbonate to carbon dioxide balance of 20:1 (Schneider et al., 1988; Baumgard and Rhoads, 2007). In the current experiment there were period ($P < 0.0001$) effects on plasma bicarbonate concentration. There was a generalised trend of increasing bicarbonate concentration for all breed ×

treatment groups. Additionally during the heat wave events bicarbonate concentrations were greatest ($P < 0.0001$) during event 3. Samples collected on d 3 of each heat wave event were higher ($P < 0.0001$) than samples collected on days 1 or 2. Bicarbonate concentration tended to be lower during heat wave events, which is consistent with the findings of Beatty et al. (2006). The authors reported that bicarbonate concentration of *Bos taurus* and *Bos indicus* heifers was significantly reduced during and after heat exposure (Beatty et al., 2006). Although Khelil-Arfa et al. (2014) reported that T_A did not affect concentration of bicarbonate, blood pH was significantly higher in heat exposed (28 °C; pH ≥ 7.35; $P \leq 0.001$) lactating cows, compared with cows at thermoneutral (15 °C) conditions.

Plasma bicarbonate concentrations were mostly within the reference range for bovines (17 to 29 mmol/L; Kaneko et al., 1997). However there were some bicarbonate concentrations between 14 and 16 mmol/L, in both period and heat wave samples. A decrease in bicarbonate concentration may be representative of metabolic acidosis (Enemark, 2008) or sub-acute ruminal acidosis (Brown et al., 2000). The diagnosis of acidosis is difficult as the clinical signs are typically subtle and are variable between individuals (Gozho et al., 2005; Enemark, 2008). Feedlot cattle can be prone to ruminal acidosis as a result of the high starch diet, resulting in shifts of microflora populations which can be associated with a decrease in rumen pH (Goad et al., 1998; Gozho et al., 2006).

5.4.4.3.2 Chloride

It is unclear the effect of heat load on plasma chloride concentration. However studies have indicated that plasma chloride concentration increases in dairy cows (Sanchez et al., 1994), *Bos taurus* steers (Beatty et al., 2006) and sheep (Srikandakumar et al., 2003) during heat load. However Scharf et al. (2010) reported that there were no breed ($P = 0.13$) or heat exposure ($P = 0.22$) influences on serum chloride (mEq/L) concentrations in Angus (thermoneutral, 101.78 ± 1.57 mEq/L; heat 101.56 ± 1.57 mEq/L) or Romosinuano (thermoneutral, 101.28 ± 1.57 mEq/L; heat 97.78 ± 1.57 mEq/L) steers. This supports the findings of Olbrich et al. (1971), concluding that there was no difference ($P > 0.05$) in serum chloride concentration between Zebu (151.0 ± 3.3 mEq/L) and Highlander (148.2 ± 3.3 mEq/L). In the current study, there was a period effect ($P = 0.003$) on plasma chloride concentration, whereby period 4 had a lower plasma chloride concentration (99.3 ± 0.4 mmol/L) compared with other periods.

Chloride concentration remained relatively stable throughout the duration of the study with most samples within the reference range for bovines (97 to 111 mmol/L; Kaneko et al., 1997). Beatty et al. (2006) indicated that plasma chloride concentrations of *Bos taurus* and *Bos indicus* heifers were

variable during heat load. Variability in plasma chloride concentration was observed in the current study. Additionally there were some chloride concentrations below the reference range, particularly during heat wave 2 where a majority (72 %) of the samples were between 72 and 96 mmol/L. Hypochloremia can be indicative of dehydration (Ohtsuka et al., 1997); the result of high plasma sodium; acute renal disorders (Divers et al., 1982); or the reduction of gastric contents (Sahinduran and Albay, 2006). However it is probable the hypochloremia observed within the current experiment is representative of dehydration and reduced DMI intake associated with high heat load.

5.4.4.3.3 Potassium

Potassium concentration is expected to remain stable as it is well maintained within the body (Scharf et al., 2010). However it is possible that plasma potassium concentrations do not reflect total potassium storage within the body, as potassium is primarily maintained within the intracellular fluid (Beatty et al., 2006). There are disagreements regarding the effect of heat load on circulating potassium concentrations, where some studies report that there is an increase (Srikandakumar and Johnson, 2004), decrease (El-Nouty et al., 1980) and no effect (Scharf et al., 2010) on plasma concentrations. In the current study, pooled plasma potassium concentration was greater ($P < 0.0001$) in period 1 compared to other periods. There was also a general trend for a higher potassium concentration for all breed × treatment groups during period 1. During heat wave events potassium concentrations were highly variable, although there were heat wave ($P = 0.006$) and sample within heat wave ($P < 0.0001$) effects on potassium concentrations.

Potassium concentrations were generally within the reference range, 3.9 to 5.8 mmol/L (Kaneko et al., 1997) and concentrations were variable. There were some samples that were < 3.9 mmol/L and > 5.8 mmol/L. Minimum potassium concentration was 3.2 mmol/L and maximum was 6.3 mmol/L. Hypokalaemia in adult cattle is defined as plasma or serum potassium concentrations < 3.9 mEq/L (Radostits and Done, 2007). Hypokalaemia is associated with several diseases as well as an inadequate potassium intake (Earle et al., 1951). Inadequate dietary potassium would be unlikely in the current experiment given the formulated diet. However there is a prevalence of hypokalaemia in sick lactating dairy cows, which is partially accounted for by a decrease in DMI (Constable et al., 2014). A reduction in DMI is more likely to account for some of the variability in plasma potassium concentrations within the current experiment, especially within heat wave event 2.

Scribner et al. (1955) reported that respiratory acidosis resulted in increasing potassium concentration in the extracellular space. Hyperventilation may indicate a compensatory response to metabolic acidosis, which is potentially associated with a bicarbonate imbalance due to an increased

respiration rate during heat load. An increase in respiration rate is associated with an increase in carbon dioxide being exhaled (Baumgard and Rhoads, 2007), this increase in respiration results in a decrease of carbon dioxide in blood. This imbalance of carbon dioxide in the blood initiates bicarbonate secretion from the kidneys that can be utilised, through saliva, to buffer and maintain rumen pH (Baumgard and Rhoads, 2007), avoiding the onset of ruminal acidosis (West, 2003). Incidentally it is difficult to determine whether the high potassium concentrations within this experiment are representative of true hyperkalemia. As there were no consistent trends of elevated potassium concentration, it is possible that a proportion of the blood cells rupture post sample collection, resulting in an increase in plasma potassium concentration.

5.4.4.3.4 Sodium

El-Nouty et al. (1980) reported a reduction ($P < 0.01$) in serum sodium concentration under heat load. However Scharf et al. (2010) reported that heat load did not affect ($P = 0.95$) serum sodium (mg/dL) concentrations. The authors identified breed × temperature ($P = 0.05$) where serum sodium concentration heat exposure of Angus increased (thermoneutral, 141.11 ± 1.31 mg/dL; heat 144.44 ± 1.31 mg/dL) and decreased in Romosinuano (thermoneutral, 143.63 ± 1.31 mg/dL; heat 140.44 ± 1.31 mg/dL) steers. In the current experiment there were no breed × treatment effects on plasma sodium concentration during period samples. There was a period ($P = 0.0008$) and treatment × period ($P = 0.01$) effects, whereby pooled sodium concentrations were lower in shaded cattle (135.9 ± 0.6 mmol/L v 139.2 ± 0.6 mmol/L); however the concentrations were within the reference range, 132 to 152 mmol/L, for bovines (Kaneko et al., 1997).

Within the current experiment there were breed × treatment effects ($P = 0.04$) during heat wave events 1 and 2 on plasma sodium concentration, particularly during heat wave 2. Nevertheless during heat wave events pooled sodium concentrations were typically lower in samples collected on d 2. This was attributed to an increase in sweating rate. Mader et al. (2010a) indicated that as T_A approaches body temperature, sweating becomes a key physiological mechanism for heat dissipation. Additionally when T_A is equal to body temperature, heat loss via evaporation becomes the predominant method of heat dissipation (Esmay, 1969), where at 32 °C evaporative heat loss accounts for 85 % of an animal's total heat loss (Avendaño-Reyes et al., 2010). Additionally heat load is associated with increases in salivation, sweating and urination (Mader et al., 2010a). Furthermore as sweat contains a higher proportion of sodium (Johnson, 1970), reductions in plasma sodium during heat load are not unexpected.

5.4.4.4 Haptoglobin

Upon tissue damage or inflammation the body responds by activating an innate systemic response to inflammation, infection, disease and/or trauma (Carroll et al., 2009; Ceciliani et al., 2012). Thus acute phase proteins are also actively involved in the repair and remodelling of damaged tissues (Carroll and Burdick Sanchez, 2014). A characteristic reaction to hyperthermia involves a shift in liver synthesis to promote the production of acute phase proteins (Carroll et al., 2009). The concentration of circulating plasma is 0.3 mg/mL to 3 mg/mL (Bertaggia et al., 2014), while in clinically healthy cattle the reference value is $\leq 0.20 \pm 0.03$ mg/mL (Nazifi et al., 2008). Although there were some samples where haptoglobin concentration was within the reference range, < 0.20 mg/mL, there were samples that were greater (maximum = 0.54 mg/mL), indicating an acute response. Haptoglobin concentrations were highly variable and no consistent trends were observed.

An increase in circulating haptoglobin is associated with an increase in pro-inflammatory cytokines (Maes et al., 1995; Langlois and Delanghe, 1996; Carroll and Burdick Sanchez, 2014). Therefore elevated plasma haptoglobin was not unexpected within the current experiment due to the variability in cytokine interleukin 6 concentrations. There was between individual and within individual variability in plasma haptoglobin. Plasma haptoglobin concentrations were lower in Angus steers during period 1 (P = 0.01). Additionally heat wave, had an effect (P = 0.0008) on plasma haptoglobin where pooled concentration increased with each heat wave (event 1, 0.23 ± 0.02 mg/mL; event 2, 0.30 ± 0.02 mg/mL, event 3, 0.31 ± 0.02 mg/mL). Carroll and Burdick Sanchez (2014) indicated that the magnitude and duration of haptoglobin response appeared to be associated specifically to a stressor. The authors concluded that haptoglobin profiles may provide informative information regarding animal health and wellbeing. There is the potential that the acute phase protein response is stressor specific, indicating that the haptoglobin profile may prove to be characteristic of specific disease (Godson et al., 1995), or stressors. Additionally Álvarez-Blasco et al. (2009) reported obese women (body mass index $\geq$ 30 kg/m^2) had serum haptoglobin concentrations that were greater than non-obese women (body mass index $\leq$ 30 kg/m^2), indicating that body composition potentially influences circulating haptoglobin. Although a trend for increasing plasma haptoglobin was not observed within this experiment, chronic low grade inflammation has been associated with metabolic conditions such as obesity related insulin resistance and diabetes (Suagee et al., 2012).

5.4.4.5 Heat Shock Protein 70

Although a HSP_{70} response was not observed within this experiment it is important to consider that HSP have been highly conserved through evolution, highlighting their biological significance. It is

understood that HSP function as intracellular chaperones or proteases where they become responsible for the assembly, stabilisation, folding and translocation of oligomeric proteins as well as the degradation of damaged protein structures (Pockley, 2003). However it is unknown whether there is a complete understanding of their molecular function. Furthermore knowledge regarding the initiation and role of HSP within the heat load response is not yet fully understood. Roy and Collier (2012) indicated that the ability of an animal to tolerate thermal challenges at the cellular level is a direct function of their ability to maintain elevated levels of circulating HSP. However Lindquist and Craig (1988) indicated that there are circumstances where exposure to heat load does not correlate with the initiation of the HSP response. Additionally attempts to identify the role of singular proteins in thermotolerance have been unsuccessful (Lindquist and Craig, 1988). However HSP are also involved in the activation of the innate immune system by increasing the concentration of cytokines interleukin 1, 6, 12 and tumour necrosis factor-alpha (Tsan and Gao, 2004).

When using HSP to determine the thermotolerance of animals it is important to consider the impact of other stressors on the expression of HSP. Gaughan et al. (2013) indicated that individual stressors may potentially have a mild impact on the animal; however a HSP response could be initiated where the animals are subjected multiple stressors. Furthermore Fader et al. (1994) concluded that caution must be maintained in the development of HSP as biomarkers, whereby individual basal levels need to be taken into consideration to prevent the misdiagnosis of stressed animals. Additionally the heat shock response is potentially further confounded by the animal's normal developmental acclimation response to the changing seasons (Fader et al., 1994). It is still yet to be determined whether circulating HSP concentration alone is able to be developed into a biomarker for heat tolerance in cattle.

