final report

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Heat load nutrition program

Final Report

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Executive Summary

Heat stress in cattle is a recognised welfare and production issue in the feedlot sector. The main objective of this research program was to provide recommendations for nutritional management of feedlot cattle during periods of high heat load during summer. We undertook two approaches in measuring and understanding the metabolic and inflammatory impacts of high heat load by running feedlot trials over summer and winter seasons, and under the controlled conditions of the climate chamber.

Our working hypothesis at the start of this program, was that high heat load causes loss of gut integrity, leading to systemic inflammation that generates more heat load and thus exacerbates the problem, resulting in further organ damage and eventual loss of homeostasis and thermoregulation. To find evidence for these events, the program conducted seven feedlot trials (of 70 – 110 days) and four climate chamber experiments of increasing heat load. The acute high heat load regimes were designed to mimic a strong heatwave event. In all trials and experiments, the cattle were on a finisher ration.

The most significant findings were obtained from the acute high heat load climate chamber experiments. The impact of an overnight large increase in daily maximum and minimum temperatures and THIs which was sustained for one to three days was captured by intensive collection of physiological measures, and biochemical and haematological parameters obtained from frequent bleeds.

As can be anticipated, the steers responded with marked increased rumen temperature, respiration rate, panting score, water usage and a dramatically reduced feed intake. The minimum and maximum rumen temperatures were a full degree higher than normal indicating high levels of accumulated heat load. Distinctive responses from the liver, kidneys, and lungs were detected during high heat load. There was no indication of organ damage, loss of gut integrity or inflammation at this stage.

The steers were also intensively studied during the four to six day recovery phase in the climate chambers. Recovery seemed to induce a slight negative thermal balance with lower than normal rumen temperature, rectal temperature, and respiration rate despite the increased feed intake. There was evidence for liver damage, and increased vulnerability to infection due to reduced white blood cell counts. Systemically, the steers are mildly acidotic. Again, there was no indication of systemic inflammation.

The use of a heat load ration and timing of its introduction to animals at risk during a predicted heatwave was tested. An industry standard heat load ration was supplied to the steers either two days prior to or on the day of onset of acute high heat load. The clear result was that change of diet at the start of a heatwave is injurious to cattle, the liver being especially impacted. The success of the final climate chamber trial ensures that the industry has a robust protocol available for comparison of other diets or rations.

Despite the limitations of the feedlot trials, it was reassuring that some of the metabolic responses observed in the high head load climate chamber experiments were captured during or after the more intensive heatwave events in the feedlot trials. The very comprehensive nature of this research program has produced a globally unique data set; there is much more to be gleaned and communicated.

The future of managing heat load in the feedlot lies with developing a heat load model based on rumen temperature as collection of this data becomes easier and cheaper. “Protecting”

the liver and moderating the acidosis during recovery are targets for nutritional or pharmaceutical interventions. We have the wherewithal and interest to tackle both.

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1 Background

The modern urbanised Australian society has become increasingly aware and interested in both the consequences of a warming environment and the welfare of production animals, particularly in the intensive livestock industries. Concurrently, there is the continued pressure for increased efficiencies and productivity. The cattle feedlot industry requires improvements that enhance both welfare and production efficiency.

Feedlot cattle are predominantly young male cattle on high concentrate rations. The most commonly observed effects of increased heat load are reduced feed intake (with consequent reduced growth rate), reduction in feed efficiency during and after a heat event, and the increased frequency of health-related issues.

The strong industry consensus is a need to have better nutritional knowledge for improved management of cattle through summer and during high heat load events. With input from industry representatives, this research program was designed to deliver new nutritional strategies for a forecast heat event and investigate the need for management of summer high risk periods or all of summer.

A highly resourced, five year program of research was undertaken to achieve this goal investigating the responses of feedlot animals over summer in experimental feedlots, or under controlled heat load events in climate chamber experiments.

Previous evidence has shown that the gut barrier function is disrupted in heat stress therefore the initial focus of this research was the effect of heat stress on gut health and integrity, and its role in inflammatory responses in the animals. The climate chamber experiments would guarantee intensive monitoring of physiological responses and intensive blood sampling for multiple analyses of metabolites, endotoxin, and metabolic and inflammatory hormones to inform of the status and dynamics of the inflammation, energy metabolism and muscle breakdown. The feedlot experiment sampling would be subjected to similar analyses, but samples would be collected less frequently and fewer physiological observations would be performed.

Additionally, once the heat stress responses were identified, altered dietary regimes could be trialled to identify optimal management strategies prior to or during anticipated high heat load events. To ensure that any recommended intervention is applied to gain the most optimal result, this program intends to understand the altered metabolic and inflammatory responses induced by high heat load in feedlot steers. Dietary or management practices may then be proposed to reduce the impact of heat stress in the Australian feedlot industry.

2 Project Objectives

2.1 Provide recommendations for nutritional management of feedlot cattle during summer, and/or during periods of high heat load, to improve productivity and welfare of feedlot cattle, through:

2.1.1 Establish the need for nutritional strategies to manage heat load through the high risk periods of summer, or all of summer feedlot studies.

2.1.2 Understand the impact of heat load conditions on immune status, and the altered metabolic and inflammatory responses induced by these conditions.

2.2 Develop new nutritional interventions and management strategies before, during and after heat load events.

2.3 Increased ongoing nutritional research capability for industry through training and mentoring of a postdoctoral fellow at the University of Queensland.

3 Methodology

3.1 Climate chamber experiments

All studies were conducted with the approval of The University of Queensland animal ethics committee (SAFS/210/13/MLA) in accordance with the guidelines described by the National Health and Medical Research Council (2013) in Australia.

On arrival at QASP, animals were housed in paddocks with access to roughage only. On entry to the feedlot (day 0), steers were vaccinated against clostridial diseases (Pfizer Animal Health, Australia), Bovine Respiratory Disease (Coopers Animal Health, Australia) and trivalent tick fever (Department of Agriculture, Fisheries and Forestry (Biosecurity Queensland), Australia). Animals were also treated for internal and external parasites (Cydectin, Fort Dodge Australia P/L, Baulkham Hills, NSW, Australia) and were weighed (non-fasted) on feedlot entry and at seven day intervals prior to entry to the rooms and after exit from the rooms, and at each blood sampling throughout the trial period. Rectal temperatures were also obtained at these times by inserting a digital thermometer (BD™, Becton Dickinson and Company, USA) into the rectal cavity.

Each steer was orally administered with a transmitting rumen temperature bolus (Smartstock, Pawnee, OK, USA) on day 0, an active RFID transmitter which communicated to a base station, and data was transcribed to a database using proprietary software (TechTrol Inc., Pawnee, OK). In trials 9-12 they also received a temperature and pH monitoring bolus (SmaXtec, Austria). Further, animals in trials 4 to 12, each had a hormone growth promotant (HGP) implant inserted (Synovex® with trenbolene acetate growth and finishing implant for steers and heifers, Zoetis).

The commencement of the feeding period (entry to feedlot pens, day 0) was staggered according to replication group to ensure that all animals were subject to identical management and feeding regimes. The animals were housed in the feedlot pens in replication groups of 12 head. The pens had an area of 162 m^2 (27 m x 6 m) with a shade footprint of 1.2 m^2/animal at midday.

The animals were fed twice daily with 50% of the daily ration fed at each feed. Refusals were removed prior to each feeding and weighed. The cattle were fed a starter ration initially and transitioned gradually to a finisher ration whilst still in the feedlot pens.

Apart from the chronic heat load trials (CC1-3), animals were then housed for 10 days in external individual pens with individual access to a water and feed trough. During this period the same procedures (feeding, weighing and observations) were conducted as in the feedlot and the animals were housed according to their future positions in the climate rooms. Feed was offered twice daily with 50% of the daily ration fed at each feed. Refusals were removed once daily and weighed.

Animals were subsequently housed in the climate controlled rooms (CCR) for the experimental phase of the trials where they were kept in individual pens (2.2 x 2.2 m^2). In the chronic and acute heat load trials (CC1-3 and CC4-6 respectively) the animals were matched as pairs by weight and feed intake levels (one animal within pair in each treatment). The cattle were allocated to one of 12 pens (three pens per room) and two treatments, hot treatment (HOT) or thermoneutral (TN), and were pairfed meaning that the ‘fed amount’ of

grain was adjusted depending on the HOT animals' intake. In the high heat load trials (CC7-8), all 12 steers were subject to the same temperature parameters however those in CC8 received a heat load feed ration due to difficulties coping with the heat.

The final climate chamber trials were the heat load diet trials (CC9-12) where cattle were subjected to one of three dietary treatments during the hot period. Dietary treatments consisted of a control diet (Diet 1), a heat load ration fed on day 1 of hot conditions (Diet 2), and a heat load ration fed two days prior to hot conditions (Diet 3).

Whilst in the feedlot pens, onsite climatic data were collected at 10 min intervals using an automated weather station (Vantage Pro, Davis Instruments, CA). Weather data collected included ambient temperature (TA; °C); relative humidity (RH; %); wind speed (WS; m/s) and direction; solar radiation (SR; W/m^2); black globe temperature (BGT; °C) and daily (0900 h) rainfall (mm) from which temperature humidity index (THI), heat load index (HLI) and accumulated heat load for shaded Angus (AHLU86) were calculated (Gaughan et al., 2008).

