Breeding Focus 2018 – Reducing Heat Stress

Edited by

Susanne Hermesch

Animal Genetics and Breeding Unit, University of New England, Armidale, Australia

Sonja Dominik

CSIRO Agriculture and Food, Armidale, Australia

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University of New England

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Animal Genetics and Breeding Unit

University of New England

Armidale NSW 2351

Australia

http://agbu.une.edu.au

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Preface

"Breeding Focus 2018 – Reducing Heat Stress" is the third workshop in the series. The Breeding Focus series was developed to provide an opportunity for exchange between industry and research across a number of agricultural industry sectors. With this goal in mind, workshops have included presentations across the livestock and aquaculture industries to take participants outside their area of expertise and encouraged them to think outside the box. This year we increased the scope even further by also inviting presentations from the cropping and horticulture industries. Since the topic of heat stress has recently gained increased attention, we will discuss a wide range of aspects associated with heat stress, such as the physiology of heat stress and phenotypic indicators, genetic approaches and industry impacts.

Heat stress in animals describes a situation where an animal is exposed to high temperatures and unable to dissipate body heat, which causes an increase in body temperature. In the short term, an animal will react to heat stress with behavioural strategies (e.g. seeking shade, panting) to reduce the heat load. With prolonged excessive heat load, feed intake is reduced and production losses occur. Under extreme circumstances, excessive heat load can lead to death. In plants, heat stress can be defined as irreversible damage to plant function and development as a consequence of hot temperatures. Environmental causes of heat stress in plants and animals include high temperatures and high humidity over a long period of time, which is exacerbated by low cloud cover and high solar radiation.

With raising average temperatures, agricultural industries are faced with the challenge to manage potential impacts of heat stress on their crops, their pasture base and welfare and production of their livestock or aquaculture species. Management strategies such as shade and irrigation are effective but costly and, depending on the severity of climatic conditions, may have limited success. Susceptibility of organisms to heat stress can vary due to factors such as age and general health, but also genetic factors, such as breed or variety. Further, as we will hear during the workshop, genetic variation exists within breeds that enables genetic approaches to address heat stress in plants and animals. Selective breeding provides a long term approach that facilitates improvement of the physiology of plants and animals to cope with excessive heat load. The challenge here is to obtain cost-effective phenotypes to describe heat stress.

The chapters of this book discuss where the current climate is trending, and outlines opportunities for the crop, orchard, livestock and aquaculture industries to describe and measure heat stress, all with the focus on genetic improvement.

We would like to thank everyone who has contributed to this event for their time and effort: the authors for their contributions to the book and presentations, the reviewers who all readily agreed to critique the manuscripts. We would like to express a special thanks to Kathy Dobos for her contributions into the organisation of this workshop and the publication. Thank you!

Susanne Hermesch and Sonja Dominik
Armidale, September 2018

Towards breeding for heat tolerance and resilience in beef cattle

Gene Wijffels[1], Megan Sullivan[2], Stephen Anderson[2], Sally Stockwell[1], Russell McCulloch[1], Suzie Briscoe[1], Joseph Olm[2], Judy Cawdell-Smith[2] and John Gaughan[2]

[1]CSIRO Agriculture and Food, St Lucia, QLD 4067, Australia

[2]University of Queensland, Gatton, QLD 4345, Australia

Abstract

"Heat stress" has grown in prominence as a production and welfare concern for most livestock production systems. High heat load affects animal growth, milk production, reproduction and health. As the average night temperature rises globally and locally, the dissipation of day time accumulated heat load from animals and infrastructure is reduced. This situation has been exacerbated by heat waves of increasing frequency, duration and intensity over recent decades.

The most obvious responses to high heat load and characteristic of heat stress are reduced feed intake, increased water consumption and, in cattle, panting. The reduced feed intake lowers the heat of fermentation in the rumen, and metabolic activity particularly in organs like the liver. This is an excellent coping strategy, but has serious consequences for growth and reproductive potential. This adaptation is about staying alive and in good health and while limiting other functions that impact on production until the situation normalises.