Using circulating HSP concentration to define heat tolerance is controversial and inconsistent as HSP are not only expressed in response to thermal stressors. Any stressor, including nutritional and psychological stressors, will incite the HSP response; therefore quantifying thermal tolerance through circulating HSP levels has the potential to be misleading. Basiricò et al. (2011) concluded that gene markers to determine the animal's ability to produce HSP may be a more reliable indicator of an animal's ability to maintain bodily functions during stressful periods. Lindquist and Craig (1988) indicated that *Drosophila spp.* studies have identified that HSP_{70} is a member of a multigene family which includes 5 to 6 copies of the HSP_{70} gene plus a singular copy of the HSP_{68} gene. Using gene marker technology to determine heat tolerance may allow for the identification of animals with genes that support heat tolerance in heat sensitive, i.e. *Bos taurus*, breeds thus

improving animal performance and wellbeing during periods of heat load (Hammond et al., 1996; Hansen, 2004). This particularly applies where these heat tolerant animals are comparable or perform better than *Bos indicus* genotypes in terms of economically important traits (Hammond et al. 1996), specifically the ability to maintain growth during hot weather. Therefore the use of gene marker technology may have the potential to provide more insightful information regarding an animal's heat tolerance and warrants further investigatory studies.

5.4.4.6 Glucose

In ruminants glucose availability is predominantly supplied by hepatic gluconeogenesis (Itoh et al., 1998). Circulating plasma glucose levels become the equilibrium between glucose production and utilisation within the body (Itoh et al., 1998), where normal basal plasma concentration in bovines is between 2.50 and 4.16 mmol/L (Kaneko et al., 1997). In the current experiment glucose concentrations are reflective of basal concentrations, whereby cattle were fed at approximately 1430 h the day prior to blood sample collection. There was a trend for increasing plasma glucose concentration over time. This was unsurprising given the high energy feed, whereby Nikkhah et al. (2008) reported high concentrate diet (forage to concentrate ratio = 38:62) increased ($P = 0.001$) the plasma glucose concentration of lactating dairy cows.

Heat load is thought to increase glucose usage by skeletal muscle (Rhoads et al., 2013a), where numerous authors have reported a decrease in circulating glucose concentration (Achmadi et al., 1993; Itoh et al., 1998; O'Brien et al., 2010; Scharf et al., 2010). However Abeni et al. (2007) found a significant association ($P < 0.001$) between plasma glucose concentration and maximum daily THI over a two year study (year 1, r = -0.48; year 2, r = -0.26). Within the current study, breed had a greater effect on plasma glucose concentration. Brahman steers tended to have higher plasma glucose concentrations than Angus steers particularly during periods 1 ($P < 0.0001$) and 2 ($P = 0.003$). Additionally during heat wave 1, there was an effect of breed × heat wave ($P = 0.04$), whereby during heat wave 1 Brahman (5.1 ± 0.3 mmol/L) steers had greater circulating glucose concentrations compared to Angus (4.5 ± 0.3 mmol/L) and Charolais (4.5 ± 0.3 mmol/L; $P = 0.008$) steers. Numerous authors have noted lower metabolic rates in Brahman cattle (Kibler and Brody, 1950; Vercoe, 1970; Frisch and Vercoe, 1977). Perhaps the initial difference in plasma glucose concentration within the current experiment is a reflection of the metabolic differences of *Bos indicus* and *Bos taurus* genotypes. This is supported within the current experiment as days on feed increased, the variability in glucose concentration between breed × treatment groups decreased.

5.4.4.7 Insulin

Insulin is an important regulator of carbohydrate and lipid metabolism (Baumgard and Rhoads, 2012b); however the main activity of insulin receptors is to prompt glucose uptake (Mehla et al., 2014), in muscle and adipose tissues. Baumgard and Rhoads (2013) suggest that insulin is typically elevated during heat load, although it is important to acknowledge that insulin response to heat load varies across studies (Achmadi et al., 1993; Itoh et al., 1998; O'Brien et al., 2010; Wheelock et al., 2010). Within the current experiment there was a defined trend of increasing plasma insulin concentration as the experiment progressed, and within each successive heat wave event. Adamiak et al. (2005) indicated that as body condition score and diet ME content increased ($P < 0.001$) plasma insulin concentration in dairy cattle, therefore it can be concluded that as feedlot cattle become heavier and fatter there is an increased likelihood of the animal becoming hyperinsulinemic.

Providing equines with high starch and high glycaemic index diets has been associated with an increased likelihood of developing insulin resistance (Hoffman et al., 2003). Insulin resistance is a metabolic condition resulting in the reduced sensitivity of insulin sensitive tissues, i.e. adipose tissue, skeletal muscle and the liver, to insulin induced glucose disposal (Treiber et al., 2005; Suagee et al., 2012; De Koster et al., 2015). Insulin resistance has been associated with persistent hyperglycemia despite increased insulin secretion (Leiva et al., 2014). This is somewhat unsurprising as plasma insulin concentration is essentially determined by plasma glucose concentration (McCarthy et al., 1977). Therefore the increased insulin concentration within this experiment may be reflective of elevated glucose concentration, and indicative of insulin resistance.

5.4.4.7.1 Glucose Insulin Ratio

Literature regarding the glucose to insulin ratio in cattle is deficient. Numerous studies have investigated the influence of heat load on glucose and insulin concentrations (Achmadi et al., 1993; Itoh et al., 1998; Abeni et al., 2007; O'Brien et al., 2010; Scharf et al., 2010; Wheelock et al., 2010; Pearce et al., 2013; Rhoads et al., 2013a). However these studies do not identify the relationship between glucose and insulin by means of glucose to insulin ratio. Within the current experiment there was a trend for decreasing glucose to insulin ratio over period ($P < 0.0001$) and consecutive heat waves ($P = 0.0002$). Glucose to insulin ratio was variable, associated with the variability of plasma glucose and insulin concentrations, resulting in treatment × breed ($P = 0.05$) and breed ($P = 0.05$) effects. Additionally during heat wave events there were effects of breed ($P = 0.02$), where Brahman steers also tended to have a greater glucose to insulin ratio. This was particularly evident

during heat wave 2 (P = 0.0097) where pooled ratio for Brahman steers was 1.3 ± 0.2, compared with 0.6 ± 0.2 and 0.8 ± 0.2 for Angus and Charolais steers respectively.

In women with polycystic ovary syndrome the fasting glucose to insulin ratio is a good measure of insulin sensitivity in obese (Legro et al., 1998) and non-obese (Ducluzeau et al., 2003) patients. A decreased glucose to insulin ratio indicates decreased insulin sensitivity, which is typically associated with increased plasma insulin concentration and somewhat normal blood glucose concentrations (Ducluzeau et al., 2003). Obese women with polycystic ovary syndrome typically have elevated fasting glucose concentrations, as a result of an increased basal hepatic glucose production, which reflects hepatic insulin resistance (Legro et al., 1998). The fasting glucose to insulin ratio reflects both insulin resistance and insulin sensitivity; however the glucose to insulin ratio appears to be a more sensitive marker of insulin resistance (Legro et al., 1998). Insulin resistance has been associated with glucose to insulin ratio values of ≤ 4.5 (Parra et al., 1994; Legro et al., 1998) and < 6.4 (Carmina and Lobo, 2004) in human patients and ≤ 10 in equines (Divers, 2008). Pooled by breed × treatment maximum (4.68 ± 2.39) and minimum (0.31 ± 0.03) glucose to insulin ratio were observed in un-shaded Brahman (period 1) and shaded Angus (heat wave 2; sample 1; d 71) steers respectively. Although not established for cattle, the low glucose to insulin ratios observed within the current experiment may be indicative of insulin resistance. However a threshold to describe insulin resistance and sensitivity in cattle is necessary.

The prevalence of insulin resistance in obesity is unknown (Ferrannini et al., 1997). Most of the literature is focused on polycystic ovary syndrome; however studies in equines highlight an association between insulin resistance and metabolic syndrome (Divers, 2008). Given the links between polycystic ovary syndrome and reproductive endocrine functions, these studies indicate that insulin resistance is associated with metabolic and endocrine parameters. Pro-inflammatory cytokines have been associated with insulin resistance whereby these cytokines impair glucose disposal by insulin sensitive tissues (Yudkin et al., 2000; Suagee et al., 2012). The association between pro-inflammatory cytokines and insulin resistance is related to the inflammation associated with obesity (Suagee et al., 2012). Given the elevated cytokine interleukin 6, glucose and insulin concentrations combined with the high energy diet, designed for weight gain and fat deposition, insulin resistance in the cattle of the current experiment does not seem unfounded.

5.4.4.8 Lipids

Heat load effects lipid metabolism, and the alteration to this metabolic pathway ensures partitioning of nutrients, derived from the diet and tissue, towards muscles (Baumgard and Rhoads, 2013).

Although the mechanisms are unclear, under heat load conditions animals do not mobilise adipose tissue triglycerides (Baumgard and Rhoads, 2012a). Failure to mobilise adipose tissue despite being in a hypercatabolic condition is potentially associated with changes in insulin homeostasis during heat load (Sanz Fernandez et al., 2015), as insulin acts as a regulator of lipid metabolism (Baumgard and Rhoads, 2012b). However limiting adipose tissue mobilisation during heat load prevents the animal from initiating glucose sparing mechanisms normally enlisted to maintain skeletal muscle synthesis (Baumgard and Rhoads, 2012a), during exposure to stressors.

5.4.4.8.1 Cholesterol

The clinically normal range of plasma cholesterol concentrations in bovines is 1.50 – 2.28 mmol/L (Kaneko et al., 1997). Results from the current experiment show that there was a general trend of increasing plasma cholesterol concentration over time. Additionally, Brahman steers tended to have higher plasma cholesterol concentration compared with Charolais and Angus steers across periods. Numerous studies have reported differences in plasma/serum cholesterol concentration between *Bos taurus* and *Bos indicus* genotypes (O'Kelly, 1968a, b; Olbrich et al., 1971; Scharf et al., 2010). O'Kelly (1968a) and O'Kelly (1968b) reported that irrespective of diet, grazing pasture versus lucerne (25% CP), *Bos indicus* had plasma cholesterol concentrations that were higher ($P < 0.05$) than *Bos taurus* genotypes. Given the ability of *Bos indicus* to survive in harsh conditions, the difference in plasma cholesterol concentration is potentially a reflection of the metabolic differences between *Bos indicus* and *Bos taurus* genotypes, possibly accounting for the differences observed within this experiment.

Studies investigating the effect of heat load on plasma/serum cholesterol concentrations are limited. Although a recent study reported down regulation of the genes fatty acyl coenzyme A reductase 1; alkylglycerone phosphate synthase; hydroxy-3-methylglutaryl- coenzyme A synthase, and cholesterol biosynthesis, sterol-C4-methyl oxidase responsible for lipid metabolism in response to heat load (THI > 104) (Mehla et al., 2014). Additionally Scharf et al. (2010) reported that heat load resulted in an increase in serum cholesterol concentration of Angus (thermoneutral, 64.11 ± 6.17 mg/dL; heat 91.55 ± 6.17 mg/dL) steers. However there was no difference in serum cholesterol concentration in Romosinuano steers at thermoneutral (58.01 ± 6.17 mg/dL) or heat exposed (64.88 ± 6.17 mg/dL) (Scharf et al., 2010). As the current experiment progressed, the number of samples where plasma cholesterol concentrations > 2.28 mmol/L increased. There is a link between fat deposition and an increase in plasma lipoprotein concentrations (Jeusette et al., 2005). Given this and in conjunction with the wide acceptance of the association of days on feed and increasing body

fat composition, the increase in plasma cholesterol concentration over time in this experiment was unsurprising.

5.4.4.8.2 Triglycerides

Under physiologically normal conditions plasma triglyceride concentrations are between 0.0 and 0.2 mmol/L in cattle (Kaneko et al., 1997). However the nutritional status of cattle can influence plasma triglyceride concentration (O'Kelly, 1968b). Given this, all plasma triglyceride concentrations within the current experiment were within the reference range for bovines. However there were breed (P = 0.002), period (P = 0.004) and breed × period (P =0.0004) effects. Pooled plasma triglyceride concentrations were higher in Angus steers during period 1 and Brahman steers during periods 2 and 4. There was a trend of increasing pooled plasma triglyceride concentration with each heat wave event (heat wave 1, 0.18 ± 0.01 mmol/L; heat wave 2, 0.21 ± 0.01 mmol/L; heat wave 3, 0.24 ± 0.01 mmol/L). Scharf et al. (2010) found that heat exposure (26 °C to 36 °C) increased (P = 0.05) serum triglyceride concentration of Angus (heat, 19.22 ± 1.88 mg/dL; thermoneutral, 15.22 ± 1.88 mg/dL) and Romosinuano (heat, 19.88 ± 1.88 mg/dL; thermoneutral, 15.64 ± 1.88 mg/dL) steers. O'Kelly (1968a) and O'Kelly (1968b) reported no differences in serum triglyceride concentration in Brahman or Brahman crossbred bulls; however in heifers the authors found that Africander × *Bos taurus* (31.2 ± 5.2 mg/ 100 mL) and *Bos taurus* (32.1 ± 0.1 mg/100 mL) genotypes had serum triglyceride concentrations that were greater (P < 0.01) than Brahman (16.4 ± 0.6 mg/ 100 mL), Brahman × Hereford (15.8 ± 3.6 mg/ 100 mL) and Africander (21.6 ± 1.8 mg/100 mL) under grazing conditions.