In the climate chambers, micro-climatic conditions (TA, ºC and RH, %) within each room were monitored at 10 min intervals over the trial period using temperature and humidity data loggers (HOBO UX100-011). Temperature and humidity were regulated according to the requirements of each trial. Temperature and THI regimes for each trial are depicted in Fig. 1 (CC1-3), Fig. 2 (CC4-6), Fig. 3 (CC7-8) and Fig. 4 (CC9-12).

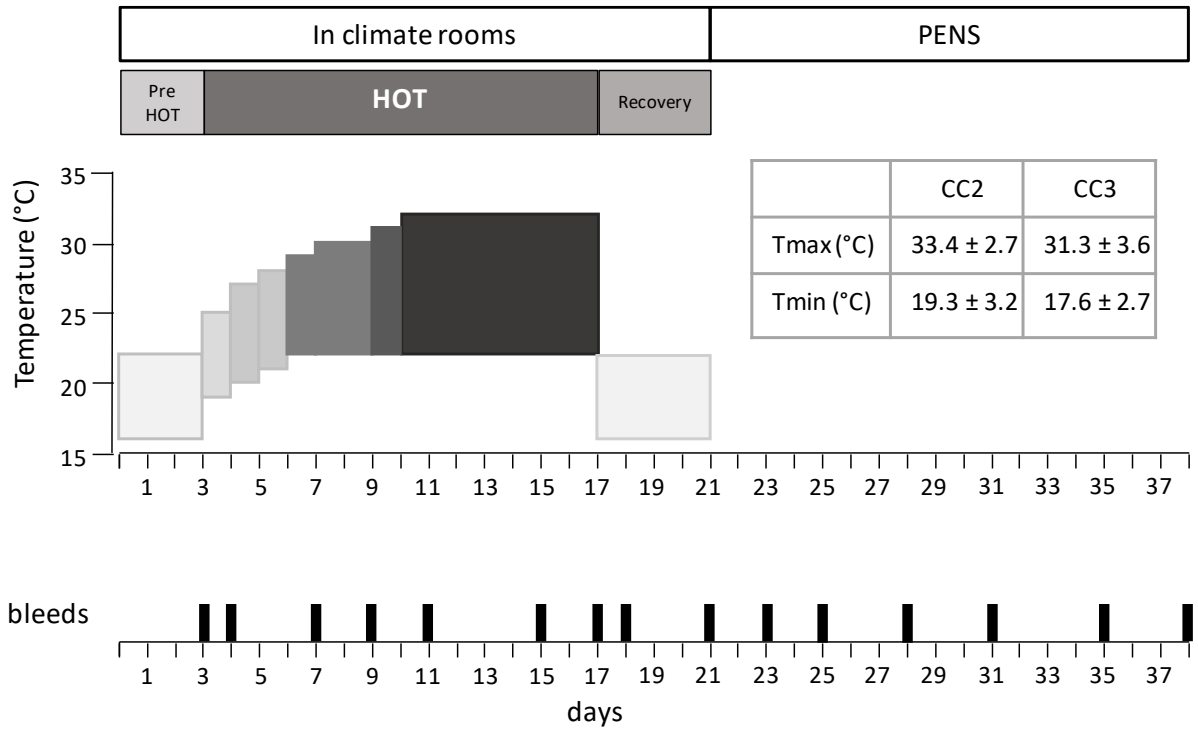

Fig. 1. The climatic regime of the chronic moderate heat load challenge (CC1-3). The range of the mean daily ambient temperature and duration of each period is depicted for the 38 days of the experiment. The PreHOT, HOT and Recovery periods were conducted in climate controlled rooms. The PENs interval occurred in outdoor feedlot pens. Only CC2 and CC3 proceeded to PENs. Blood sampling days are indicated also.

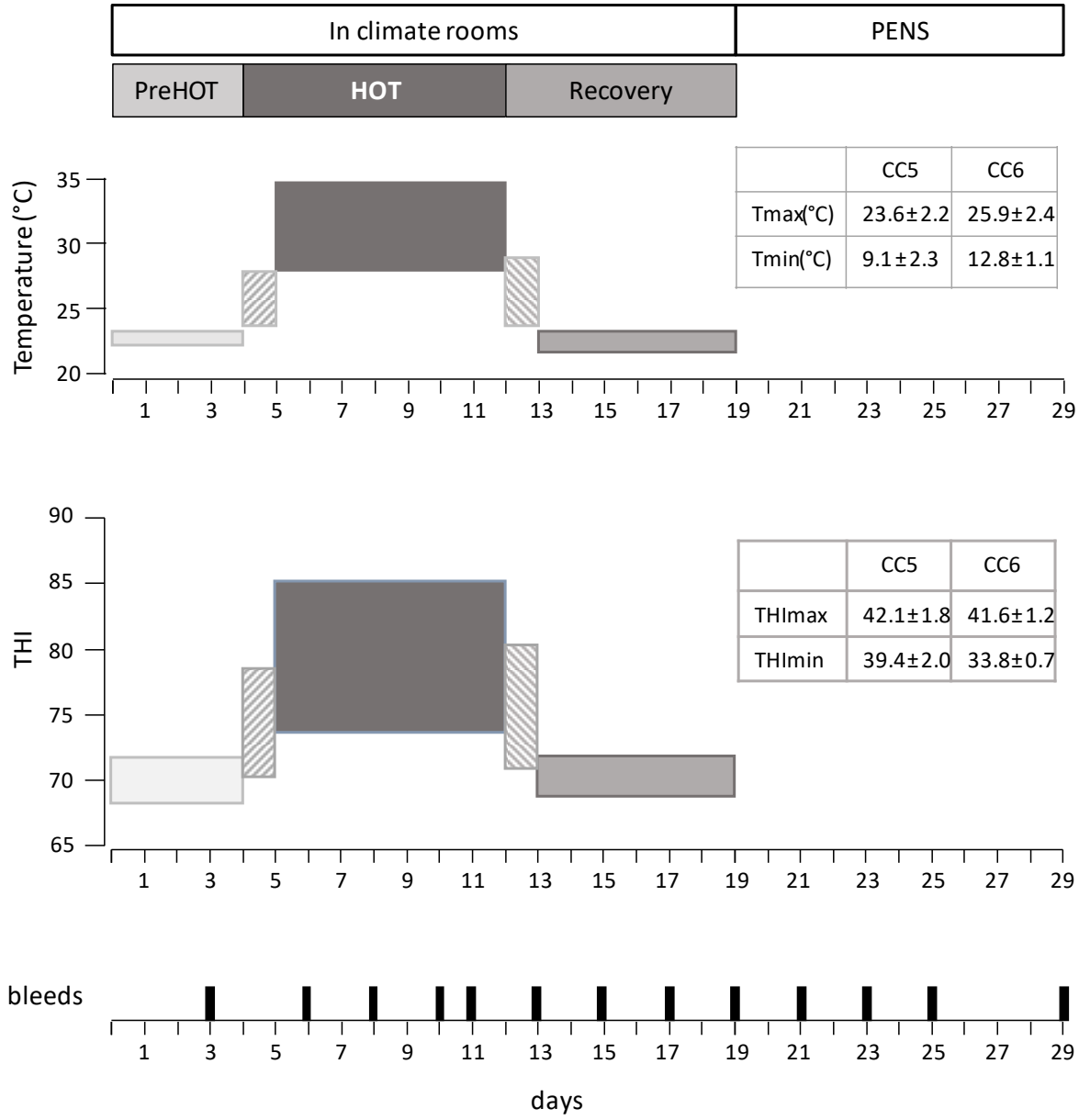

Fig. 2. The climatic regime of the acute moderate heat load challenge (CC4-6). The range of the mean daily ambient temperature and Temperature-Humidity Index (THI) and duration of each period is depicted for the 29 days of the experiment. The PreHOT, HOT and Recovery periods were conducted in climate controlled rooms. Only CC5 and CC6 proceeded to PENs. The PENs interval occurred in outdoor feedlot pens where the climatic conditions (mean ± SD) for each replicate is presented in the inserted tables. Blood sampling days are indicated also.