So, is the heat resilient phenotype simply an animal that keeps eating during high heat load regardless of the consequences, or the animal that copes well, preserves homeostasis and recovers quickly? In this paper we report our finding that aim to address this question.

Introduction

Research on the effects of heat stress in cattle has been ongoing since at least the 1940's (Bianco, 1965). The literature is replete with reports of field trials where conditions are difficult (impossible) to control and replicate, with different diets, breeds or breed composites, ages, weights, gender, and in many studies are of high producing dairy cows, in pregnancy or stage of lactation etc. Comparing results is problematic because of the multitude of conditions used; there is no standardised methodology. Earlier studies in climate controlled facilities were hampered by limited ability to control ambient temperature, and often no control of humidity

and wind speed, and inability to provide diurnal temperature and humidity cycling. Moreover, studies tended to focus on a few variables, for example, coat colour, hair type, electrolyte balance, a suite of hormones, or a subset of immune functions (see reviews, by Beede and Collier, 1986; Bernabucci *et al*., 2010; Baumgard and Rhoads, 2012). While much more has been done in the temperate dairy cow which is very vulnerable to heat stress, there is limited understanding of the impacts of high heat load on beef cattle, particularly growing beef cattle, at a systemic level. Furthermore, the pathway to recovery and how to ensure recovery from heat stress is less understood. At this point in time, we simply cannot identify a heat tolerant or heat resilient animal.

Our work aims to make a contribution to understanding both the heat stress response, and assess amelioration of heat stress through nutrition and management interventions and genetics. Our focus in understanding heat stress and heat resilience has been the feedlot steer, specifically, the black Angus steer, on full finisher ration at 60 - 100 days-on-feed (in the feedlot). The challenges of the feedlot environment are the diet which is high in metabolisable energy, pen density, and with any intensive livestock housing situation, reduced dissipation of heat and a higher risk of the spread of pathogens. Overall the feedlot sector has improved infrastructure and management to reduce heat load in cattle (shade, orientation to prevailing winds, dietary management, vaccinations and customised weather forecasting). The industry is also developing nutritional and/or management practices to further ameliorate heat stress in lot fed cattle. In this context, we are conducting research to comprehensively investigate the metabolic and endocrine impacts of heat stress and how heat stressed animals recover. This will enable us to differentiate between animals of varying heat tolerance and provide objective evidence for the efficacy of interventions to reduce heat stress. This report highlights some of our current findings.

Assessing the Impact of Increased Heat Load on Feedlot Steers

We have developed two protocols, a Moderate Heat Load regimen and a High Load regimen, to assess the impacts of two levels of heat load. For both regimens the animals were subjected to 4 consecutive periods. The first three periods were conducted in climate controlled rooms; preHOT: acclimation in climate controlled rooms in individual pens in thermoneutral (TN) conditions, HOT: when the steers were subjected to a heat load challenge period; Recovery@ TN: recovery in TN conditions. Finally, in the fourth period, the steers were returned to feedlot outside pens (PENS) (see Figure 1). Live weights were 450 - 500 kg on entry to the climate controlled rooms, and the animals were maintained on finisher ration throughout (supplied twice daily) with *ad libitum* access to water. The steers' physiological responses were monitored intensively (e.g. respiration rate, movement, fecal score, rumen temperature) during the preHOT, HOT and Recovery@TN periods, and blood samples were collected frequently to determine haematological, metabolic, endocrine and inflammatory changes.

The Moderate Heat Load regimen consisted of two treatment groups, a Control group and a thermally challenged group. During the HOT period, the thermally challenged cohort was

subjected to a moderate but constant heat load for five days with maximum ambient temperature of 35°C (see Figure 1A). The Control group was maintained at TN conditions throughout the experiment but was fed restricted during the HOT period. Two replicates were conducted with 6 head per treatment group in each replicate. The PENS period for the Moderate Heat Load regimen was 10 days, with blood collected on alternate days. These animals were further grown out in feedlots for another 2 weeks but blood samples were not collected.