Results from the current experiment suggest little change in triglyceride concentrations, although there were some statistically significant effects, the plasma triglyceride concentrations were within the reference range for cattle. An early study in pigs suggests that in tropical environments plasma concentration of triglycerides is elevated (Christon, 1988). Hot T_A decreases oxidative reactions resulting in limited utilisation of fatty acids for energy, thus favouring hepatic synthesis of triglycerides (Christon, 1988). Additionally exposure to a hot environment increases adipose tissues lipoprotein lipase (Christon, 1988), indicating that animals have an increased capacity to uptake and store intestinal and hepatic derived triglycerides (Baumgard and Rhoads, 2012a) when experiencing heat load. In rats it has been shown that plasma insulin is a mediator of triglyceride production and secretion, where a decrease in insulin concentration results in lower triglyceride secretion and reduced removal from circulation (Iwai et al., 1989). Therefore the increase in circulating insulin and triglycerides is likely to be representative of an increase in alternative energy supplies during heat load periods. However it remains unclear what effect summer weather has on plasma

triglyceride concentrations, as the literature suggests that there have been limited investigations into this area.

5.4.5 Conclusion

These results suggest that haematological parameters are closely interrelated. These relationships indicate that metabolites and metabolic hormones are key participants in stress and immune interactions (Carroll and Burdick Sanchez, 2014). Furthermore the interactions of these haematological parameters in response to stressors ensure animal survival, although there are relatively large gaps in the knowledge regarding the metabolic and biochemical changes that occur during heat exposure (Rhoads et al., 2013b). The mechanisms in which haematological factors influence other parameters remains somewhat undefined and there are many inconsistencies within the literature. Nevertheless haematological parameters can be sensitive indicators of thermal stress in cattle (Mitlöhner et al., 2002).

Chapter 6

General Discussion, Implications of Research and Conclusions

6.1 Introduction

If the forecasted climate change predictions are accurate, global warming is likely to have a significant impact on the stability and sustainability of intensive livestock production enterprises worldwide. In Australia, the mean ambient temperature ($\mathbf{T_A}$) has been increasing linearly by 0.19 °C per decade, between 1970 and 2006 (Murphy and Timbal, 2008). Numerous species are likely to be negatively impacted by the changing global environment (Hennessey et al., 2007). Many species have adaptations to cope with short term climate variability, i.e. seasonal changes; however these adaptations may not be successful for long term viability (Hennessey et al., 2007). Regardless of climate change and the predicted changes to the thermal environment, summer conditions will continue to incite heat load responses in intensive animal production systems worldwide.

The impact of hot climatic conditions, particularly heat wave events, on intensive animal enterprises worldwide highlights the importance of predictive forecasting and measurement of these conditions to allow for further development of information on managing livestock through these significant events. Moreover, Gaughan et al. (2010b) indicated that with forecasted climate change, significant economic losses are likely; particularly if current management protocols are not modified to reflect the predicted shift in climatic conditions. The ability to predict the effects of forecasted hot climatic conditions on livestock is important to producers, especially in terms of welfare and performance (Gaughan et al., 2008b).

The ability to predict hot climatic conditions is of particular importance in feedlot enterprises where the general public perception is becoming increasingly concerned about the welfare of cattle during summer. However describing the responses of animals to their thermal environment is somewhat difficult due to the dynamic relationship that exists between the animal and their environment (DeShazer et al., 2009). Key factors in describing the dynamic nature of this relationship are based on understanding the biophysical interactions that exist (DeShazer et al., 2009). This involves having an appreciation of the cumulative effects of the animals' environment, both thermal and physical, on behavioural, physiological and haematological responses of animals to hot environmental conditions.

6.2 General Considerations

Performance based selection of livestock has been used for numerous decades. In future years, producers will continue to select replacement breeding stock based on individual performances for traits that are deemed economically important. The selection pressures placed on animals has the ability to influence the genetic composition throughout successive generations. Rhoads et al.

(2013a) stated that whilst genetic improvement programs continue to place emphasis on the economically significant traits, there is the potential that this will decrease thermotolerance due to the relationship that is observed between animal productivity and increasing metabolic heat production.

Arias and Mader (2011) indicated that daily water intake increased at a temperature humidity index (**THI**) value of 67.2. The authors suggested that the threshold of 67.2 may have represented the THI value at which cattle were activating thermoregulatory mechanisms to cope with heat load (Arias and Mader, 2011). However the livestock safety weather index indicates that a THI value of between 70 and 74 is the lower threshold where cattle are experiencing no stress. This highlights that responses to heat load are exceptionally varied, between not only groups of animals, but also individual animals, and may also be indicative of the change in genotype through performance based selection. Therefore there will be a requirement to constantly re-evaluate predictive climatic models, i.e. heat load index (**HLI**), to account for the changes in animal genotype and phenotype as determined from performance based selection.

6.3 New Technologies in the Measurement of Body Temperature

Body temperature is considered to be a reliable indicator of thermal balance. However defining core body temperature is somewhat problematic as no clear definition of core body temperature is available. Numerous methods have been used as an estimate of core body temperature, i.e. tympanic (Davis et al., 2003; Mader et al., 2010a), abdominal (Lefcourt and Adams, 1996; Gaughan et al., 2010a), rumen (Ipema et al., 2008; Rose-Dye et al., 2011; Mohammed et al., 2014), and rectal (Gaughan et al., 1999; Gaughan et al., 2008a). However how these estimates of core body temperature relate to actual core body temperature has not been elucidated.

During periods of high heat load an increase in core body temperature can be considered as the function of the amount of heat gained by the animal from the thermal environment. However an animal's normal metabolic processes, i.e. digestion and locomotion, also result in an increase in core body temperature and therefore the diurnal pattern exhibited. Verwoerd et al. (2006) showed that cattle are able to isolate their body temperature from the thermal environment during moderate temperatures. When conditions become hot, however, cattle are no longer able to cope with increasing ambient conditions. Heat accumulation and dissipation from the body is constantly adjusting, however as ambient heat load increases, above a given threshold which is largely species specific, heat accumulation becomes greater than dissipation resulting in an increase in core body temperature.

Traditional methods of measuring body temperature involve relocating animals to specifically designed handling facilities (Rose-Dye et al., 2011). The relocation of animals to handling facilities may subsequently result in an increase in body temperature, due to the initiation of the stress response from handling as well as locomotion, potentially falsifying body temperature observations. Due to increasing animal welfare concerns, non-invasive methods of obtaining body temperature that are fast, efficient and reliable need to be investigated. However to be considered as an alternative method of determining body temperature, new technologies need to be not only rapid and reliable but also have a strong association with other validated measures of body temperature (Johnson et al., 2011).

6.3.1 Rumen Temperature

The use of rumen temperature data is discussed within Chapters 3 and 5. The collection of rumen temperature data allowed for real time evaluation of each individual's thermal status to be undertaken. Results from both experiments indicate that rumen temperature follows a diurnal rhythm. Generally rumen temperature was increasing, between 0800 h and 2000 h, and decreasing, between 2000 h and 0800 h. These data suggest that rumen temperature increases and decreases with increasing and decreasing ambient conditions. Additionally the rumen temperature of shaded cattle showed less variation, i.e. smaller differences between maximum and minimum rumen temperature, than un-shaded cattle. These results indicate that the availability of shade was an important factor in improving the animals' ability to cope with heat load. However the greatest influence on the regulation of rumen temperature within that experiment was genotype. As a general rule Brahman steers did not have much variation in rumen temperature compared with Angus and Charolais breeds. This indicates that breed, rather the thermoregulatory abilities of *Bos indicus* cattle, had an influence on changes in rumen temperature. Further investigation into the mechanisms that *Bos indicus* cattle utilise to regulate body temperature are warranted.

Rumen boluses were programmed to transmit rumen temperature recordings to a database at 10 minute intervals throughout the duration of experiments discussed within Chapters 3 (128 d) and 5 (130 d). At each transmission of rumen temperature the previous 11 data points (110 minutes) were also enumerated, thereby minimizing potential data loss. However data losses occurred during the experiments discussed in Chapters 3 and 5. Raw data acquisition, i.e. prior to enumerating previous 11 data points (110 minutes), were 13.9 % and 30.5 % for data presented within Chapters 3 and 5 respectively. These data losses were attributed to cattle spending prolonged periods of time mulling around concrete water troughs and lying at the back of pens and behind concrete water troughs, potentially disrupting the connection between the receiver and the rumen boluses. Additionally

there were also some issues with longevity of the rumen boluses where in both experiments boluses failed. Subsequent research from our colleagues' lab in the US using rumen boluses from the same distributor, has had data acquisition rates of approximately 70 % to 80 %. There are some key differences in the feedlot where The University of Queensland (**UQ**) research feedlot water troughs are located at the back of pens and pens are made with steel RHS versus water troughs located at the front of pens and wooden posts and 2.5 cm steel cabling as pen materials in the US. Data loss in the UQ studies were attributed to cattle spending prolonged periods i) around water troughs and ii) in sternal recumbency towards the back of the pens. The location of the water troughs potentially explains the substantial difference in data acquisition with US pens having water troughs located closer to receivers. However it is unclear the impact that steel structures have on the transmission of data. Rose-Dye et al. (2011) used rumen boluses sourced from the same distributor as described within Chapters 3 and 5. The authors did not report the system frequency or any data loss (Rose-Dye et al., 2011). However in an Australian study, Beatty et al. (2008) used temperature telemeters (Datamet, Potchefstroom, South Africa), operating within the 150 to 152 MHz frequency. However data losses were not reported by the authors. It is possible the 915 to 928 MHz frequency used within the current experiments is not optimal and adjusting the operating radio frequency to suit Australian conditions may improve the data acquisition rates.

The mean differences between rumen temperatures and rectal temperatures were small using both real time (0.16 ± 0.02 °C) and hourly mean (0.13 ± 0.02 °C) temperature (Chapter 3). Additionally in Chapter 5 the mean difference between rumen temperature and rectal temperature was 0.06 ± 0.06 °C, where the majority of the data (n = 89) is situated within the 95 % confidence interval (upper limit +1.23 °C; lower limit -1.12 °C). Although there were a limited number (n = 93) of data points available from the experiment reported in Chapter 5, the use of rumen temperature was supported by the findings reported in Chapter 3 where 834 and 892 data points available for analysis using real time and hourly mean rumen temperature respectively. The determination of the relationship between rumen temperature and rectal temperature was primarily determined by the availability of rumen temperature data within the hour of obtaining rectal temperature. The data reported in Chapter 5 were based on 36 animals representing 3 breeds, i.e. 12 Angus, 12 Charolais and 12 Brahman steers; whilst data presented in Chapter 3 were based on observations from 80 Angus steers. Additionally data were collected under fasting conditions in Chapter 5 where cattle were fed once daily at approximately 1430 h, whilst data reported in Chapter 3 were collected under non-fasting conditions where the cattle were fed at approximately 0700 h and rectal temperature measurements were recorded between 0730 h and 1200 h. Considering the number of animals within both experiments and the difference in feeding schedules, the small difference observed

between rumen temperature and rectal temperature demonstrates that rumen temperature can be used as a proxy for core body temperature.

Further investigation is required to determine the true nature of the relationship between ambient conditions and rumen temperature. Additionally the relationship between rumen temperature and rectal temperature needs to be determined across different hours of the day to ensure that rumen temperature is a suitable proxy of core body temperature. The impact of water intake on rumen temperature also warrants further investigation as water intake is known to result in dramatic, but temporary, decreases in rumen temperature (Beatty et al., 2008; Bewley et al., 2008b). Additionally the amount of time it takes for rumen temperature to return to basal temperature post water intake is unknown. The influence of water consumption on rumen temperature would depend on the frequency of intake along with water temperature and amount of water consumed at each time point.

In future studies, given that body temperature was assessed in all animals via the same methodologies, i.e. rumen temperature, variability in the temperature changes are relative to the individuals and groups within the studies, thereby supporting that particular methodology. Thus it may be concluded that rumen temperature is a functional estimate of core body temperature and therefore rumen temperature can be used to measure and quantify heat load in feedlot cattle. By using remote sensing technologies, long term data is able to be collated and used to evaluate an animal's body temperature without inciting alterations to body temperature through animal handling. Furthermore access to real time body temperature data has the potential to provide useful information to quantify an animal's thermal status, therefore allowing for effective alleviation management of feedlot cattle. Although prior its commercial use improvements are required to ensure the reliability of the devices used within the rumen.

6.3.2 Infrared Thermography

There are limitations to the use of infrared thermography images as discussed within Chapter 4. Within the experiment infrared thermography images were collected whilst animals remained unrestrained in pens, in order to reduce handling stress and evaluate the usefulness of infrared thermography images in a field setting. In comparison, George et al. (2014) reported technicians physically retraining cattle and sheep within working facilities to ensure animals remained completely motionless during infrared thermography image collection. Therefore, within the experiment described in Chapter 4 there were some inconsistencies between infrared thermography images collected at each observation, i.e. not all infrared thermography images were of equal

distance from the steers. Additionally as steers were unrestrained within pens, infrared thermography images were obtained from shaded and un-shaded areas, likely impacting on the determination of body surface temperature. Furthermore each infrared thermography image was analysed manually allowing room for manual errors to occur, i.e. zones may not be identical across images, when determining body surface temperature. Additionally there is no defined standard for the assessment of infrared thermography images described within the literature. To gain a complete understanding of body surface temperature obtained by infrared thermography, the analysis of infrared thermography images should be conducted by each individual pixel; therefore the development of an automated technique would be beneficial. Furthermore the determination of body surface temperature relies to some extent on a 2 dimensional shape and this does not account for the natural curvature of the animal's body. It remains unclear what impact this has on the determination of body surface temperature. However infrared thermography images must be taken perpendicular to the animal; therefore there must be some assumed errors in the body surface temperature derived from the images.