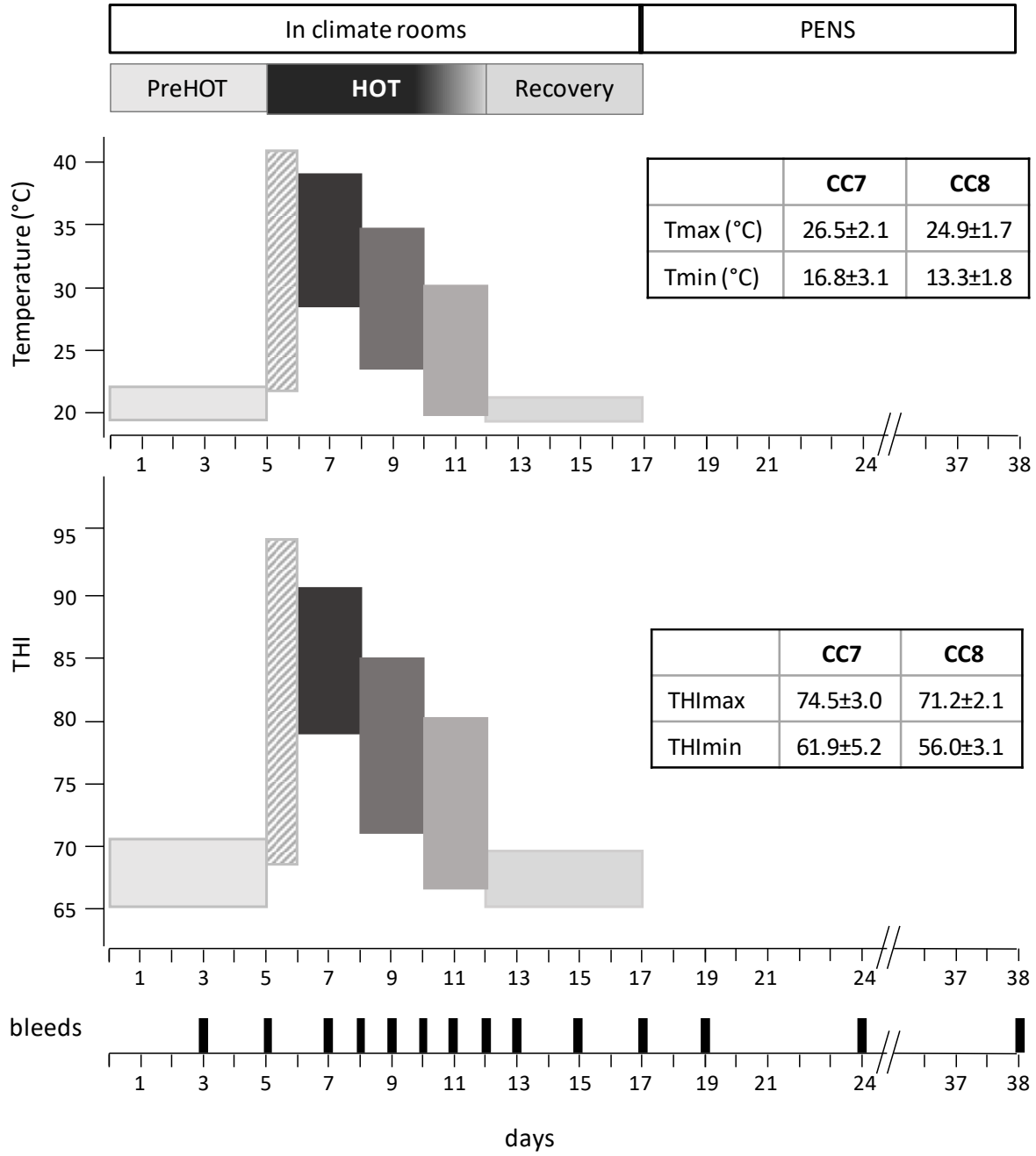

Fig. 3. The climatic regime of the high heat load challenge (CC7 and 8). The range of the mean daily ambient temperature and Temperature-Humidity Index (THI) and duration of each period is depicted for the 38 days of the experiment. The PreHOT, HOT and Recovery periods were conducted in climate controlled rooms. The PENs interval occurred in outdoor feedlot pens where the climatic conditions (mean ± SD) for each replicate is presented in the inserted tables. Blood sampling days are indicated also.

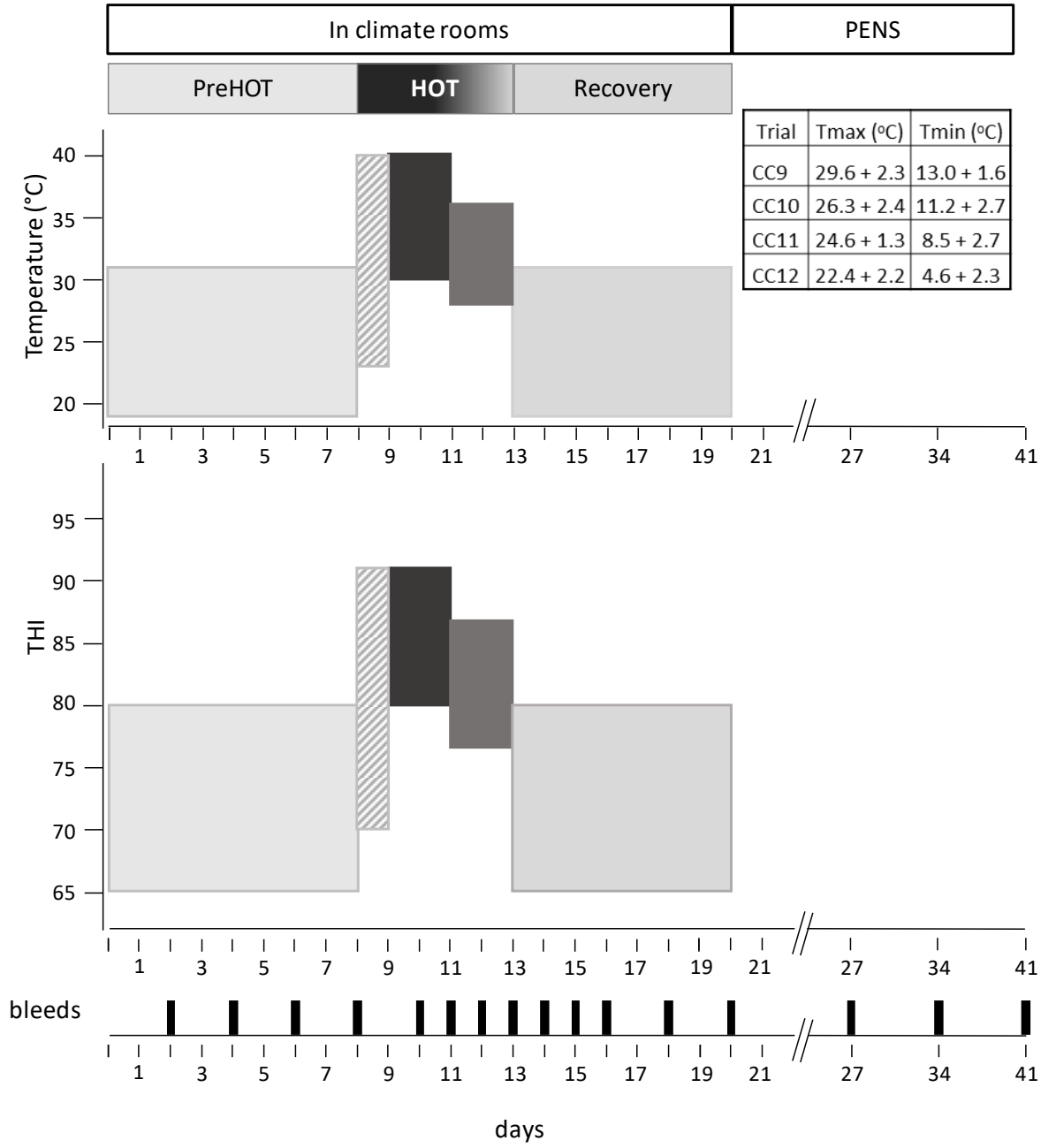

Fig. 4. The overall climatic regime of the high heat load challenge imposed during the CC9-12 trials. The range of the ambient temperature and Temperature-Humidity Index (THI), and duration of each period is depicted for the 41 days of the experiment. The PreHOT, HOT and Recovery periods were conducted in climate controlled rooms. The PENs interval occurred in outdoor feedlot pens; the climatic conditions (mean ± SD) for each replicate is presented in the inserted tables. The PENs mean maximum and minimum temperatures for each cohort are based on Gatton BoM weather station data. Daily maximum and minimum THI data was not available. Blood sampling days are indicated also.

Physiological data was obtained on all animals in the feedlot pens, individual pens and climate controlled rooms. The physiological status of cattle was determined using panting scores, respiration rate, posture (standing/lying), animal surface temperature, head position, activity (eating/drinking/ruminating/no activity), temperament (agitated/calm/depressed) and

pen position. Observations in the feedlot were carried out three times daily, increasing in frequency once the animals were in individual pens or in the climate controlled rooms to two hourly intervals or hourly intervals during the hot periods.

Blood samples were obtained from all animals in all studies at seven day intervals while in the feedlot pens or in the individual pens. In the climate controlled rooms, samples were taken at varying intervals depending on the trial. These are depicted in Fig. 1-4. Samples were collected from each steer by jugular venepuncture using 10 mL vacutainer tubes containing either K_3-ethylenediaminetetraacetic acid (K_3-EDTA) or lithium heparin (BD, Franklin Lakes, NJ) for whole blood and plasma preparation. Plasma was separated from cells within two hours of collection by centrifugation at 1400 × *g* (2500 rpm) at 4°C for 10 min. Plasma was frozen and stored at -80˚C until assayed. Biochemical and haematological analysis was performed on the whole blood or plasma by a commercial pathology company (Veterinary School Diagnostic Services (VSDS), UQ, Gatton – CC1-6; IDEXX Laboratories, Brisbane – CC7-12). Stored plasma was analysed by enzyme linked immunosorbent assay (ELISA) for cytokines (IL1β, IL6, IL10, TNFα, IFNγ), endocrine markers (T3, T4 and TSH), haptoglobin (stress indicator), adiponectin, leptin and prolactin. Other assays were performed to determine insulin and endotoxin (LPS) levels in the plasma.