For the High Heat Load regimen, there was only a single treatment (that is, there was no thermoneutral feed restricted Control group). The cattle were subjected over the HOT period to three days high heat load with sudden onset, but then and stepped down in intensity over the following four days (see Figure 1B). Two replicate experiments with 12 head each were conducted. The PENS period for the High Heat Load regimen was 21 days, with blood collected at 1 and 3 weeks after exit from the climate controlled rooms.

Haematological and plasma biochemical profiles were performed by a commercial provider (IDEXX), while measurement of plasma hormones, inflammatory markers, and glutamine were made in our laboratory with commercially available and in-house reagents. These assays were performed on all plasma samples.

To simplify analyses, we compared the data for each of the three periods in the climate rooms, PreHOT, HOT (or TN for the Control), Recovery@TN, and the final period in outdoor PENS (as indicated in Figures 1A and B). Average daily gain (ADG) was calculated from live weight obtained prior to entry to the climate rooms (approx. day -5), at exit from the rooms, and twice during the PENS period (Figure 2). Having collected data from the heat challenged group and control group in the Moderate Heat Load regime, we were able to analyse the differences in responses between these two cohorts, as well as contrast the PreHOT measures for each group with the subsequent periods (Figures 2 and 3). For the High Heat Load regimen, the PreHOT measures were contrasted with the subsequent periods only.