Some studies suggest that infrared thermography may be used to determine thermal balance (Montanholi et al., 2008; Weissenböck et al., 2010; Brown-Brandl et al., 2012; Rowe et al., 2013; Nascimento et al., 2014). As discussed within Chapter 4 there appears to be no relationship between rumen temperature and body surface temperature. It is important to acknowledge that the body surface temperature determined via infrared thermography does not provide an absolute measure of core body temperature.

There is the potential that the measurement of body surface temperature can be used to determine the heat flow from the animal. Body surface temperature is dependent on the interactions between ambient climatic conditions, metabolic heat production, blood flow and coat properties, i.e. which is likely to have insulative properties (Tattersall and Cadena, 2010). Additionally subcutaneous fat is an insulator and slows heat dissipation (Bernabucci et al., 2010). The primary cardiovascular response of many species during heat exposure is vasodilation at the periphery, whereby an increase in blood flow to the skin promotes heat dissipation (Leon and Helwig, 2010). During the experiment discussed in Chapter 4 infrared thermography images were able to visualise what appear to be capillary beds on some occasions, which may be representing an increase in blood flow to the periphery. Although difficult to depict on exported images (Figure 6.1), it is probable that the ability to visualise the capillary beds is influenced by the individual's coat characteristics, i.e. thick and woolly versus fine and glossy, and sub-cutaneous fat deposition.

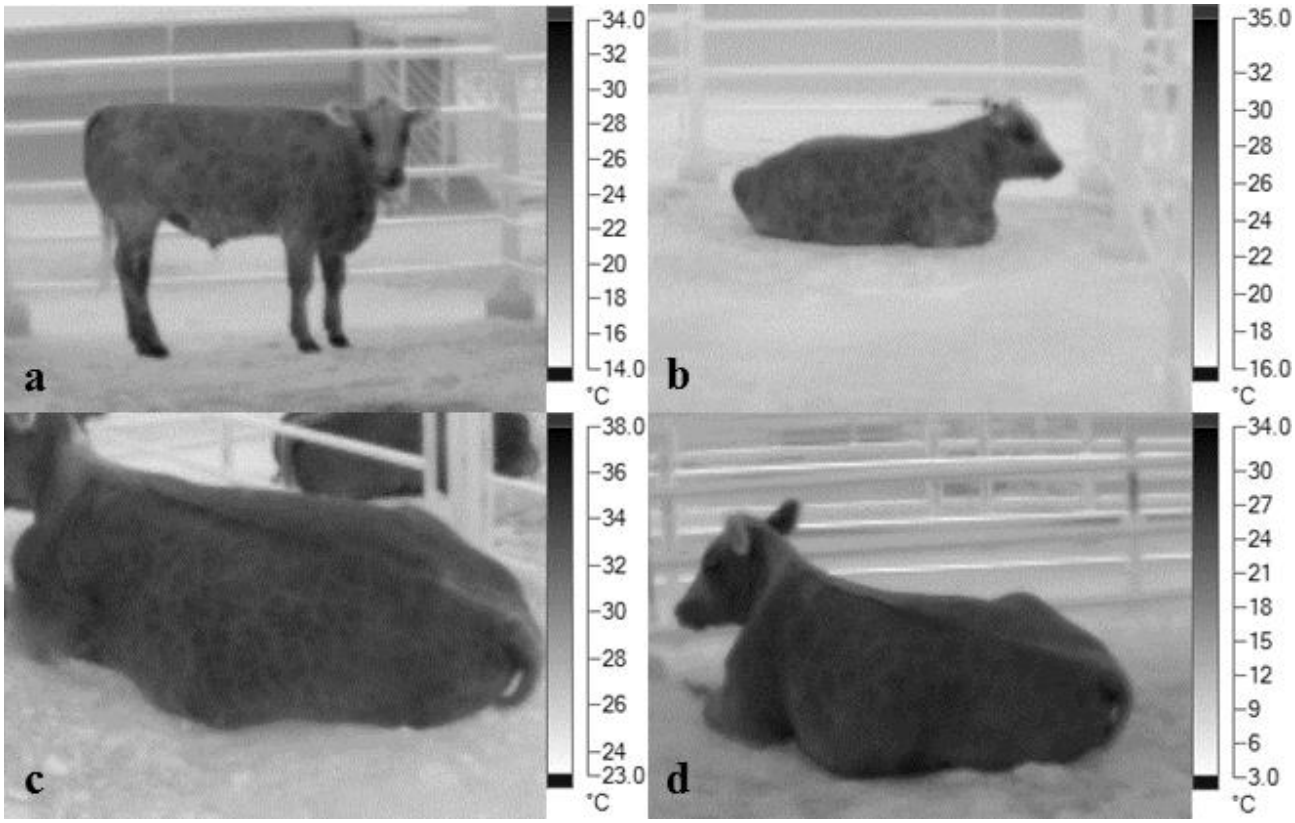


Figure 6.1 a, b, c and d: Infrared thermography images visualising capillary beds in Angus steers

The development of a model using a combination of body surface temperature and an estimation of core body temperature, i.e. rumen temperature, may provide an opportunity to further develop knowledge regarding thermal exchange. For example if core body temperature is greater than body surface temperature and body surface temperature is greater than T_A, it could be concluded that the animal would be dissipating heat to the environment. However it is important to exercise caution in the development of a thermal exchange model as not all sections of the animal's body are going to accumulate or dissipate heat at the same frequency. There is the potential that if the animal was standing in the sun and T_A is 'cool' the animal is likely to be accumulating heat along the dorsal surfaces but may in fact be dissipating heat from the ventral surfaces, being the underbelly and legs.

Body surface areas also influence thermal exchange, whereby thermal windows have been identified for numerous species (Tattersall and Cadena, 2010). These thermal windows are important regulators of thermal exchange (Tattersall and Cadena, 2010), and these areas typically receive greater cutaneous blood flow (Weissenböck et al., 2010). In mammalian species these thermal windows have been identified as the regions on the head including the ears and nose, as well as the lower legs and feet (Klir and Heath, 1994; Tattersall and Cadena, 2010; Weissenböck et al., 2010). Observations throughout infrared thermography image processing for Chapter 4 appear

to show that the thermal windows in cattle appear to be primarily the eyes and nose (Figure 6.2) and lower limbs; although further investigations would be required to clearly define thermal windows in cattle. Additionally the question arises as to whether the thermal windows are comparable in all breeds of cattle, i.e. *Bos taurus* versus *Bos indicus*.

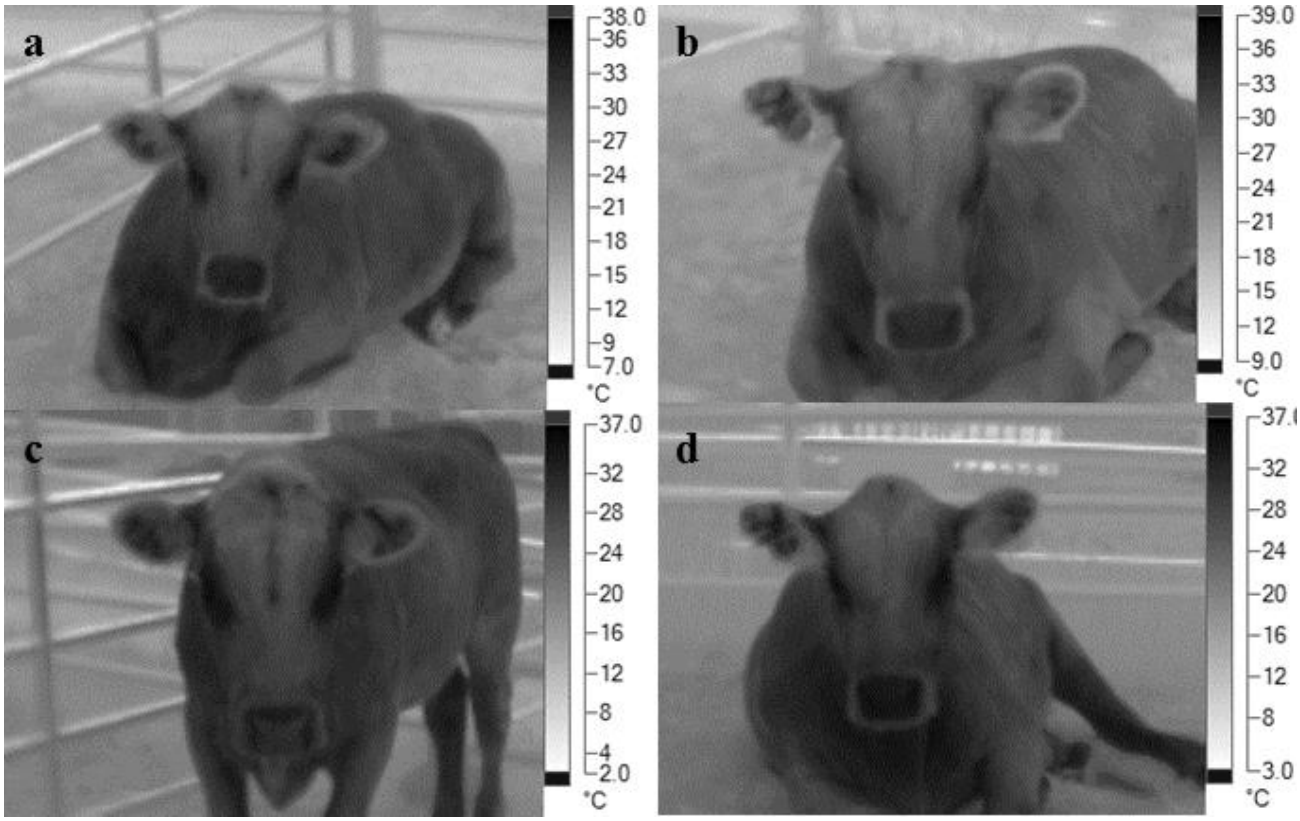


Figure 6. 2: a, b, c and d: Infrared thermography images visualising potential thermal windows of the eyes and nose in Angus steers

The activation of thermal windows in African elephants (*Loxodonta africana*) appears to be regulated by an increased arterial blood flow to specific regions of the ear (Weissenböck et al., 2010). Klir and Heath (1994) reported that foxes (*Vulpes vulpes*) have thermal windows that account for approximately 33 % of the animal's total body surface area. Additionally the authors indicated that foxes were able to actively control heat flow from the body's surface (Klir and Heath, 1994). These thermal windows have the potential to identify whether the animal was dissipating heat, as inactivated thermal windows would be indicative of the animal conserving metabolic heat (Weissenböck et al., 2010). Tattersall et al. (2009) indicated that the beak of the toucan (*Ramphastos toco*) was a thermal window, whereby the authors estimated that activation of the thermal window could be responsible for approximately 500 % of heat dissipation. It is unlikely that cattle would be able to dissipate similar heat loads through thermal windows but it is probable that these thermal windows are a key physiological mechanism, in the regulation of core body temperature. If a thermal exchange model was able to be developed that accurately describes

thermal exchange through the use of infrared thermography and core body temperature measurements, it could potentially have correction factors that would allow for application across numerous species and breeds.

6.4 Behavioural Responses

Animals have developed techniques that minimise the effect that the thermal environment has on the body as a whole. Observations of cattle behaviour can be used to quantify animal responses to heat load (Mitlöhner et al., 2001a). In response to increasing thermal loads, cattle will initiate purposeful behavioural changes. Cattle behaviours during heat load, particularly panting score, can be used as a viable alternative to using body temperature to assess the heat load status of cattle (Brown-Brandl et al., 2006b; Mader et al., 2006; Gaughan et al., 2008b; Gaughan and Mader, 2014). These behavioural adaptations are the animal's first response to increasing thermal loads. Assessment of behavioural responses can provide information about animal comfort and the need to implement mitigation strategies. However it is important to consider that cattle are capable of acclimating to their thermal environment. Acclimation can be considered as a within lifetime process whereby continuous exposure to a particular stressor, i.e. hot climatic conditions, results in biological adjustments, thereby increasing the fitness of that individual animal to survive in those conditions (Horowitz, 2001). Therefore the behavioural responses of cattle will potentially differ with repeated exposure to heat load.