In the heat load diet trials (CC9-12), samples for blood gases and pH measurements were also obtained at each bleed and analysed using a Siemens RAPIDPoint® 500 blood gas analyser. Blood gases (pH, pCO_2, pO_2), electrolytes (Na^+, K^+, Ca^{++}, Cl^-), metabolites (glucose, lactate) and co-oximetry (tHb, fO_2Hb, fCOHb, fMetHb, fHHb), along with HCO_3^-, Base Excess (BE), Anion Gap (AnGap), Haematocrit (Hct) and Osmolality (mOsm), in extracted whole blood were measured using the RAPIDPoint® 500 within 30 min of the blood being drawn from the animal.

Several statistical analyses were performed on the data from the climate chamber trials. In CC7-8, a mixed model ANOVA was used for statistical analysis for physiological parameters and rumen temperature where individual steers were used as experimental units and time as a repeated measurement. Individual 10 min T_{RUM} were converted to individual hourly means. The model included fixed effects such as treatment, day and time of day. For feed intake individual steers were used as experimental units. Mean daily feed intake for each steer were analysed using a GLM (Minitab 17, Minitab, Inc). The model included fixed effects such as treatment and day. Treatment differences are termed significant when p values ≤ 0.05.

In CC9-12, for rumen pH and temperature measurements, a repeated measures model with a first order auto-regressive error structure (SAS) was used. The model included pen, replication group, diet, day, hour and animal ID. For respiration rate a repeated measures model was used with the model including pen, replication group, diet, day, diet x day and Diet x hour of day. For dry matter intake a repeated measures model with a first order ante-dependence covariance structure was used. The model was selected based on the Akaike Information Criterion (AIC) and fitted using the SAS MIXED procedure. Denominator degrees of freedom for statistical tests were calculated using the Kenward-Roger method. For live weights and carcass measurements the GLM procedure (SAS) was used. The model included replication group and diet. For the plasma variables, mean and SEM were calculated for each diet for all days (days 2 – 20, and the three bleeds in feedlot pens (days 88, 95 and 103 DOF) and for each period PreHOT, HOT, Recovery and PENs and each diet. Two-way ANOVA was used to determine significant differences between diets.

At the end of the climate chamber trials (end of feedlot phase after climate controlled facility exit) all cattle were slaughtered at a commercial abattoir. The whole carcass was examined for gross normality and rumen boluses were recovered. In CC1-6, tissue samples were also obtained. The heart, liver, rumen, intestine, lung, kidney, pancreas, bladder, spleen, skeletal muscle and fat tissue were assessed for gross pathology. Samples were collected from each of these organs and fixed in 10% neutral buffered formalin for histopathology or snap frozen in liquid nitrogen for RNA analysis. Rumen fluid and urine samples were also collected.

In addition, all animals in the first group (CC1) had biopsy samples taken of liver, subcutaneous fat, longissimus dorsi muscle and semitendinosus muscle for RNA and histology analysis on exit from the climate controlled rooms.

3.2 Feedlot Trials

3.2.1 Gatton

All studies were conducted with the approval of The University of Queensland animal ethics committee (SAFS/210/13/MLA) in accordance with the guidelines described by the National Health and Medical Research Council (2013) in Australia.

As with the climate chamber experiments, steers were vaccinated on entry to the feedlot however hormonal growth promotants were not used in the feedlot trial studies. The cattle were weighed (non-fasted) on entry and at seven day intervals for the duration of the trials, including at each blood sampling. Similarly, rectal temperatures were also obtained at these times.

Steers were allocated to each pen based on initial liveweight, equalising total pen weight across the number of pens used within each trial (Gatton summers 1,2 and winter, eight pens, 10 head/pen; Gatton summer 3, eight pens, 12 head/pen). The pens had an area of 162 m^2 (27 m x 6 m) with a shade footprint of 1.2 m^2/animal at midday.

Rumen boluses (Smartstock, Pawnee, OK) were orally administered via a custom designed applicator to all animals in all trials. Rumen temperatures were transmitted and recorded at 20 min intervals. Rectal temperatures were obtained from all animals at seven day intervals.

Cattle in the feedlot commenced on a starter ration through to day 16, then transitioned to a finisher ration over 10 days for the remainder of the study. Refusals were removed and weighed daily with average consumption per pen and per animal calculated. For summer trials 1 and 2, and the winter trial, cattle were fed twice daily at approximately 0700 h and 1630 h daily. In Gatton summer 3 however, half of the cohort (four pens, 48 animals) were fed their entire daily ration at 0930 h while the other half received their ration at 1330 h. Feed composition is described in more detail in MS Report 3 -Section 3.4 p17 and MS Report 6 - Section 7.6.1.2 p57. Feed intake data was used to determine performance parameters ((dry matter intake (DMI); average daily gain (ADG); feed conversion rate (FCR)). Water intake was not monitored. Water temperature in all troughs was monitored at 10 min intervals using data loggers.

Onsite climatic data were collected at 10-30 min intervals using an automated weather station located beside the feedlot. Weather data collected included ambient temperature (TA; °C); relative humidity (RH; %); wind speed (WS; m/s) and direction; solar radiation (SR; W/m^2); black globe temperature (BGT; °C) and daily (0900 h) rainfall. From these data temperature humidity index (THI), heat load index (HLI) and accumulated heat load (AHLU86) for shaded Angus were calculated (Gaughan et al., 2008). Ambient temperature and relative humidity were also monitored under the shade structure in a pen in the middle of all feedlot pens.

The physiological status of the cattle was assessed using panting scores, pen location (shade, no shade, feed bunk, water trough), posture (standing, lying) and animal disposition (calm, relaxed, agitated). The number of animals ruminating at each observation time was also recorded. Data was collected at intervals between 0600 and 1800 h each day. Video cameras were set up in front of the pens to allow for 24 h assessment of the cattle. The cameras allowed all areas of the pens to be viewed.

Blood samples were obtained from all animals in all studies at 7 d intervals. Samples were collected from each steer by jugular venepuncture using 10 mL vacutainer tubes containing either K_3-ethylenediaminetetraacetic acid (K_3-EDTA) or lithium heparin (BD, Franklin Lakes, NJ) for whole blood and plasma preparation as previously described. Plasma was frozen and stored at -80˚C until assayed. Biochemical and haematological analysis was performed on the whole blood or plasma by a commercial pathology company. The Veterinary School Diagnostic Services, UQ, Gatton assayed Gatton winter trial and summers 1 and 2 samples; whereas IDEXX Laboratories, Brisbane dealt with the Gatton summer 3 samples. Plasma assays were conducted as described for the climate chamber trials.

In statistical analysis, the mean percentage of cattle, on a per group basis, under the shade or in the sun were evaluated. Mean panting score (MPS), pen surface temperature (PST), rumen temperature, HLI categories and AHLU categories and their interactions were used in the data analysis. Hourly (hour) bolus temperature data were also evaluated on a per group mean. For statistical analysis pens were used as experimental units, except for rumen temperature where individual animals were used as experimental units. A mixed model ANOVA (SAS Inst., Inc., Cary, NC) was used to analyse the long sequences of measurements for animal data, with time as a repeated measurement and individual animals as subjects. Statistical differences were analysed using a Tukey-Kramer test.

DMI, ADG and FCR were analysed using a repeated measures model (PROC MIXED, SAS Inst., Inc., Cary, NC). The model included the effects of treatment and week as fixed effects, with pen as a random effect. Least squares means were estimated for treatment and week effects. Differences between treatments were separated using PDIFF procedure of SAS. Pair-wise comparisons of means were carried out for dry matter intake, average daily gain and feed conversion rate. Treatment differences are termed significant when p values ≤ 0.05.

Carcass attributes were analysed using a mixed model (PROC MIXED, SAS Inst., Inc., Cary, NC). The model included the effect of treatment as a fixed effect, with pen and animals as a random effects. Least squares means were estimated for treatments and the different considered significant when $p \leq 0.05$. Some discrete measures (meat colour and butt shape) were analysed using a chi-square test of association.

At the end of the trials all cattle were slaughtered at a commercial abattoir. Rumen temperature boluses were recovered and the whole carcass was examined for gross normality and all major internal organs were assessed for gross pathology. Full MSA carcass assessment was conducted for all animals. In Gatton summer 1 and 2 and the Gatton winter trials, two animals from each pen were selected based on performance and physiological characteristics (gain and condition response) to have tissue samples collected as described for the climate chamber trials.

3.2.2 Nebraska

All studies were conducted with the approval of The University of Nebraksa animal ethics committee.

Similar to the feedlot trials conducted at Gatton, three trials were completed in Nebraska, US by the University of Nebraska, two during summer and one in late autumn. In the first summer and autumn trials, 80 steers were housed in eight feedlot pens. They were bled fortnightly over a 10 (summer 1, 6 bleed time points) or 14 week period (winter, eight bleed time points). In the second summer trial, 96 steers were housed in the feedlot and bled fortnightly over a 12 week period (seven bleeds).

Body temperature monitoring boluses were inserted into the rumen on the first day of the trial and were set to record at 10 min intervals throughout the duration of the study. Panting scores and respiration rates for the summer trials were taken every weekday between 2 and 3 pm. Panting scores were given for each pen and any animal with a score of three or more was recorded.