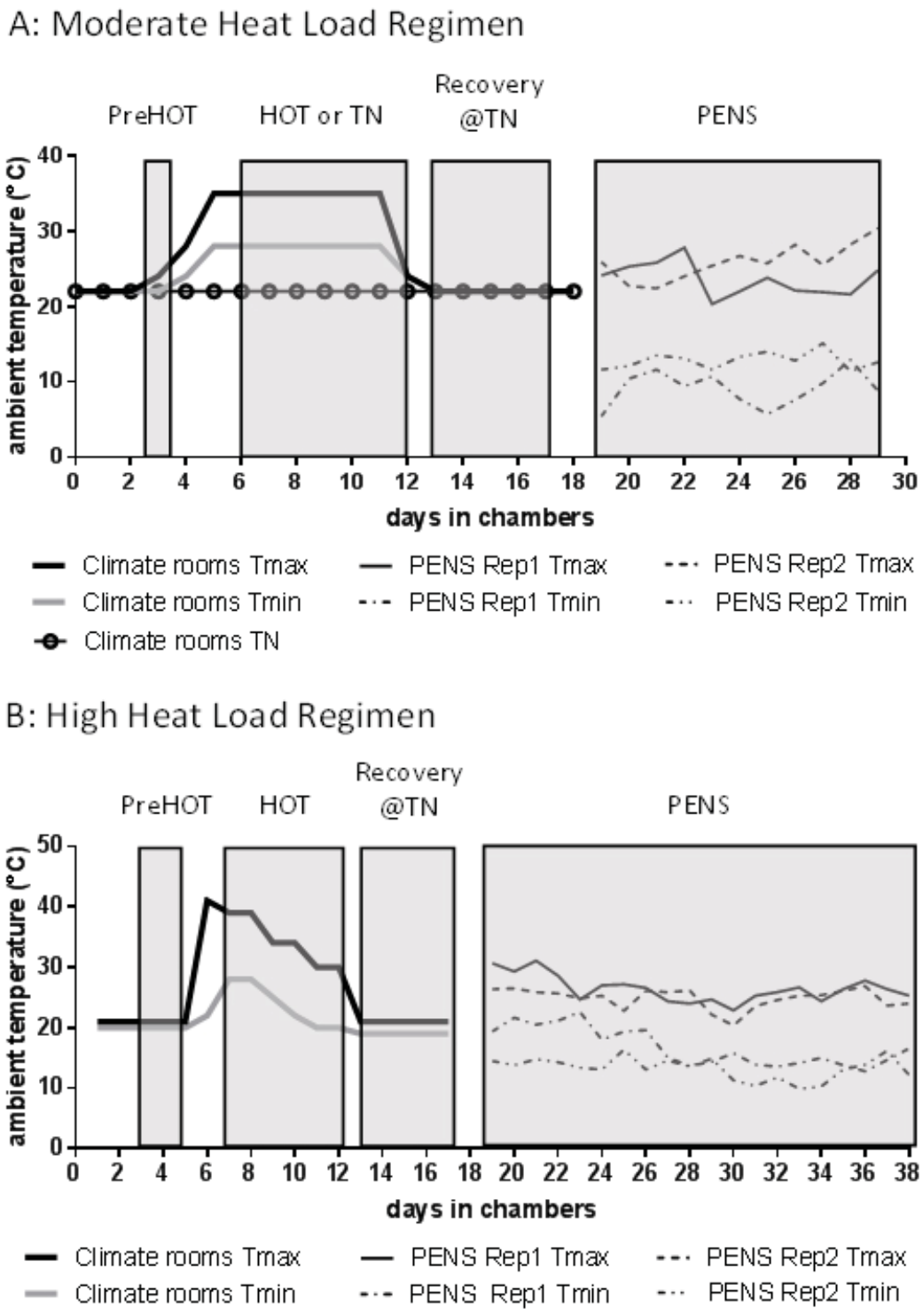

Figure 1. The maximum and minimum ambient temperatures (Tmax and Tmin) of the Moderate and High Heat Load regimens. The temperature range for each regimen during the three periods in the climate controlled rooms (PreHOT, HOT, Recovery@TN) and for each replicate (Rep1 and Rep2) while in PENS. A. The Moderate Heat Load regime. The Control group was maintained at constant thermoneutral conditions (Climate rooms TN). B. The High Heat Load regime. TN: thermoneutral; Rep: Replicate.

Systemic Responses to Increased Heat Load

Feed Intake and Growth

The average daily feed intake during the HOT period for the Moderate and High Heat Load trials were 33% and 48% less than the preHOT feed intake respectively (Figures 2A and B). The feed restricted Control group had a feed intake that was 21% lower than the preHOT period. The ADG at the end of the climate room periods was close to zero for all groups (Figures 2C and D). However, ADG can be affected by water consumption and retention in animals with high heat load, in addition to faecal and urinary excretion, feed intake, gut fill and feed utilisation. On average, ADG for all groups recovered to PreHOT levels after the first week in PENS.

Metabolic and Endocrine Responses

Along with the sudden reduction in feed intake, the elevated rumen and rectal temperatures, and respiration rate in the thermally challenged groups presented a typical heat stress response for cattle. The frequent blood sampling enabled a comprehensive assessment of metabolic and endocrine changes. In Figure 3, the trajectories of the means during the four periods for a selection of metabolites and hormones are shown. Generally, changes seen as a consequence of thermal challenge during the Moderate Heat Load regimen were repeated during the High Heat Load regime. However, when there were ambiguous responses detected in the Moderate Heat Load regime, the responses obtained with the High Heat Load thermal challenge were clear cut.