6.4.1 Shade Utilisation

Providing shade for feedlot cattle alters the microclimate within the pen providing an area for cooling (Mitlöhner et al., 2002), supporting the regulation of core body temperature. The advantage of shade structures is that the application is passive, where animals are able to utilise shaded areas voluntarily (Eigenberg et al., 2005). It has been well established that the provision of shade is advantageous for feedlot cattle, particularly in *Bos taurus* breeds. The strong expression of shade seeking behaviours *Bos indicus* within the experiment discussed in Chapter 5, indicate that these cattle were seeking relief from hot weather conditions. Shade utilisation showed a marked increase between HLI categories moderate (HLI 70.1 $\leq$ 77) and hot (HLI 77.1 $\leq$ 86). Gaughan et al. (2004b) reported that un-shaded Angus heifers showed a preference for shade usage when HLI $\geq$ 83.

Shade utilisation was only calculated on animals within the shaded treatment. However steers within the un-shaded pens were observed expressing shade seeking behaviours from the shade footprint of other animals and from structures around the pen, i.e. fence lines, feed bunks and water troughs. This is supported by the findings of Mitlöhner et al. (2001b), Castaneda et al. (2004) and

Gaughan and Mader (2014). Whilst these findings reiterate that it is impossible to completely remove access from shade footprints in feedlot pens, these results also highlight the importance of shade provision for feedlot cattle. This is particularly important as public perception of animal welfare standards is becoming increasingly apprehensive. Although animal performance between un-shaded and shaded groups did not differ, there were significant differences in rumen temperature and behavioural expressions between treatment groups.

There is also some conjecture regarding the amount of shade, m^2/animal, required to offset the impact of heat load (Clarke and Kelly, 1996; Mitlöhner et al., 2002; Sullivan et al., 2011). Therefore further investigation into the amount of shade, m^2/animal, required to offset the impact of heat load would be advantageous for commercial industry. Furthermore in future years the provision of shade for summer fed feedlot cattle in commercial industry should be strongly endorsed for feedlots located in tropical and sub-tropical regions.

6.5 Haematological Responses

Hyperthermia as a result of heat load can compromise cellular function and result in physiological changes (Hansen, 2004) as it promotes oxidative stress (Lacetera et al., 2006). The increase in oxygen pressure within the blood due to an increase in respiration rate might be the cause of alteration of oxidative status (Bernabucci et al., 2002). However due to the complex and dynamic relationships that exist between the body's stress response and immunological functions, defining the responses of these biological markers can be somewhat difficult to interpret (Carroll and Burdick Sanchez, 2014). This is especially important given the interrelationship of stressors, particularly within a feedlot where cattle can be exposed to environmental, nutritional and social stressors, combined with the variability of individual responses. To develop a clear understanding of biological responses to heat load it is imperative that knowledge regarding these responses and endocrine biomarkers is advanced. Additionally, as identified throughout the literature and further highlighted by Rhoads et al. (2013a), there are inconsistencies in our knowledge regarding the haematological changes that occur during heat load. However it must be acknowledged that the responses initiated by the body are fundamental to ensure survival.

The literature regarding haematological responses is predominantly focused on lactating dairy cattle and it is important to consider the physiological differences between beef cattle and lactating dairy cows, i.e. energy demands for growth versus lactation. Therefore there is limited knowledge regarding the responses of growing beef cattle, the advancement of knowledge in these areas is

required. Developing an understanding of the haematological responses of feedlot cattle will allow for improved mitigation strategies to be established and implemented within commercial industries.

There are limitations to the haematological parameters discussed within Chapter 5. Essentially blood samples were collected monthly with additional samplings occurring during forecasted heat wave events. Unfortunately the forecast material supplied by the forecasting service did not always meet the criteria of heat wave events. For these experiments a heat wave was defined as 3 or more consecutive days where the maximum HLI for the reference animal, a un-shaded Angus < 100 days on feed HLI threshold for heat accumulation = 86, was ≥ 86 combined with a maximum accumulated heat load ≥ 50 during daylight hours (0600 and 1800 h). Whilst the heat wave events may not have conformed to this definition, climatic conditions during these events were still sufficient to elicit a heat load response. Steers were sampled within the same conditions and therefore the haematological responses become relative to this experiment. However as steers were housed in groups of six, feed intake was not measured individually. Differences in haematological responses may be representative of variations in DMI, but this could not be determined in the current study due to the lack of individual feed intake data. It then becomes important to consider the net impact that DMI and heat load have on changes in circulating haematological parameters, indicating that individual studies are necessary to gain a complete understanding of the heat load response. Individual variably in haematological parameters were observed within the experiment and discussed in Chapter 5. Using a single time point sample is potentially a reflection of the stress response associated with animal handling rather than the overall health status (Carroll and Burdick Sanchez, 2014). Therefore it remains unclear whether the blood sampling intervals within the current experiment were sufficient to identify haematological responses to heat load. The importance of sampling at frequent intervals has been previously demonstrated (Schneider et al., 1988). Future studies need to incorporate a frequent sampling schedule. Additionally the small numbers within breed × treatment groups further confound the results discussed within Chapter 5. Ideally these studies would be conducted with a greater sample size, although it is difficult to quantify the numbers required due to the individual variability observed.

6.5.1 Acute Phase Proteins and Pro-Inflammatory Cytokines

It has been suggested that feedlot diets reduce the structural integrity of the intestinal epithelium (Suagee et al., 2012). The acute phase protein response is activated by pro-inflammatory cytokines by the release from macrophages and monocytes from the source of inflammation or trauma (Carroll et al., 2009). Cytokine interleukin 6 is an important regulator of localised and systemic inflammatory responses (Xing et al., 1998). Carroll and Burdick Sanchez (2014) reported that an

increase in circulating haptoglobin is associated with an increase in cytokine interleukin 6. Therefore the increased circulating haptoglobin and cytokine interleukin 6 concentration within this experiment may be indicative of reduced gut integrity in these cattle. However, it is important to consider the impact of other potential stressors, i.e. environment, nutrition and psychological, on haematological responses. Additionally sub-clinical aliments, i.e. ruminal acidosis, will also affect circulating haematological concentrations. Furthermore it is unclear the effect of the length and/or degree of heat load exposure on the longevity of negative implications on the body, i.e. damage to the visceral organs and the permanency of this damage.

6.5.2 Insulin

Significant reductions in DMI are typically associated with hypoinsulinemia (Baumgard and Rhoads, 2012b). Despite marked reduction in DMI, basal insulin levels are typically elevated during heat load (Itoh et al., 1998; Baumgard and Rhoads, 2007; Wheelock et al., 2010). The increase in insulin concentration appears to be associated with an increase in insulin secretion from the pancreas, rather than a reduction in the rate of utilisation (Baumgard and Rhoads, 2007). Mehla et al. (2014) reported that insulin receptors are highly up-regulated 4, 12 and 48 hours post exposure to severe heat load (THI > 104). Therefore there is the potential that an increase in insulin is an evolutionary adaptation to heat load (Baumgard and Rhoads, 2012b). The increase in insulin during periods of heat load appears to be an adaptation to ensure animal survival (O'Brien et al., 2010; Baumgard and Rhoads, 2012b). However the mechanisms responsible for enhanced insulin action and its specific purpose during heat load is unknown and remains perplexing. It remains unclear as to whether the elevated insulin concentrations described within Chapter 5 are due to summer weather conditions or the interrelationship between diet type, weight gain and insulin resistance. Given the elevated cytokine interleukin 6, glucose and insulin concentrations combined with the high energy diet, designed for weight gain and fat deposition, insulin resistance in the cattle of the current experiment does not seem unfounded.

6.6 Heat Load Index Thresholds

Currently all accredited feedlots (n = 450) in Australia are required to undertake a heat stress risk assessment evaluation at the commencement of the heat load season, i.e. at the beginning of summer (November). Updating and refining the HLI model will improve the ability of the model to predict the impact of climatic conditions on feedlot cattle. Improving the robustness of the model will ultimately improve the ability of livestock managers to deal with heat load events and prepare appropriate abatement strategies. Whilst the current heat load model that is used in the prediction service is informative and successfully forecasts impending climatic conditions, the effect of night

time conditions on heat accumulation and dissipation from cattle appears to be inconsistent, particularly during heat wave events. The accumulated heat load thresholds may require re-evaluation to ensure the model remains an accurate representation of the conditions the animals are experiencing, especially during heat waves.

The current accumulated heat load model accounts for the duration of exposure above the HLI upper threshold, i.e. HLI_{86} for un-shaded Angus < 100 days on feed, and recovery period where conditions HLI is below the lower threshold, HLI_{77} for un-shaded Angus < 100 days on feed (Gaughan et al., 2008b). During heat wave events where accumulated heat load does not abate overnight, i.e. accumulated heat load $\neq 0$, the HLI threshold at which the cattle begin to accumulate heat load may actually be lower than the current model accounts for; however further investigation is require to quantify this. Within the experiment discussed in Chapter 5, a heat wave event lasting 6 days the rumen temperature of un-shaded Angus appears to increase at a lower accumulated heat load threshold with each consecutive day. This suggests that the accumulated heat load threshold for this genotype was decreasing as the heat wave progressed and would suggest that the accumulated heat load model needs to be adjusted to account for conditions where accumulated heat load does not abate overnight, warranting further investigations.

During the development of the model Gaughan et al. (2008b) acknowledged that where night time conditions are insufficient to allow for night time heat dissipation, cattle will enter the subsequent day with an accumulated heat load. Furthermore during the development of the HLI model, thresholds and adjustments were identified which allows for numerous animal factors and management strategies to be incorporated within the model, whereby the accumulated heat load threshold value for un-shaded and shaded Brahman < 100 days on feed were defined as 96 and 100 respectively. However Gaughan et al. (2008b) acknowledged that there was not sufficient data where HLI > 95 to provide a definitive HLI threshold for these animals, indicating that further investigation in this area is required. Therefore it is difficult to assess and define the thermoregulatory abilities of *Bos indicus* breeds, particularly in reference to the regulation of core body temperature. Due to the harsh environment, both climate and nutritionally, Australia's cattle population is predominantly *Bos indicus*; therefore there is a greater need to understand the thermoregulatory abilities of these genotypes. Whilst the current HLI model accounts for genotype and days on feed, it does not account for metabolic differences between and within breeds, nor does it take into consideration the different heat content of grains fed to cattle. Numerous authors have noted lower metabolic rates in Brahman cattle (Kibler and Brody, 1950; Vercoe, 1970; Frisch and Vercoe, 1977), where Frisch and Vercoe (1977) indicated that the fasting metabolism of Hereford ×

Shorthorn steers was 6 to 10 times greater than Brahman × Hereford-Shorthorn steers. A lower fasting metabolism in Brahman can potentially be explained by two mechanisms i) these animals have a lower energy requirement for basal metabolic functions or ii) these animals are more energy efficient (Vercoe, 1970). The production of less heat from basic metabolic functions has advantages in the maintenance of core body temperature and overall thermal equilibrium. As a result the less heat produced by basal metabolic functions results in a decrease in the amount of heat required to be dissipated. Potentially this may be the cause of the limited variability in rumen temperature of both shaded and un-shaded Brahman steers observed within Chapter 5.

Regional HLI forecasts are provided by Katestone Environmental (http://chlt.katestone.com.au/) provide a powerful management tool for commercial producers. However the forecasting system does not account for local and microclimate conditions. Differences between forecasted and onsite UQ ambient conditions during a heat wave event 2 as described in Chapter 5 were observed. This highlighted the importance of onsite weather stations and climate monitoring of the HLI and accumulated heat load within individual feedlots. Therefore it is important that persons responsible for the management and welfare of feedlot cattle are observing cattle responses to the thermal environment as the indices developed are a guide and are not absolute. Furthermore it is particularly important that persons observing and managing livestock understand the behavioural, particularly panting, responses of cattle to heat load and are prepared to implement alleviation strategies when required. Misdiagnosis of heat load behaviours and the unreliability of forecasted climatic conditions could potentially lead to significant mortalities within a commercial feedlot.

6.7 Future Directions

Providing a clear and concise definition of the biological mechanisms of how heat load influences animal health, wellbeing and performance is crucial for the advancement of mitigation strategies (Rhoads et al., 2013a). However providing and identifying clear definitions are difficult as many of the basic biological mechanisms affected by heat load still remain unclear. In order to provide commercial producers with beneficial mitigation strategies, a better understanding of physiological responses and endocrine biomarkers that are indicative of an appropriate stress response is required (Carroll and Burdick Sanchez, 2014). Furthermore the problems with identifying the effect of heat load on these biological mechanisms are further confounded as the response to heat load is highly variable.

The response of each individual animal to hot climatic conditions is dependent on a number of individual characteristics; including genotype, coat characteristics, health and days on feed.

Additionally more information is required to understand the impact of management decisions, i.e. feed restrictions during heat load and the provision of shade, on physiological responses and endocrine biomarkers and their association with naturally occurring differences in stress and immune function in beef cattle (Carroll and Burdick Sanchez, 2014). Additionally a significant body of the literature regarding this is based on studies specific to dairy cows, mice or other species. Given the difference in production status between dairy cows and feedlot cattle it is unclear as to the application of this research to feedlot cattle. Therefore there is a need to engage in these research areas as it specifically applies to feedlot cattle. Defining the biological mechanisms at which thermal stress alters animal metabolism may potentially provide direction for the development of innovative mitigation strategies (Johnson et al., 2015), that are suitable and economically viable for commercial producers.