The feed ration mix for the first summer and the winter trial is described in MS Report 3 - Section 3.5 p58. The second summer Nebraska pen trial proceeded as a supplements trial testing the efficacy of two different classes of feed additives promoted as having rumen/intestinal protective effects. The complete compositions of these rations are given in MS Report 5 Section 7.2 p27.

In all three Nebraska trials, blood samples were collected by venepuncture into K_3-EDTA containing vacutainers and analysed for both haematological (14) and biochemical (24) parameters at commercial diagnostic laboratories in the USA. Harvested plasma was also sent to Brisbane in dry ice for further assay.

At slaughter, body tissue samples of the rumen, lung, heart, liver, kidney and small intestine were collected, fixed in 10% formalin and then set in wax blocks in preparation for shipment to Australia and histological examination.

Averages of variables of all animals in each trial were statistically compared using paired t-tests to determine significant differences between the trials.

4 Results

4.1 Climate chamber experiments

4.1.1 High heat load climate chamber study - CC7 and 8

4.1.1.1 Introduction

The purpose of these two chamber trials, CC7 and CC8, was to determine the appropriate conditions in chambers to induce high heat load in grain fed HGP implanted cattle, and to investigate the altered performance, physiology and metabolism during and after exposure to such conditions. The inflammatory status was assessed also. To maximise the number of animals under this regime, no pairfed thermoneutral controls were included. Moreover, the anticipated very low feed intake during HOT conditions that would have been imposed on a thermoneutral cohort would have provoked a stress response in its own right and led to animal management problems.

4.1.1.2 Climate regime

A schematic of the regime of the high heat load chamber trial conditions is presented in Fig. 5 below. Five days in thermoneutral conditions (PreHOT) were followed by an overnight transition to three very hot days, and then two steps down in temperature and THI over the subsequent four days. After these seven days of very hot to hot conditions (HOT), the animals recovered in thermoneutral conditions for five days (Recovery). They then exited the chamber and were housed externally in feedlot pens (PENs). The average weather conditions while in PENs is given in Fig. 5 also.

The climatic regime of the two trials varied slightly in that during the HOT period for CC7, the maximum temperature on days 6 and 7 was kept at 41°C, followed by a day at 38°C (day 8) before stepping down to ~35°C for two days and ~30°C for two days (see Fig. 6A below). The precise regime for the HOT period for CC8 consisted of a single day at 41°C, two days at ~38°C, two days between 34-35°C and two days at ~30°C. The rise in the overnight minimum temperatures over days 5 and 6 for CC8 was moderated also. As seen in Fig. 6B, there was little effect on maximum or minimum THI with these adjustments.

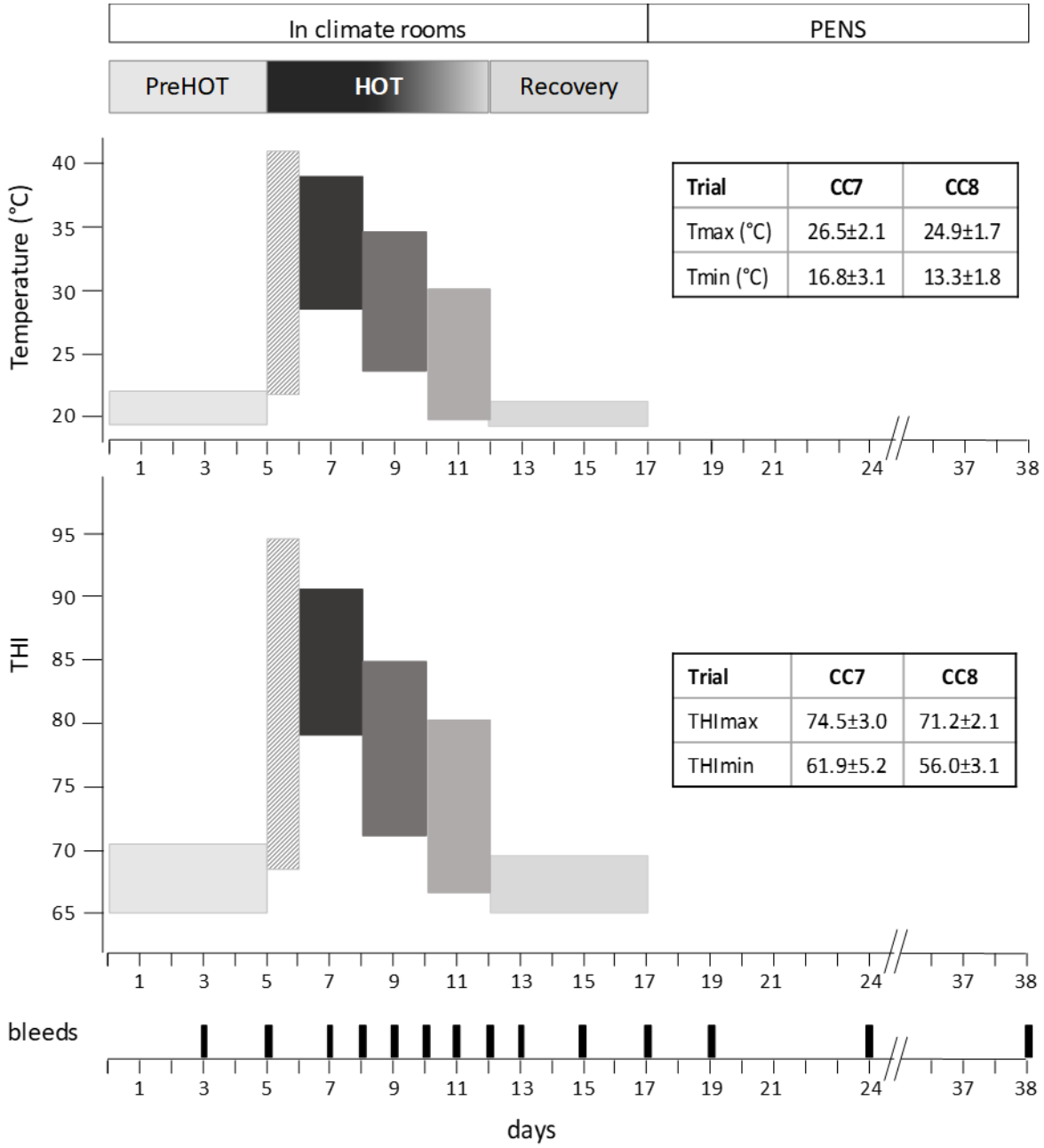

Fig 5. The overall climatic regime of the high heat load challenge imposed during the CC7 and CC8 trials. The range of the ambient temperature and Temperature-Humidity Index (THI), and duration of each period is depicted for the 38 days of the experiment. The PreHOT, HOT and Recovery periods were conducted in climate-controlled rooms. The PENs interval occurred in outdoor feedlot pens; the climatic conditions (mean ± SD) for each replicate is presented in the inserted tables. Blood sampling days are indicated also.