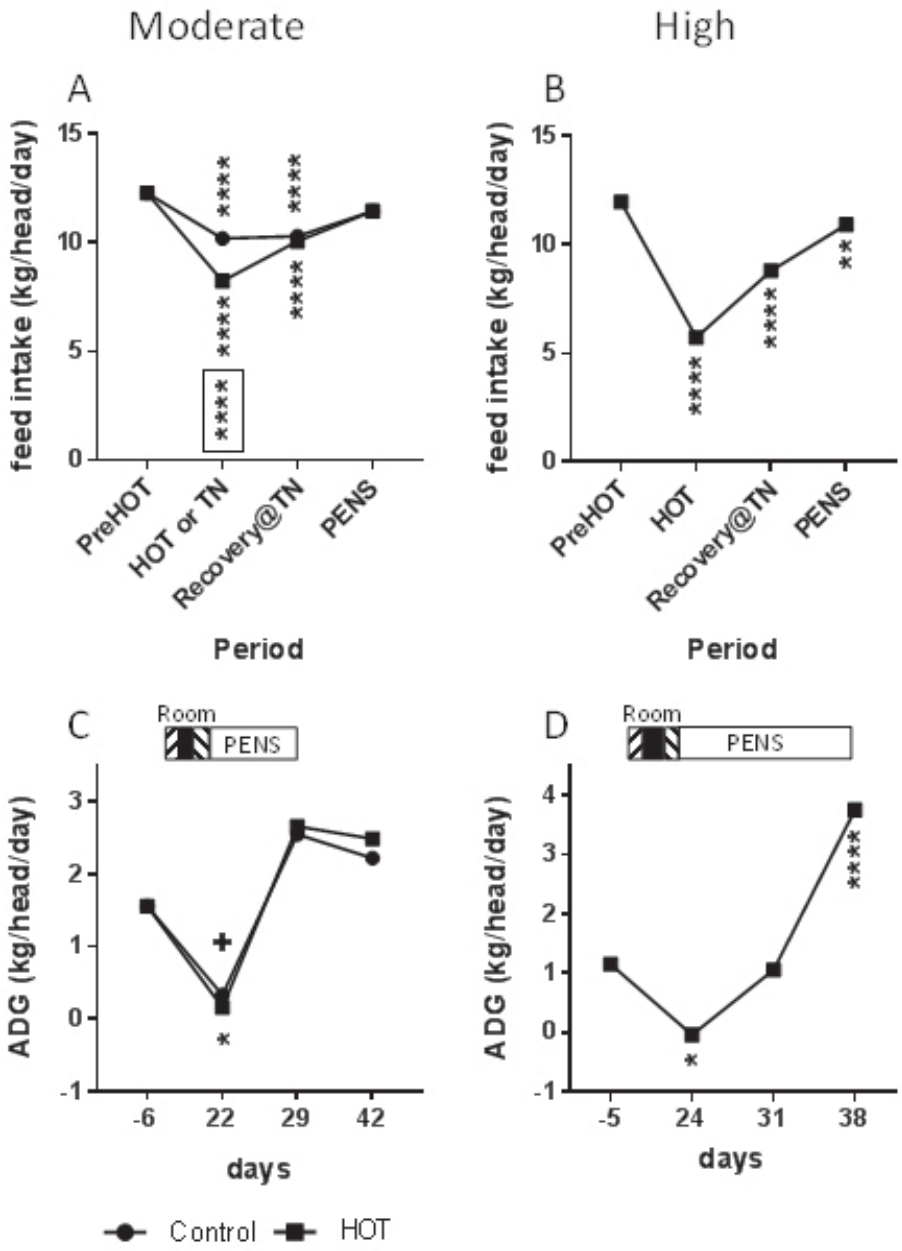

*Figure 2. The mean daily feed intake (A) and Average Daily Gain (ADG) (B) of the steers before, during and after thermal challenge by the Moderate and High Heat Load Regimens. Feed intake was measured daily. ADG was derived from live weights taken prior to entry to, and on exit from the climate controlled facility, and during PENS on the days indicated. The intervals in the climate controlled rooms (Room) and pens (PENS) are shown. Statistically significant differences are indicated by the asterisks. The asterisks close to the plotted means indicate significant difference from the preHOT mean, whereas the boxed asterisks positioned above the x-axis indicate significant difference between the HOT (thermally challenged) and Control groups (Moderate Heat Load regimen only). +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001. Moderate and High Heat Load regimens are given in Figure 1. The Control group which was run for Moderate Heat Load experiment only were kept in thermoneutral conditions but feed restricted during the HOT period.*