Although not explored here, high energy concentrates fed to finishing feedlot cattle have the potential to increase core body temperature (Cho et al., 2014). The heat increment for feedlot cattle is considered as high (35 to 70 % of ME) however this is dependent on the balance of nutrients within the diet (Blackshaw and Blackshaw, 1994). In Australia commercial feedlots typically process their own ration. There is the potential that there is significant nutrient variability between Australian producers. Given that quality of nutrition is likely to have an impact on individual heat load, it would be warranted to investigate the impact of heat increment due to diet and nutrient composition differences on the overall heat load response of feedlot cattle. Furthermore it is likely that the nutrient balance will have an effect on the haematological responses of feedlot cattle.

Data presented in Chapter 5 indicated maximum rumen temperature recorded during the experiment was 43.7 °C. Knowledge is limited regarding the effects of heat load and high rumen temperature on rumen microflora populations (Tajima et al., 2007). There are some disagreements within the literature in regards to the effect of rumen temperature on the functionality of rumen microflora. However it has been shown that some rumen protozoa cannot withstand temperatures above 40 °C (Hungate, 1966). Tajima et al. (2007) reported that a combination of high T_A (33 °C) and RH (80 %) had profound effects on the composition and diversity of microflora within the rumen; however the authors indicated that there is limited knowledge on the effect of heat load on rumen microflora populations (Tajima et al., 2007). Thus the true impact of heat load on the overall health and wellbeing of the animal remains unknown. Further investigations are warranted to i) *understand influence of heat load conditions on rumen temperature and the associated impact on microflora populations;* and ii) *ascertain the overall impact hot conditions and elevated rumen temperature has on nutrient partitioning and integrity of the ruminant gut.* The latter is particularly important as

it is possible that feedlot diets, i.e. high starch content, reduce the structural integrity of the intestinal epithelium (Suagee et al., 2012), which may be further confounded by heat load due to the redistribution of blood flow from the internal organs to the extremities for heat dissipation. Additionally the integrity of the gut during heat load has the potential to have major implications in welfare and performance of feedlot cattle during thermal challenges. Furthermore this highlights the potential for investigations into feed additives to support gut integrity of feedlot cattle during heat load, to ensure structural integrity throughout feeding.

The experiments conducted within this thesis have highlighted possible future directions in heat load research, in summary;

- Develop an understanding of the relationship between rumen temperature and weather conditions as well as climatic models;
- Further analysis on the relationship between rectal temperature and rumen temperature at varying times to the day to ensure that rumen temperature is a suitable proxy of core body temperature;
- Develop an understanding of the impact of changes in rumen temperature on the thermal status of cattle;
- Investigate the effect of water intake on rumen temperature;
- Develop an understanding of the thermoregulatory mechanisms of *Bos indicus* cattle, with particular emphasis on the regulation of body temperature;
- Investigate the influence of heat load conditions on rumen temperature, and
    a. The associated impact on microflora populations and rumen health; and
    b. The overall impact hot conditions and elevated rumen temperature has on nutrient partitioning and integrity of the gut;
- Investigate the impact of heat increment due to diet and nutrient composition differences on the overall heat load response of feedlot cattle;
- Investigate feed additives to reduce the impact of heat load and support gut integrity;
- Investigate the use infrared thermography in the potential development of a thermal exchange model;
- Investigate the potential of heat shock proteins, and other biological parameters, as potential biomarkers for thermotolerance; and,
- Investigate the inter-relationship between cytokine interleukin 6, glucose and insulin, insulin action and subsequent potential of insulin resistance in feedlot cattle.

6.8 Conclusion

The impact of hot conditions cannot be completely removed where animal production occurs in tropical and sub-tropical regions. With the implementation of management strategies, producers are able to reduce the impact of heat load. However whilst scientific research continues to advance knowledge regarding cattle responses to heat load, studies also need to be conducted under commercial conditions to provide a real world understanding of the implications that heat load has for commercial producers. The key focus of heat load research should remain primarily on developing effective management strategies to support animal comfort and performance during heat load, from a commercial perspective. It is particularly important to remain focused towards commercial implications, as it is the commercial producers within the intensive animal industries, irrespective of species, that are enduring the economic and social burden of heat load. However it is also important to recognise that there is a need to use a multidisciplinary approach when investigating the responses of cattle to heat load, as clearly the biological systems within the body are convincingly interrelated and are associated with animal survival. The development of a comprehensive understanding of the factors that influence heat load, both environmental and animal, allows for innovative mitigation strategies to be established and implemented during heat related stress events, thus improving animal survivability and welfare during these events.

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Appendices

Appendix 1: Interactions (*P* values) for feeding during months

Item	Month[1]				
	November	December	January	February	March
Breed	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.002
Treatment	0.009	< 0.0001	0.01	0.10	0.79
Hour	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Day	< 0.0001	< 0.0001	0.98	< 0.0001	0.02
HLI	0.16	< 0.0001	< 0.0001	0.67	< 0.0001
Breed × Treatment	0.10	0.48	0.26	0.22	0.41
Breed × Hour	0.02	0.47	0.43	0.19	0.79
Breed × HLI	0.20	0.30	0.46	0.56	0.05
Treatment × HLI	0.98	0.61	0.004	0.19	0.08
Treatment × Hour	0.76	< 0.0001	0.38	0.92	0.78
Breed × Day	0.67	0.87	0.09	0.78	0.11
Hour × Day	< 0.0001	< 0.0001	0.09	0.02	0.24
Breed × Treatment × Hour	< 0.0001	0.48	0.006	0.20	0.76
Breed × Treatment × HLI	0.24	0.38	0.36	0.41	0.89
Breed × Hour × Day	0.60	0.77	0.15	0.54	0.34

[1]November 2012, d 2 to d 31; December 2012, d 32 to 62; January 2013, d 63 to 93; February 2013, d 94 to d 121; and March 2013, d 122 to d 152

Appendix 2: Interactions (*P* values) for feeding during heat waves

Item	Heat Wave[1]			
	1	2	3	4
Breed	0.003	0.57	0.50	0.50
Treatment	0.007	0.06	0.16	0.16
Hour	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Day	0.06	0.09	0.05	0.05
HLI	< 0.0001	< 0.0001	0.02	0.02
Breed × Treatment	0.86	0.99	0.52	0.52
Breed × Hour	0.35	0.58	0.73	0.73
Breed × HLI	0.55	0.04	0.18	0.18
Treatment × HLI	0.003	0.19	0.16	0.17
Treatment × Hour	0.09	0.33	0.007	0.007
Breed × Day	0.78	0.53	0.81	0.81
Hour × Day	0.50	0.63	0.12	0.12
Breed × Treatment × Hour	0.29	0.58	0.96	0.96
Breed × Treatment × HLI	0.75	0.38	0.47	0.47
Breed × Hour × Day	0.83	0.51	0.15	0.15

[1] event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147

Appendix 3: Interactions (*P* values) for drinking during months

Item	Month[1]				
	November	December	January	February	March
Breed	0.32	0.10	0.005	0.35	< 0.0001
Treatment	0.02	< 0.0001	< 0.0001	0.08	0.07
Hour	0.50	0.003	< 0.0001	0.05	0.24
Day	0.23	0.54	0.07	0.30	0.12
HLI	< 0.0001	0.40	0.21	0.58	0.006
Breed × Treatment	0.58	0.34	0.02	0.05	0.27
Breed × Hour	0.31	< 0.0001	0.73	0.23	0.06
Breed × HLI	0.02	0.29	0.11	0.79	0.004
Treatment × HLI	0.78	0.16	0.03	0.08	0.74
Treatment × Hour	0.97	0.05	0.10	0.81	0.68
Breed × Day	0.35	0.36	0.95	0.12	0.01
Hour × Day	< 0.0001	0.09	0.57	0.29	0.35
Breed × Treatment × Hour	0.64	0.68	0.40	0.10	0.03
Breed × Treatment × HLI	0.27	0.37	0.84	0.78	0.32
Breed × Hour × Day	0.18	0.34	0.04	0.11	0.95

[1]November 2012, d 2 to d 31; December 2012, d 32 to 62; January 2013, d 63 to 93; February 2013, d 94 to d 121; and March 2013, d 122 to d 152

Appendix 4: Interactions (*P* values) for drinking during heat waves

Item	Heat Wave[1]			
	1	2	3	4
Breed	0.93	0.02	0.24	0.03
Treatment	1.00	0.19	0.80	0.82
Hour	0.0008	0.07	0.29	0.008
Day	0.005	0.28	0.48	0.37
HLI	< 0.0001	0.33	0.61	0.01
Breed × Treatment	0.59	0.05	0.44	0.23
Breed × Hour	0.29	0.6	0.44	0.50
Breed × HLI	0.96	0.06	0.34	0.18
Treatment × HLI	0.42	0.18	0.70	0.13
Treatment × Hour	0.17	0.57	0.17	0.25
Breed × Day	0.23	0.21	0.86	0.91
Hour × Day	0.03	0.10	0.90	0.14
Breed × Treatment × Hour	0.15	0.43	0.57	0.63
Breed × Treatment × HLI	0.46	0.05	0.97	1.00
Breed × Hour × Day	0.65	0.36	0.77	0.22

[1] event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147

Appendix 5: Interactions (*P* values) for ruminating during months

Item	Month[1]				
	November	December	January	February	March
Breed	< 0.0001	< 0.0001	< 0.0001	0.0004	0.03
Treatment	0.51	0.62	0.16	0.75	0.34
Hour	< 0.0001	< 0.0001	0.98	0.53	0.04
Day	0.005	< 0.0001	< 0.0001	0.001	< 0.0001
HLI	< 0.0001	0.40	< 0.0001	< 0.0001	< 0.0001
Breed × Treatment	0.75	0.05	< 0.0001	< 0.0001	0.04
Breed × Hour	0.81	0.26	0.44	0.10	0.45
Breed × HLI	0.02	0.98	0.68	0.55	0.15
Treatment × HLI	0.01	0.85	0.45	0.53	0.83
Treatment × Hour	0.01	0.79	0.29	0.86	0.89
Breed × Day	< 0.0001	0.06	0.10	0.21	0.01
Hour × Day	0.36	< 0.0001	0.002	0.60	0.008
Breed × Treatment × Hour	0.76	0.64	0.53	0.91	0.22
Breed × Treatment × HLI	0.35	0.48	0.04	0.66	0.09
Breed × Hour × Day	0.09	0.35	0.82	0.35	0.54

[1]November 2012, d 2 to d 31; December 2012, d 32 to 62; January 2013, d 63 to 93; February 2013, d 94 to d 121; and March 2013, d 122 to d 152

Appendix 6: Interactions (*P* values) for ruminating during heat waves

Item	Heat Wave[1]			
	1	2	3	4
Breed	0.24	0.07	0.82	0.05
Treatment	0.71	0.32	0.13	0.70
Hour	0.85	0.90	0.03	0.26
Day	0.53	0.05	0.12	0.90
HLI	0.01	0.001	< 0.0001	0.10
Breed × Treatment	0.98	0.005	0.0005	0.22
Breed × Hour	0.41	0.12	0.33	0.28
Breed × HLI	0.90	0.92	0.31	0.13
Treatment × HLI	0.87	0.28	0.38	0.36
Treatment × Hour	0.38	0.84	0.97	0.17
Breed × Day	0.16	0.43	0.95	0.46
Hour × Day	0.13	0.99	0.25	0.17
Breed × Treatment × Hour	0.72	0.06	0.32	0.50
Breed × Treatment × HLI	0.88	0.41	0.11	0.65
Breed × Hour × Day	0.52	0.45	0.73	0.57

[1] event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147

Appendix 7: Interactions (*P* values) for standing during months

Item	Month[1]				
	November	December	January	February	March
Breed	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment	0.009	0.01	< 0.0001	0.22	0.006
Hour	< 0.0001	0.14	0.68	0.46	0.80
Day	< 0.0001	0.36	< 0.0001	0.60	0.004
HLI	< 0.0001	< 0.0001	< 0.0001	0.36	0.18
Breed × Treatment	0.11	0.0004	< 0.0001	0.02	0.008
Breed × Hour	0.37	0.64	0.14	0.23	0.39
Breed × HLI	0.58	0.41	< 0.0001	0.19	0.02
Treatment × HLI	0.13	0.32	0.18	0.49	< 0.0001
Treatment × Hour	0.48	0.005	0.04	0.10	0.02
Breed × Day	0.03	0.72	0.15	0.43	0.003
Hour × Day	< 0.0001	0.003	0.83	0.23	0.004
Breed × Treatment × Hour	0.43	0.43	0.94	0.66	0.78
Breed × Treatment × HLI	0.65	0.23	0.44	0.50	0.01
Breed × Hour × Day	0.15	0.99	0.05	0.95	0.02

[1]November 2012, d 2 to d 31; December 2012, d 32 to 62; January 2013, d 63 to 93; February 2013, d 94 to d 121; and March 2013, d 122 to d 152