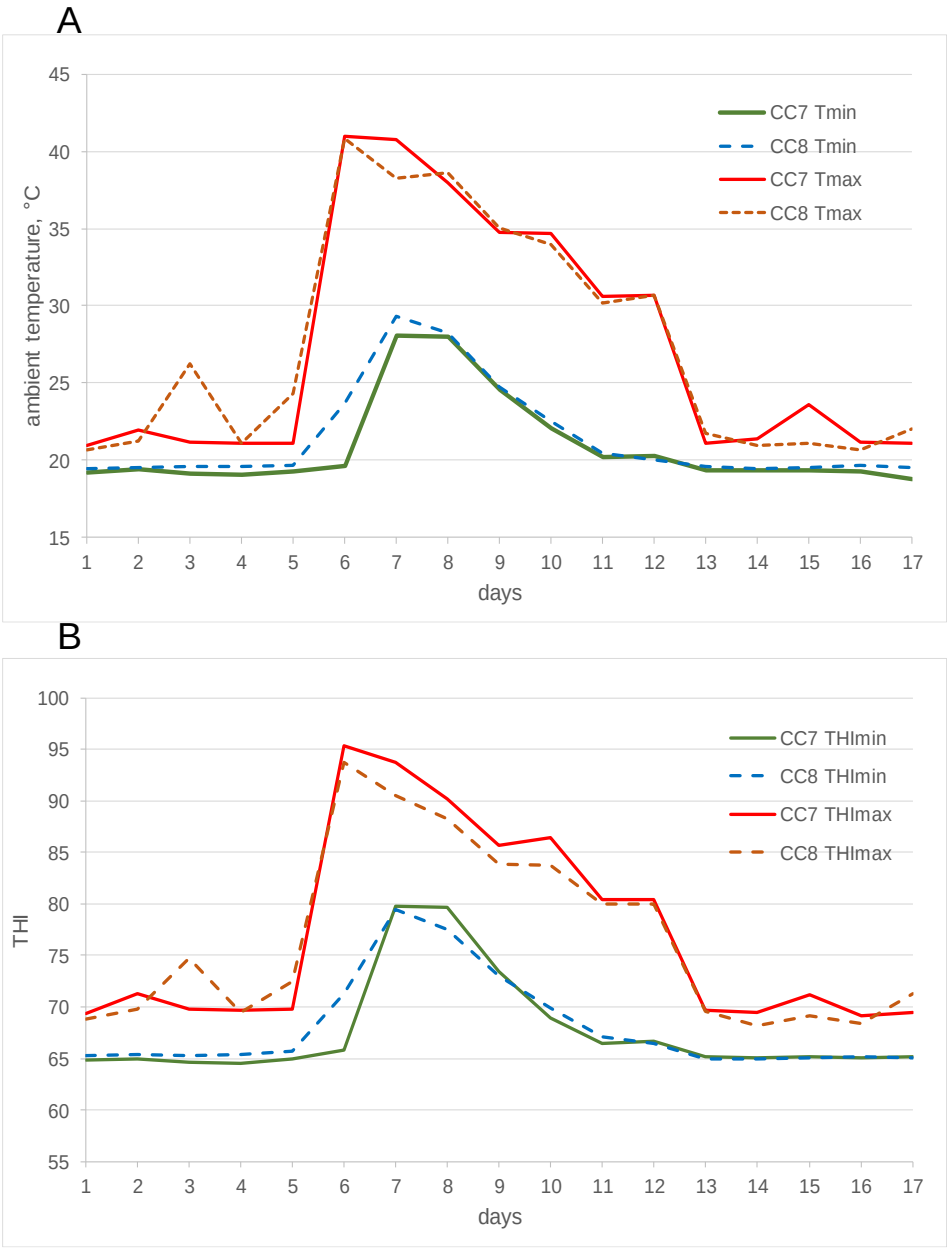

Fig. 6. The climatic regimes for CC7 and CC8 were slightly different during HOT (days 6-12). Panel A. Daily maximum and minimum temperatures for each trial during PreHOT (days 1-5), HOT, and Recovery (days 13-17). Panel B. Daily maximum and minimum THI for each trial.

4.1.1.3 Animal Performance

Of the 24 steers that entered the climate chambers, two head from each cohort of 12 steers did not proceed through the entire trial. The four steers were removed from the climate control chambers within the first two HOT days, due to severe heat load responses and not being able to cope with the climatic conditions. Two animals suffered a fatal response to conditions and two were removed when they were assessed as not coping with conditions.

4.1.1.4 Feed intake

When exposed to HOT, DMI decreased in CC7 (PreHOT, 10.1 ± 0.29 kg/head/day; HOT, 5.0 ± 0.24 kg/head/day; Recovery, 7.6 ± 0.14 kg/head/day) and CC8 (PreHOT, 10.7 ± 0.28 kg/head/day; HOT, 5.3 ± 0.27 kg/head/day; Recovery, 8.1 ± 0.14 kg/head/day) (Fig. 7). There were no differences in DMI when comparing the two trials. As HOT conditions commenced, the DMI of both treatment groups decreased and reached a minimum on day 7 (CC7) and day 8 (CC8). The feed intake began to steadily increase from day 9 but had not returned to PreHOT levels by day 17. On the last 5 days in PENs, only the CC7 animals regained a mean DMI close to that of the PreHOT days (Fig. 7).

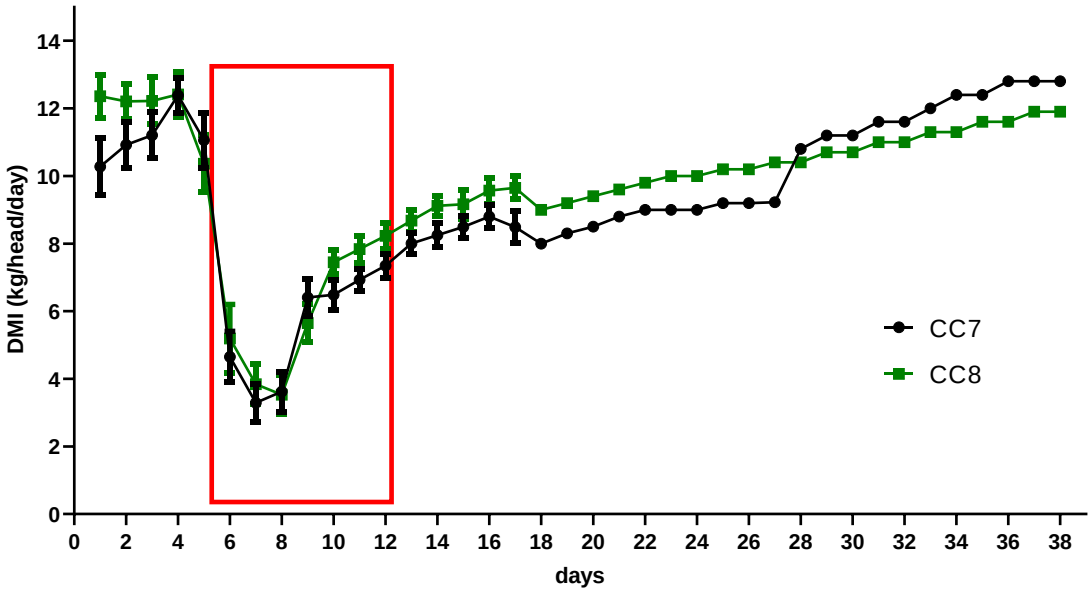

Fig. 7. Mean (± SEM) feed intake of the CC7 and 8 trials when housed in climate control chambers. Days 1-5 are PreHOT, days 6-12 are HOT (indicated by the red box), days 13-17 are Recovery, days 18-38 are in PENS.

When the DMI values for both trials are combined, the PreHOT mean DMI was 10.4 kg/head/day. This fell by approximately 50% to 5.1 kg/head/day during HOT and rose in Recovery and PENs to 7.8 and 8.4 kg/head/day respectively (Fig. 8).

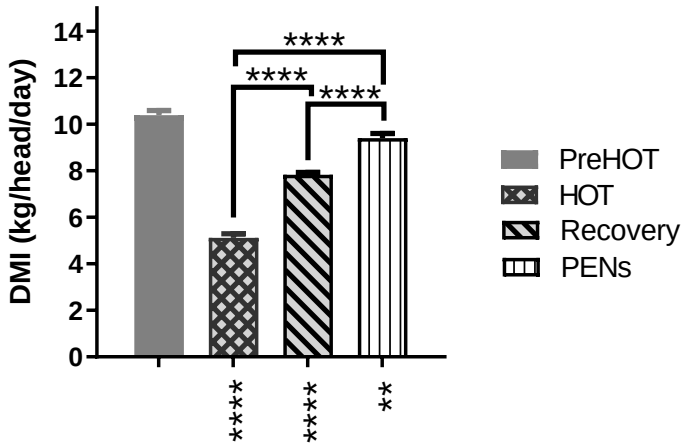

Fig. 8. Mean DMI (± SEM) of the CC7 and 8 cohorts during PreHOT and in response to thermal challenge (HOT), and during Recovery and PENs. The asterisks under the x-axis indicate statistically significant difference with PreHOT. **, p <0.01; ****, p <0.0001.

4.1.1.5 Live weights

Due to a malfunction of the scales in the race adjoining the climate chambers, live weight was not recorded during the days in the climate chambers (days 1-17). An indication of impact of the thermal challenge on live weight gain can be obtained from the weight data collected when the animals were in feedlot pens. Fig. 9 shows the mean live weights from CC7 and 8 obtained five days prior to entry to the chambers (day -5), and 1, 2 and 3 weeks after exit (days 24, 31 and 38). At day -5, the mean live weight was 603.4 kg/head, and not different from the mean live weights for days 24 and 31 (602.2 and 609.6 kg/head respectively). Only on day 38 was a significant weight gain evident (p <0.05) with a mean of 641.1 kg/head.

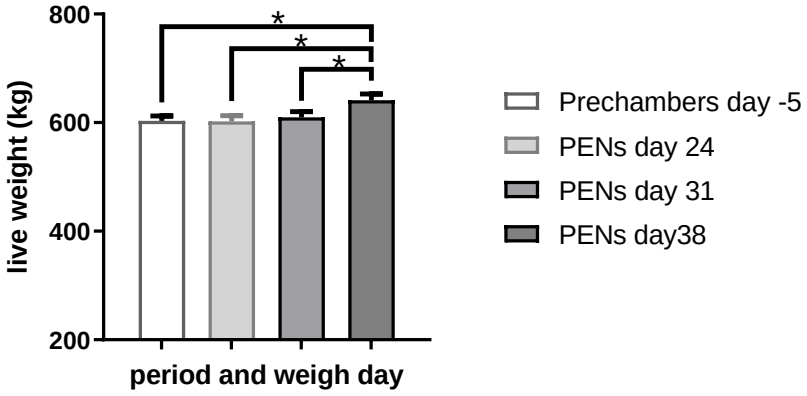

Fig. 9. Mean live weights (± SEM) of all animals of the CC7 and 8 cohorts on day -5 (prior to entry to the climate chambers), and days 24, 31 and 38 of the trial. The animals exited the chambers on day 17 and were returned to feedlot pens. *, p <0.05.