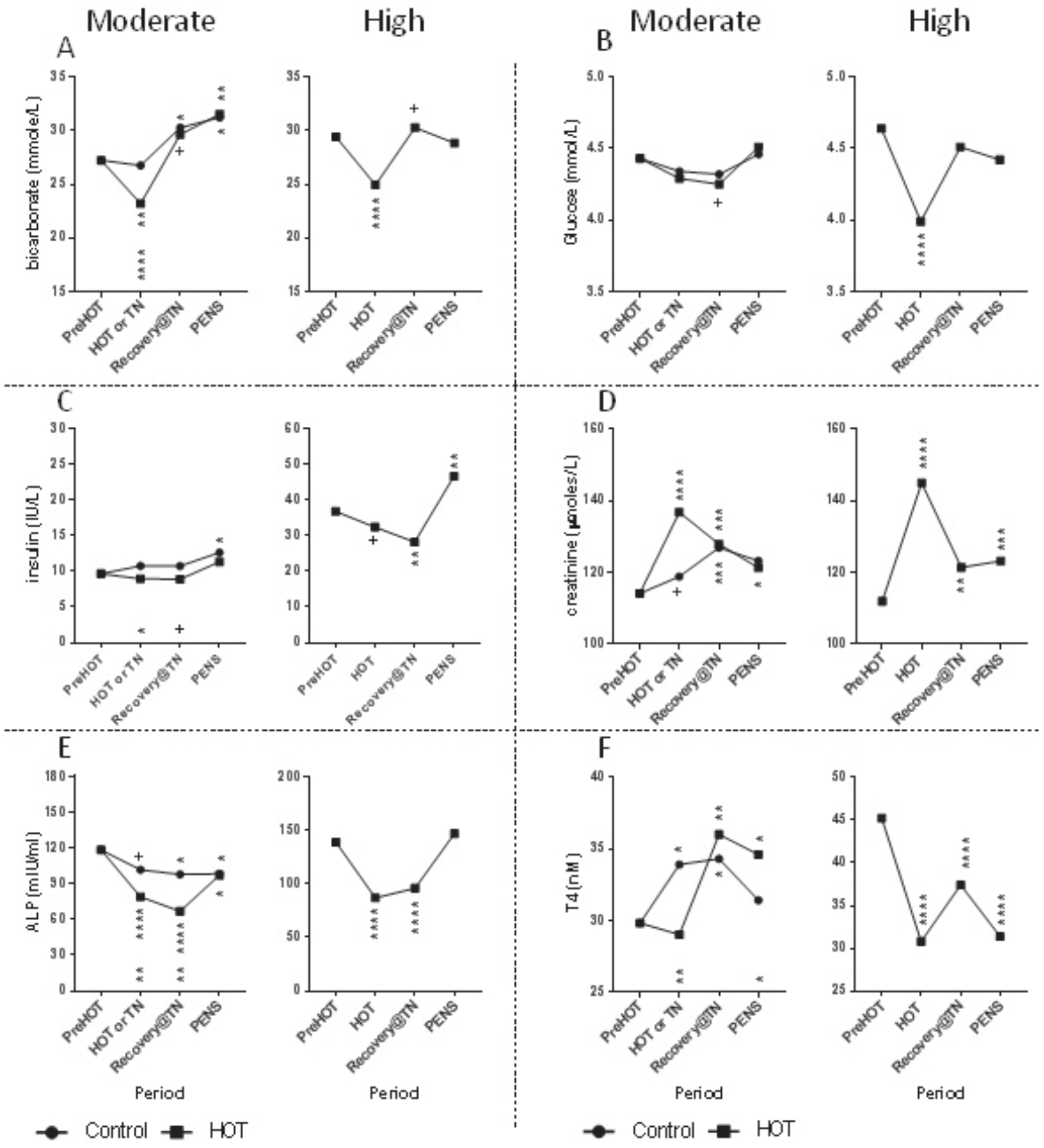

*Figure 3. The mean concentration of selected plasma metabolites and hormones, the mean plasma activity of alkaline phosphatase (ALP) during the three periods in the climate controlled rooms and in PENS during the Moderate and High Heat Load regimens. A. Bicarbonate. B. Glucose. C. Insulin. D. Creatinine. E. Alkaline phosphatase (ALP). F. T4 (Thyroxine). The asterisks close to the plotted means indicate significant difference from the preHOT mean, whereas the boxed asterisks positioned above the x-axis indicate significant difference between the HOT (thermally challenged) and Control groups (Moderate Heat Load regimen only). +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001. Moderate and High Heat Load regimens are given in Figure 1. The Control group which was run for Moderate Heat Load experiment only were kept in thermoneutral conditions but feed restricted during the HOT period.*