Appendix 8: Interactions (*P* values) for standing during months

Item	Heat Wave[1]			
	1	2	3	4
Breed	< 0.0001	0.19	0.08	0.79
Treatment	0.68	0.001	0.46	0.34
Hour	0.32	0.03	1.00	0.06
Day	0.22	0.02	0.32	0.001
HLI	< 0.0001	< 0.0001	0.002	0.80
Breed × Treatment	0.47	0.50	0.79	0.75
Breed × Hour	0.83	0.97	0.39	0.61
Breed × HLI	0.88	0.03	0.02	0.57
Treatment × HLI	0.14	0.24	0.03	0.003
Treatment × Hour	0.86	0.03	0.92	0.04
Breed × Day	0.67	0.30	0.10	0.29
Hour × Day	0.56	0.03	0.82	0.0001
Breed × Treatment × Hour	0.69	0.84	0.87	0.97
Breed × Treatment × HLI	0.58	0.23	0.56	0.59
Breed × Hour × Day	0.98	0.40	0.13	0.71

[1] event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147

Appendix 9: Interactions (*P* values) for lying during months

Item	Month[1]				
	November	December	January	February	March
Breed	< 0.0001	< 0.0001	0.0003	< 0.0001	< 0.0001
Treatment	0.13	0.11	< 0.0001	0.006	0.001
Hour	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Day	0.16	0.03	< 0.0001	0.008	0.04
HLI	< 0.0001	0.001	0.11	< 0.0001	< 0.0001
Breed × Treatment	0.29	< 0.0001	0.0006	0.17	0.08
Breed × Hour	< 0.0001	< 0.0001	0.007	< 0.0001	0.30
Breed × HLI	0.04	0.75	0.04	0.30	0.60
Treatment × HLI	0.42	0.29	< 0.0001	0.55	0.0004
Treatment × Hour	0.52	0.64	0.42	0.35	0.04
Breed × Day	0.01	0.77	0.22	0.77	< 0.0001
Hour × Day	< 0.0001	0.19	0.86	0.03	0.07
Breed × Treatment × Hour	0.34	0.69	0.12	0.50	0.12
Breed × Treatment × HLI	0.70	0.26	0.73	0.20	0.20
Breed × Hour × Day	0.52	0.77	0.68	0.97	0.46

[1]November 2012, d 2 to d 31; December 2012, d 32 to 62; January 2013, d 63 to 93; February 2013, d 94 to d 121; and March 2013, d 122 to d 152

Appendix 10: Interactions (*P* values) for lying during heat waves

Item	Heat Wave[1]			
	1	2	3	4
Breed	0.02	0.07	0.45	0.72
Treatment	0.31	< 0.0001	0.95	0.50
Hour	< 0.0001	< 0.0001	0.05	0.01
Day	0.28	0.09	0.85	0.07
HLI	< 0.0001	0.33	< 0.0001	0.0009
Breed × Treatment	0.50	0.29	0.57	0.85
Breed × Hour	0.12	0.96	0.06	0.37
Breed × HLI	0.74	0.03	0.02	0.49
Treatment × HLI	0.39	0.002	0.002	0.0002
Treatment × Hour	0.37	0.76	0.26	0.09
Breed × Day	0.81	0.50	0.02	0.97
Hour × Day	0.53	0.21	0.07	0.02
Breed × Treatment × Hour	0.66	0.85	0.67	0.67
Breed × Treatment × HLI	0.68	0.37	0.92	0.41
Breed × Hour × Day	0.99	0.92	0.50	0.72

[1] event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147

Appendix 11: Interactions (*P* values) for shade utilisation during months

Item	Month[1]				
	November	December	January	February	March
Breed	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Hour	0.005	< 0.0001	< 0.0001	0.02	0.03
Day	< 0.0001	0.51	0.59	0.07	< 0.0001
HLI	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Breed × Hour	0.001	< 0.0001	0.49	0.01	< 0.0001
Breed × HLI	0.0003	0.002	0.0004	0.004	0.59
Breed × Day	0.002	0.07	< 0.0001	< 0.0001	0.04
Hour × Day	0.65	0.37	0.002	0.97	0.67
Breed × Hour × Day	0.09	0.21	0.64	0.75	0.04

[1]November 2012, d 2 to d 31; December 2012, d 32 to 62; January 2013, d 63 to 93; February 2013, d 94 to d 121; and March 2013, d 122 to d 152

Appendix 12: Interactions (*P* values) for shade utilisation during months

Item	Heat Wave[1]			
	1	2	3	4
Breed	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Hour	< 0.0001	0.30	0.78	0.004
Day	0.002	0.11	0.02	0.55
HLI	0.03	< 0.0001	< 0.0001	< 0.0001
Breed × Hour	0.004	0.61	0.06	0.05
Breed × HLI	0.07	0.17	0.08	0.77
Breed × Day	0.32	0.08	0.16	< 0.0001
Hour × Day	0.62	0.003	0.41	0.18
Breed × Hour × Day	0.98	0.59	< 0.0001	0.32

[1] event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147

Appendix 13: Interactions (*P* values) for mean panting score during months

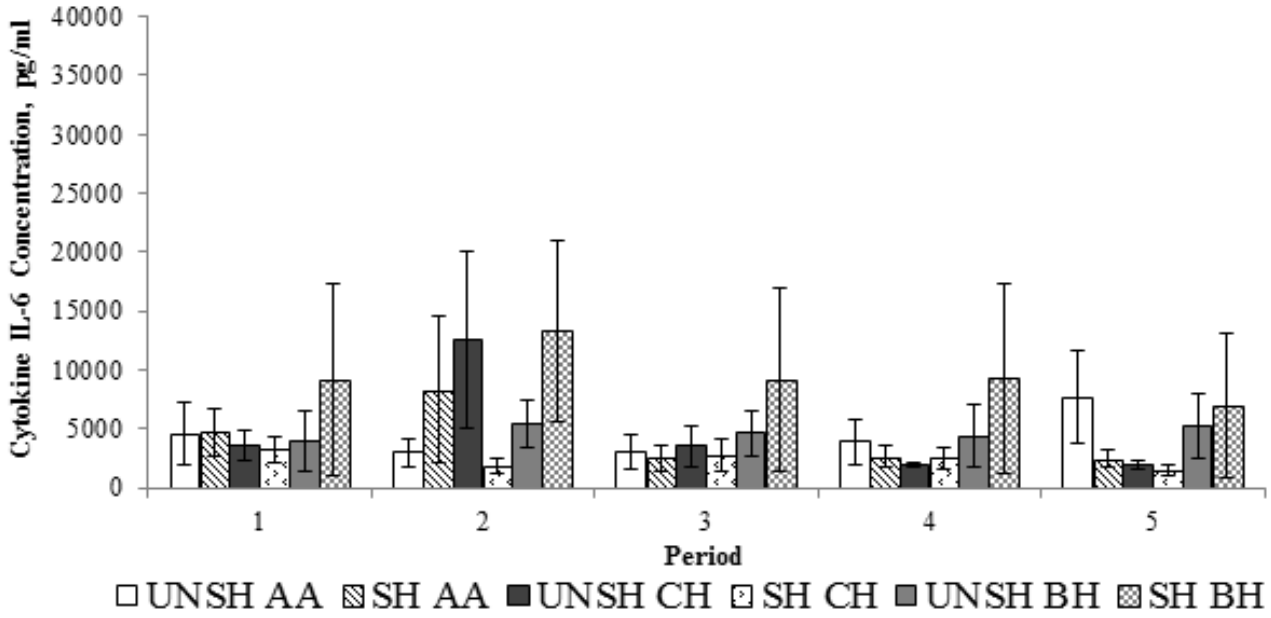
Item	Month[1]				
	November	December	January	February	March
Breed	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment	0.003	0.05	0.0009	0.0007	< 0.0001
Hour	0.91	0.83	0.08	0.45	0.72
Day	< 0.0001	0.006	0.001	0.26	< 0.0001
HLI	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0005
Breed × Treatment	0.58	0.30	0.19	< 0.0001	0.02
Breed × Hour	0.19	0.10	0.40	0.07	0.18
Breed × HLI	0.13	0.003	< 0.0001	0.02	0.005
Treatment × HLI	0.72	0.67	0.29	0.95	0.65
Treatment × Hour	0.48	0.40	0.23	0.80	0.67
Breed × Day	0.56	0.67	0.001	0.55	0.03
Hour × Day	0.07	0.58	0.02	0.94	0.27
Breed × Treatment × Hour	0.99	0.98	0.86	0.08	0.47
Breed × Treatment × HLI	0.99	0.83	0.74	0.54	0.69
Breed × Hour × Day	0.99	0.78	0.03	0.25	0.75

[1]November 2012, d 2 to d 31; December 2012, d 32 to 62; January 2013, d 63 to 93; February 2013, d 94 to d 121; and March 2013, d 122 to d 152

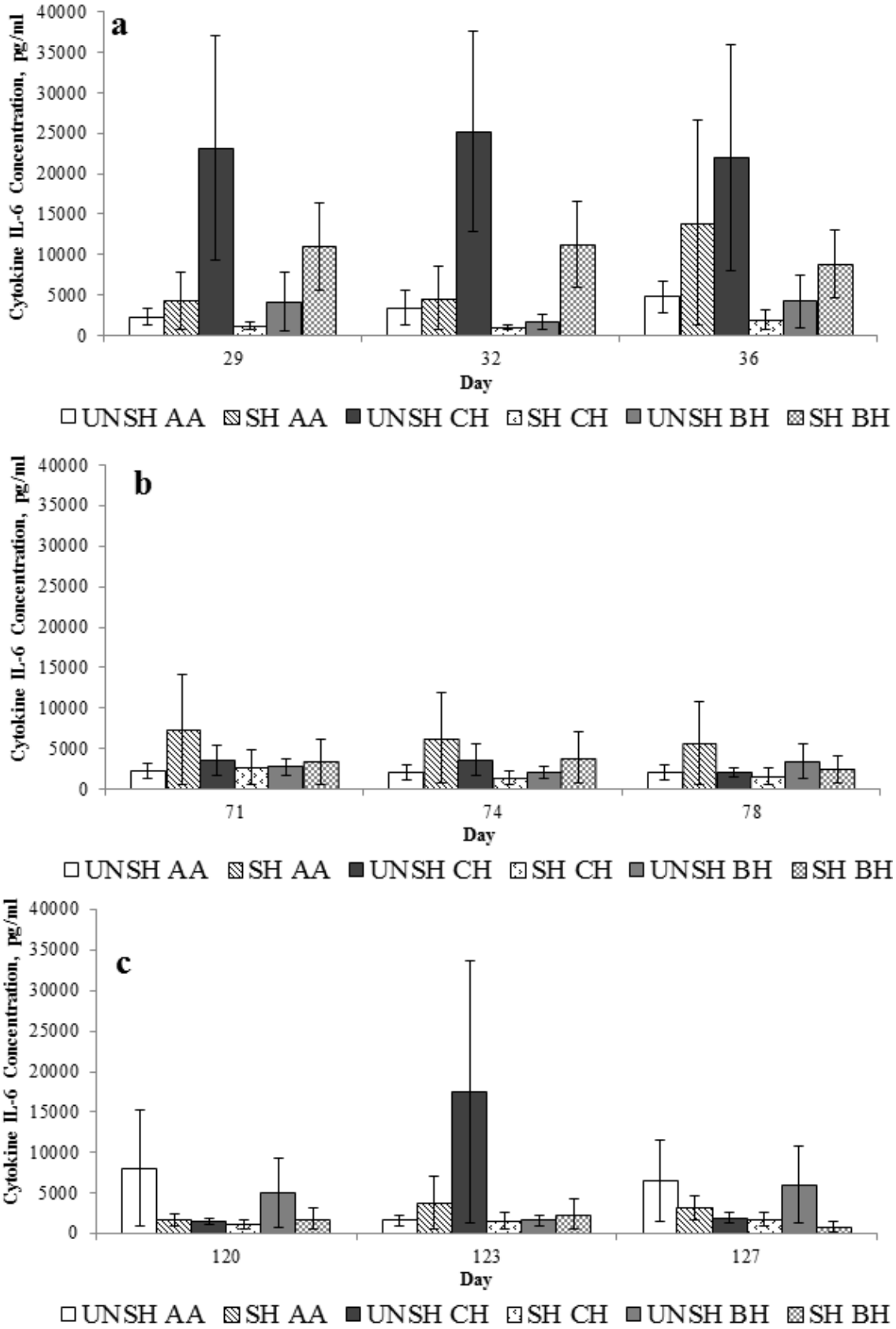
Appendix 14: Interactions (*P* values) for mean panting score during heat waves

Item	Heat Wave[1]			
	1	2	3	4
Breed	< 0.0001	< 0.0001	0.005	0.06
Treatment	0.23	0.13	0.006	0.08
Hour	0.23	0.47	0.28	0.19
Day	0.04	0.84	0.85	0.11
HLI	0.0008	0.002	0.006	0.38
Breed × Treatment	0.55	0.56	0.05	0.78
Breed × Hour	0.42	0.39	0.83	0.64
Breed × HLI	0.27	0.004	0.36	0.08
Treatment × HLI	0.47	0.71	0.22	0.95
Treatment × Hour	0.57	0.78	0.67	0.73
Breed × Day	0.04	0.81	0.90	0.71
Hour × Day	0.23	0.09	0.27	0.24
Breed × Treatment × Hour	0.78	0.96	0.28	0.94
Breed × Treatment × HLI	0.98	0.80	0.62	1.00
Breed × Hour × Day	0.81	0.22	0.57	0.73