4.1.1.6 Physiological responses

Respiration Rate

Mean respiration rate (RR) for both trials increased during HOT when compared with PreHOT ($p < 0.001$) and Recovery ($p < 0.001$) (Fig. 10). When exposed to HOT, the average RR increased for CC7 (PreHOT, 57.4 ± 1.4 bpm; HOT, 111.5 ± 1.7 bpm; Recovery, 48.0 ± 1.7 bpm). Mean RR for CC8 also increased when exposed to HOT (PreHOT, 78.4 ± 2.4 bpm; HOT, 114.8 ± 1.4 bpm; Recovery, 55.3 ± 1.9 bpm). Average RR was greatest during the first HOT day ($p < 0.001$) (day 6) when THI was at its maximum over the 17 day period. There were no differences in mean RR between CC7 and CC8 at any of the observational times between CC7 and CC8 ($p > 0.05$).

Both trials followed a similar pattern throughout the experimental period with significant increased RR during HOT. With abatement of HOT conditions and the return to thermoneutral conditions in Recovery, the RR of all cattle returning to levels similar to PreHOT.

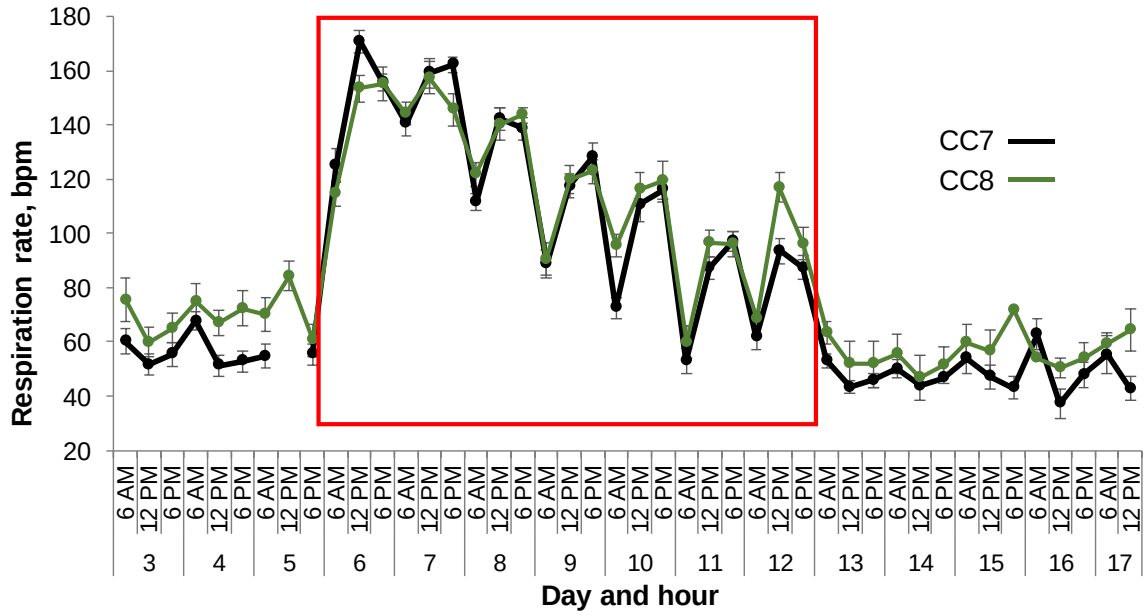

Fig. 10. Mean (± SEM) respiration rate (breaths per minute, bpm) of CC7 and CC8 trials at 6 am, 12 noon and 6 pm on days 3-17 in the climate control chambers. Days 1- 5 are PreHOT, days 6-12 are HOT (indicated by the red box), and days 13-17 are Recovery.

During HOT, the mean midday respiration rate for all animals was 127.5 bpm, and clearly significantly different to the midday means of the PreHOT and Recovery periods (Fig. 11). While the mean of the Recovery period was lower than the PreHOT mean, it was not significantly different.

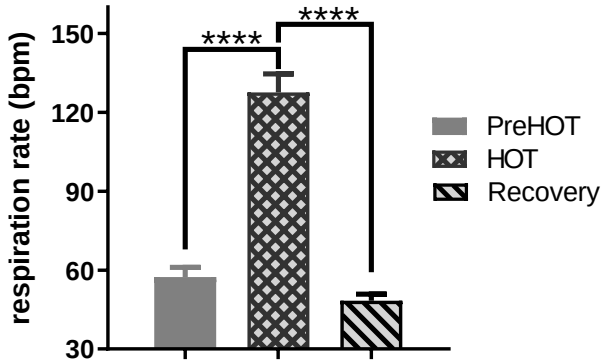

Fig. 11. The mean respiration rate (± SEM) at midday (12 noon) of all animals of the CC7 and 8 cohorts during days 3 and 4 of PreHOT, during HOT and Recovery. ****, p <0.0001.

Panting score

The mean panting score of the cattle followed a similar trend to that of RR with the mean maximum panting score of 2.85 for both trials obtained at 1500 h on day 6. The mean panting score decreased over time and returned to levels lower in Recovery compared to PreHOT (Fig. 12). The cattle in CC8 presented a slightly lower panting score across days 6, 7 and 8, however this difference is not significant (p >0.05).

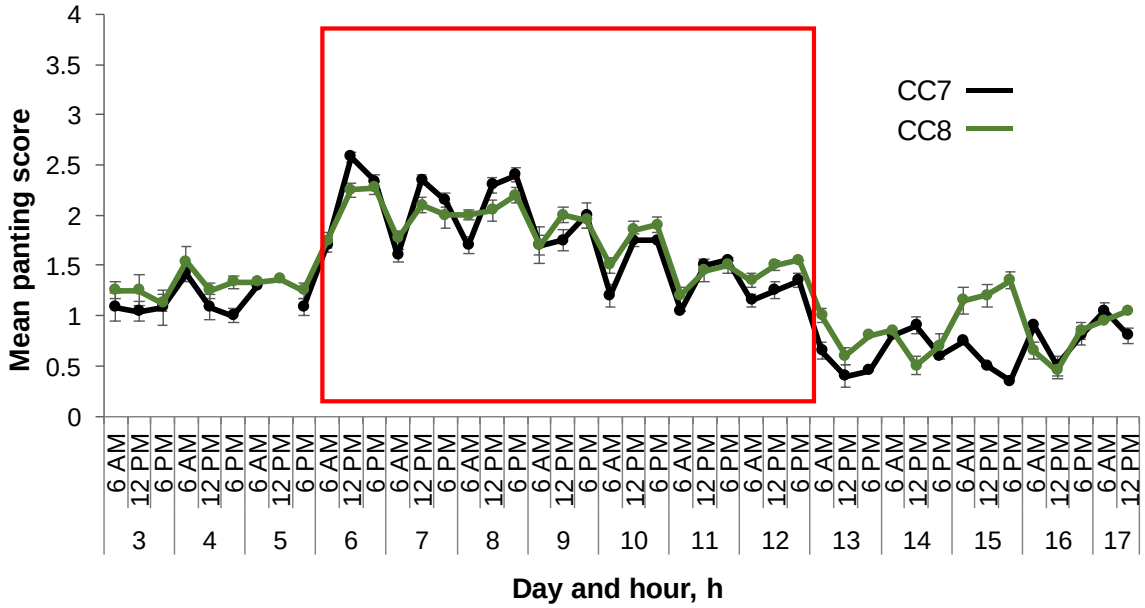

Fig. 12. Mean (± SEM) panting score of CC7 and CC8 at 6 am, 12 noon and 6 pm on days 3-17 in the climate control chambers. Days 1-5 are PreHOT, days 6-12 are HOT (indicated by the red box), days 13-17 are Recovery.

Rectal Temperature

As with the respiration rates, the two trials produced a very similar rectal temperature response and were not significantly different from each other on any day and overall (Fig. 13). The maximum rectal temperature was achieved on day 7; with means of 40.15°C and 40.26°C for CC7 and CC8 respectively. Mean rectal temperatures fell quickly over the next four days. On days 11 and 12, and during Recovery (days 13-17), the mean rectal temperatures in both cohorts were below the PreHOT means (Fig. 13). Only in PENs did the rectal temperatures return to PreHOT readings. The small rise in mean rectal temperature experienced by CC8 on day 23 (21st April 2017) was not due to a late season heatwave.

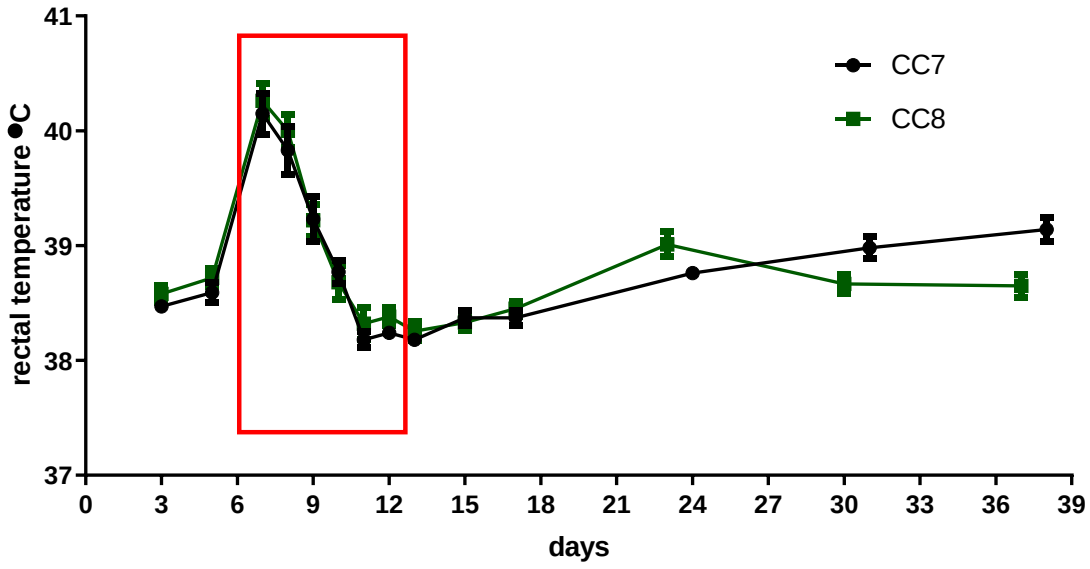

Fig. 13. Mean rectal temperatures (± SEM) of CC7 and CC8 trials when housed in climate control chambers and later in feedlot pens. Days 1- 5 are PreHOT, days 6-12 are HOT (indicated by the red box), days 13-17 are Recovery, and days 18-38 are in PENs.