Reduced plasma bicarbonate levels are typical of a heat stress response as the increased respiration rate and panting increases removal of CO_2 from the blood (Figure 3A). The Control animals did not have this response, and both the thermally challenged cohorts returned to normal plasma bicarbonate concentrations once the thermal conditions moderated. The perturbations to energy metabolism in heat stressed cattle were less clear. The plasma glucose concentrations of thermally challenged cohort in the Moderate Heat Load regimen were essentially the same as the Control group during any period, that is, despite the reduced feed intake, both cohorts maintained their plasma glucose levels (Figure 3B). There was no overt plasma insulin response during in either cohort in the Moderate Heat Load regimen (Figure 3C) although the Control group tended to have higher plasma insulin concentration (~20% higher) during feed restriction and in Recovery. However, with the increased heat load induced by the High Heat Load regimen, plasma glucose levels fell on average by 14% during the HOT period (relative to PreHOT), and plasma insulin levels tended to be lower during HOT relative to the PreHOT period mean (Figure 3C). Interestingly, the plasma insulin in this cohort fell further during the Recovery period, when plasma glucose had recovered to its preHOT concentration. This may suggest some dysregulation during the recovery period. Perturbations of glucose and insulin responses have been reported in dairy cows under moderate and high heat load (Wheelock *et al*., 2010; Baumgard *et al*., 2011).

Creatinine, is a byproduct of muscle metabolism. Plasma creatinine is freely filtered by the kidney into the urine. With increased heat load, plasma creatinine levels are consistently raised (Figure 3D), possibly implicating altered renal filtration and/or muscle metabolism. There was a rise in plasma creatinine in the Control group during Recovery also, when it converged with the thermally challenged group's plasma creatinine level. The fall and delayed recovery of plasma alkaline phosphatase (ALP) activity in both thermally challenged cohorts (Figure 3E) is indicative of reduced liver and/or bone metabolism. Reduction in plasma ALP activity is found consistently during heat stress in dairy cows (Abeni *et al*., 2007). Using more specific markers of bone turnover, we now have good evidence for decreased bone formation and increased bone mobilisation as a response to high heat load exposure (unpublished results).

The thyroid produces and releases two hormones, T3 (triiodothyronine) and T4 (thyroxine), and both have major effects on metabolic rate and thus endogenous heat production. T4 is converted to more active T3 mainly in the liver. As shown in Figure 3F, the feed restricted Control animals of the Moderate Heat load regimen responded with increased plasma T4 and T3 concentrations (unpublished results) which was sustained during Recovery. No such rise occurred in the thermally challenged group in this regimen but a rapid rise in plasma T3 followed in Recovery. In contrast, the High Heat Load regimen induced an obvious fall in plasma T4 and T3 concentrations (relative to preHOT) with a limited rise in Recovery (Figure 3F).

Concluding Comments

The examples of response during heat load and recovery presented here are indicative of the systemic impacts of the heat stress response in cattle, and the magnitude of the response seen with high heat load. The feed restricted thermoneutral Control group has been critical to our understanding of how heat affects animals independent of a reduction feed intake. That is, differences between animals with heat load and reduced voluntary feed intake versus animals with enforced feed restriction.

While our focus has been the black Angus grain fed steer, a next step would be to see how heat tolerant taurine breeds such as Senepol and Romosinuano differ in their responses to our heat load challenge regimens. Scharf *et al*., (2010) has shown that 12 month old Romosinuano steers did not experience raised plasma creatinine during a thermal challenge similar to our Moderate Heat Load regime. A mutation in the prolactin receptor gene has been implicated in conferring heat tolerance and the "slick" hair phenotype in these breeds (Littlejohn *et al*., 2014; Porto-Neto *et al*., 2018). As a result there is keen interest in gene editing this mutation into Angus and dairy breeds to test the heat tolerance of this mutation in a different genetic background.

The heat load challenge regimens we have developed are assisting us to measure the efficacy of various nutritional and management interventions in amelioration of heat stress. Equally, these challenge regimens could be applied to examining the heat load tolerance of different cattle breeds, and the progeny of elite sires. Our aim is to enable the Australian beef industry to increase the heat tolerance and resilience in the national beef herd.

Acknowledgement

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