[1] event 1, d 48 to d 52; event 2, d 71 to d 76; event 3, d 92 to d 94; and event 4, d 144 to d 147



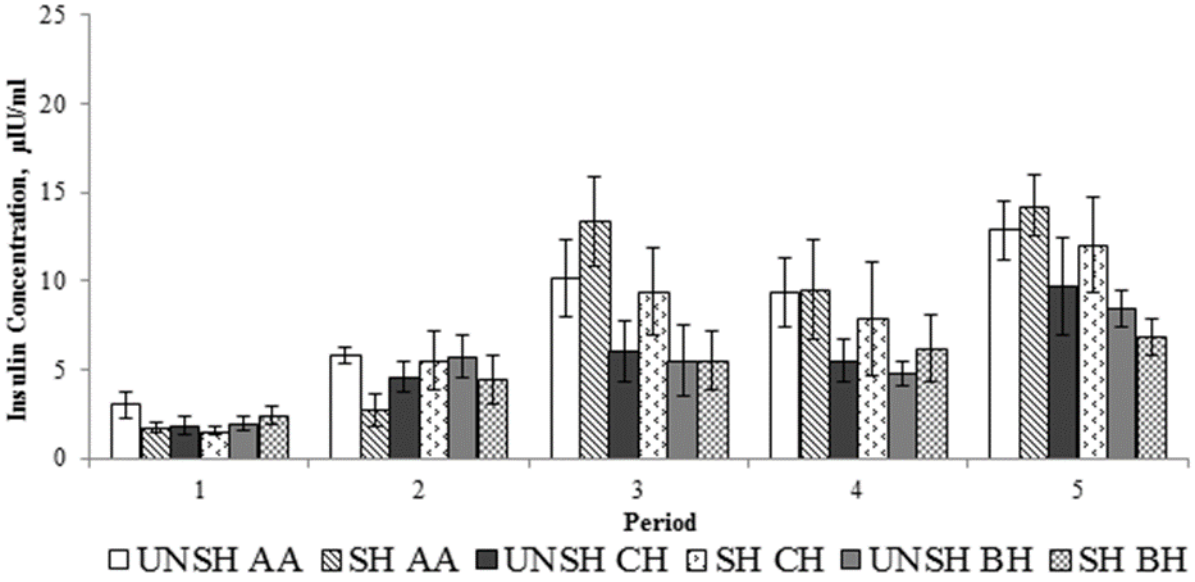
Appendix 15: Plasma cytokine interleukin-6 (IL-6; pg/mL) concentrations for period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers



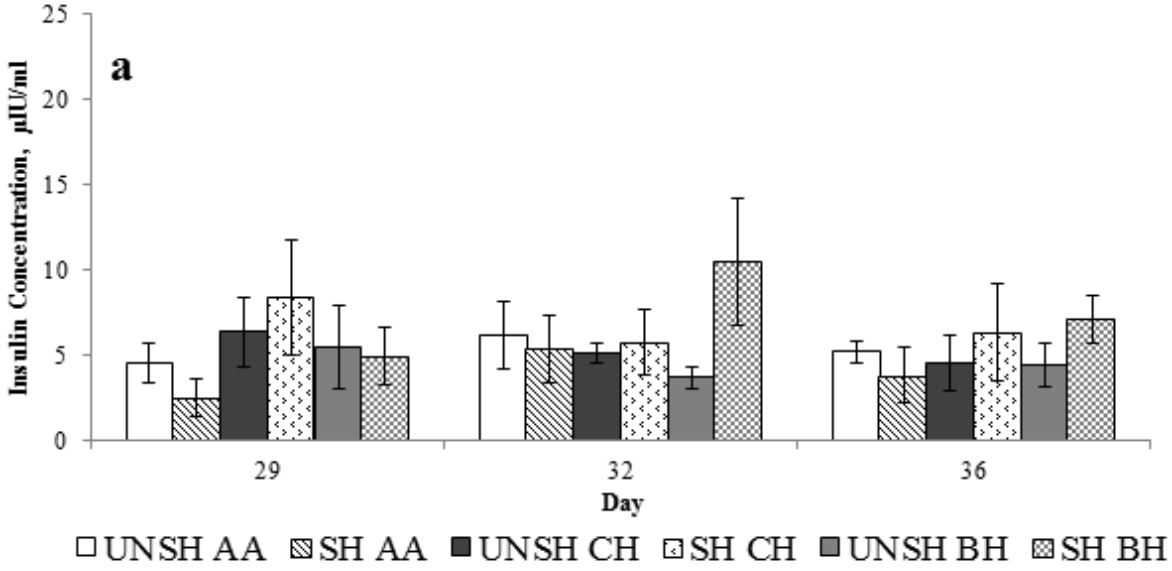
Appendix 16: Plasma cytokine interleukin-6 (IL-6; pg/mL) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

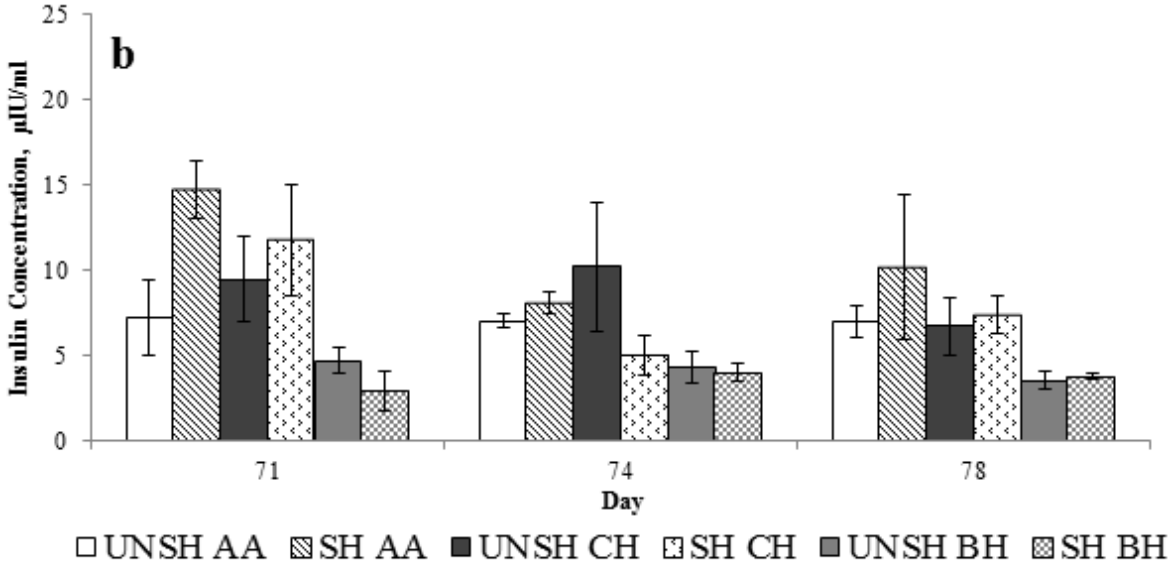
Appendix 17: Individual optical density (OD, nm) of heat shock protein 70 (HSP70) during period 1 (d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers

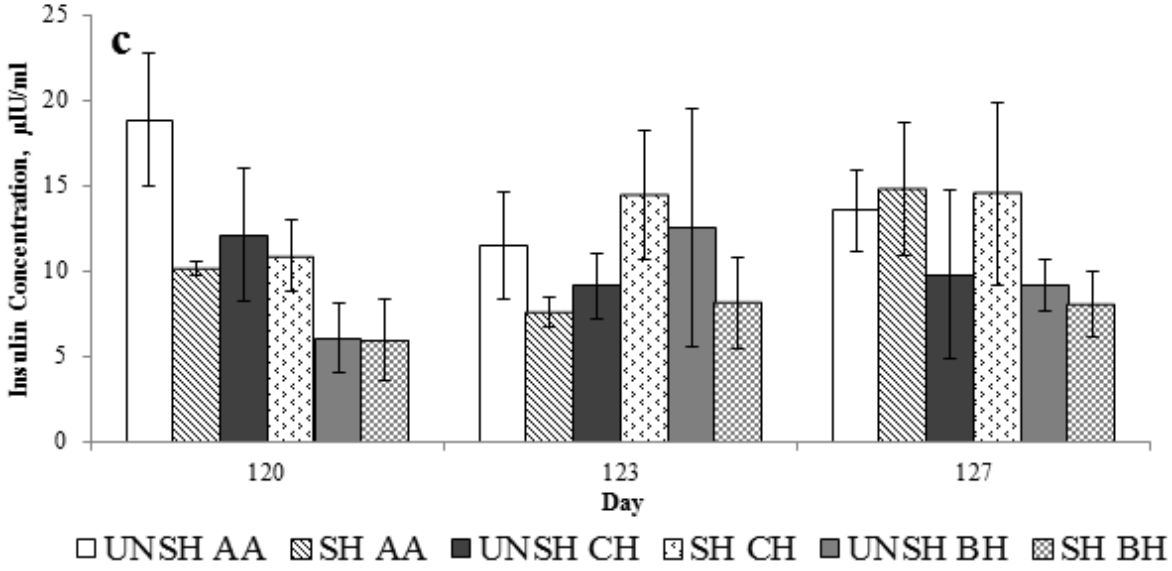
Animal ID	Treatment	Breed	HSP_{70} OD				
			1	2	3	4	5
2066	UNSH	AA	-0.03	-0.09	0.00	-0.01	-0.08
2067	UNSH	AA	-0.26	-0.06	-0.06	0.07	-0.11
2072	UNSH	AA	-0.02	0.10	-0.07	-0.09	0.03
2073	UNSH	AA	-0.07	0.02	0.02	-0.02	-0.07
2078	UNSH	AA	0.21	0.05	-0.16	-0.08	-0.02
2088	UNSH	AA	-0.21	-0.09	0.00	0.00	-0.02
2074	SH	AA	0.03	0.27	-0.21	-0.04	0.00
2075	SH	AA	0.78	0.04	0.13	-0.04	-0.05
2077	SH	AA	-0.01	0.10	0.08	0.03	-0.04
2081	SH	AA	1.34	1.92	0.24	0.12	0.02
2082	SH	AA	0.32	0.16	0.05	-0.04	-0.10
2086	SH	AA	-0.01	-0.07	-0.20	-0.24	-0.08
2065	UNSH	CH	-0.07	-0.07	-0.06	-0.03	-0.25
2069	UNSH	CH	-0.01	-0.17	-0.24	-0.11	-0.11
2079	UNSH	CH	-0.08	-0.01	-0.06	-0.09	-0.03
2080	UNSH	CH	-0.01	-0.17	-0.24	-0.11	-0.11
2091	UNSH	CH	-0.01	0.07	-0.14	0.12	-0.16
2083	UNSH	CH	-0.02	-0.01	-0.02	-	-
2070	SH	CH	0.00	-0.21	0.06	0.04	0.08
2076	SH	CH	0.38	0.76	-0.16	-0.04	-0.14
2084	SH	CH	-0.19	-0.07	-0.27	-0.03	-0.08
2087	SH	CH	-0.25	-0.02	-0.07	-0.21	0.00
2089	SH	CH	-0.01	-0.06	-0.05	-0.04	-0.11
2092	SH	CH	0.00	-0.03	-0.26	0.17	-0.08
2100	UNSH	BH	-0.09	-0.21	-0.05	-0.09	-0.12
2101	UNSH	BH	0.00	-0.02	0.01	-0.07	0.03
2103	UNSH	BH	-0.07	-0.20	-0.27	-0.06	-0.09
2106	UNSH	BH	0.60	0.21	0.27	0.51	0.36
2093	UNSH	BH	1.08	0.22	0.51	-	-
2094	SH	BH	0.20	0.00	0.10	0.32	0.11
2095	SH	BH	-0.01	-0.27	-0.10	-0.23	-0.02
2096	SH	BH	1.21	0.13	0.17	0.04	0.05
2098	SH	BH	0.00	-0.03	-0.09	-0.28	-0.28
2099	SH	BH	-0.05	-0.01	0.02	-0.20	-0.12
2105	SH	BH	-0.07	-0.26	-0.06	-0.09	-0.09



Appendix 18: Plasma insulin (µIU/mL) concentrations for period 1(d 8 and 9), period 2 (d 36 and 37), period 3 (d 64 and 65), period 4 (d 99 and 100) and period 5 (d 127 and 128) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH)







Appendix 19: Plasma insulin (µIU/mL) concentrations for heat waves a) 1 (d 29, 32 and 36); b) 2 (d 71, 74 and 78); and c) 3 (d 120, 123 and 127) for un-shaded Angus (UNSH AA), un-shaded Charolais (UNSH CH), un-shaded Brahman (UNSH BH), shaded Angus (SH AA), shaded Charolais (SH CH) and shaded Brahman (SH BH) steers