Overall, the mean rectal temperature on days 3 and 5 (PreHOT) was 38.59°C (Fig. 14). During the HOT period it rose to a mean of 39.11°C. The mean rectal temperature in HOT was significantly higher than all other periods. In Recovery, the mean rectal temperature at 38.33°C was significantly lower than the mean of all the other periods (Fig. 14). The mean rectal temperature in PENs was 0.5°C higher than the PreHOT mean.

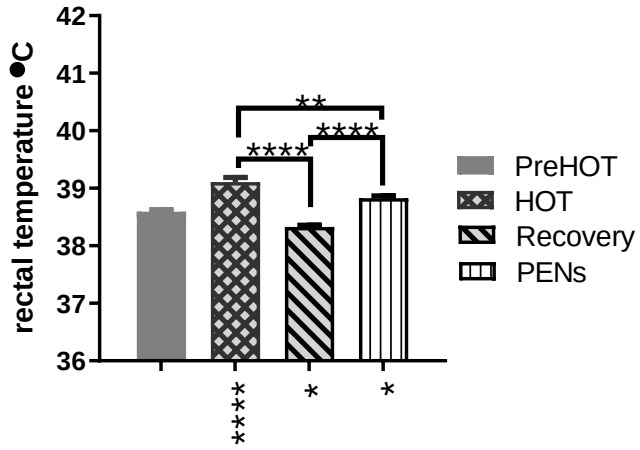

Fig. 14. The mean rectal temperatures (± SEM) of all animals of the CC7 and 8 cohorts during days 3 and 4 of PreHOT, HOT, Recovery and in PENS. The asterisks under the x-axis indicate statistically significant difference with PreHOT. *, p <0.05; **, p <0.01; ****, p <0.0001.

Rumen Temperature

When exposed to HOT, rumen temperature increased in CC7 (PreHOT, 38.5 ± 0.02ºC; HOT, 39.4 ± 0.03ºC; Recovery, 38.4 ± 0.02ºC) and CC8 (PreHOT, 39.0 ± 0.01ºC; HOT, 39.7 ± 0.02ºC; Recovery, 38.5 ± 0.01ºC) (Fig. 15). There was a slight difference between the rumen temperature of the trials, with CC8 on average presenting with a 0.33ºC higher temperature than CC7 over the 17 days in chambers. However, there were no significant differences in rumen temperature for hour and day for both trials ($p < 0.0001$). The animals in both trial groups followed a similar daily diurnal trend with mean rumen temperature reaching a maximum at 1500 h and a minimum at 0700 h during HOT (Fig. 15). Both trials returned to a mean rumen temperature in Recovery slightly below levels of PreHOT.

When looking at the mean maximum and minimum rumen temperatures by period, the mean maximum rumen temperature during HOT was 1.0°C higher than the PreHOT mean, and 1.3°C degree higher than the Recovery mean (Fig. 16). The changes in the mean minimum temperatures were not so extreme; the HOT mean minimum rumen temperature was 0.6°C higher than the PreHOT mean, and 0.88°C higher than the Recovery mean. While the Recovery mean maximum and minimum rumen temperatures were consistently lower than those of the PreHOT period, there was no significant difference between the two periods at this level.

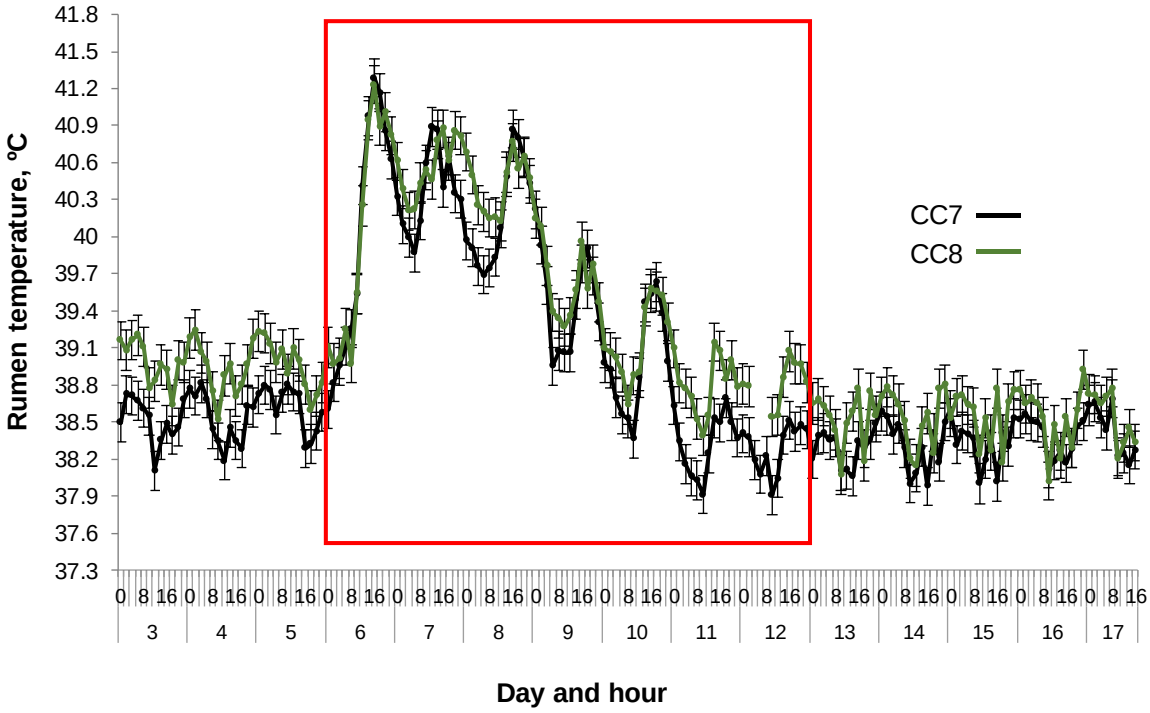

Fig. 15. Hourly mean rumen temperature (± SEM, ºC) of CC7 and CC8 when housed in climate control chambers. Days 1- 5 are PreHOT, days 6-12 are HOT (indicated by the red box), and days 13-17 are Recovery.

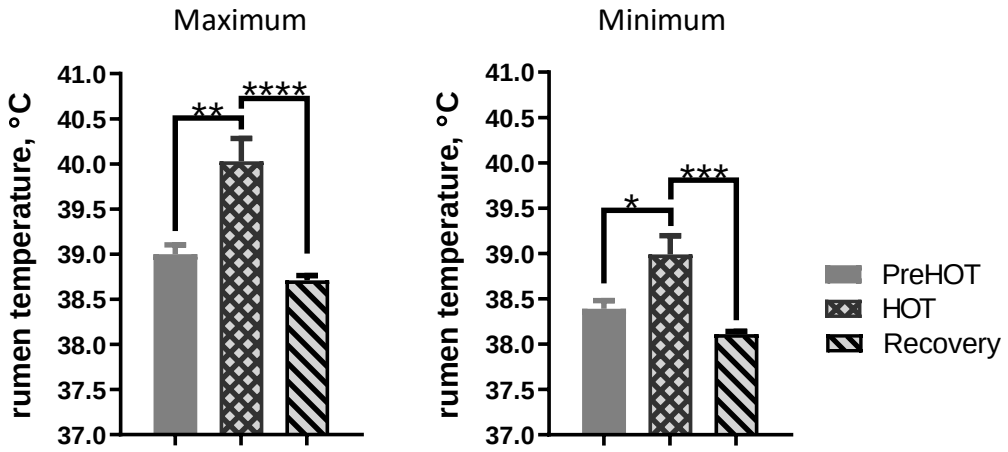

Fig. 16. Mean maximum and minimum rumen temperatures (± SEM) of all animals of the CC7 and 8 cohorts during PreHOT, HOT and Recovery. *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

4.1.1.7 Metabolic Responses

Where the data from CC7 and 8 bleeds were highly correlated and not significantly different between the trials (at the level of paired and unpaired t-tests), the data has been combined for the analyses below.

Bicarbonate blood buffering

The mean plasma bicarbonate concentration experienced a rapid decrease with the onset of HOT (Fig. 17A). Day 9 recorded the lowest concentration at ~23% lower than the PreHOT mean (days 3 and 5). Recovery seemed to occur in two stages, the first phase on days 11 and 12, followed by a climb to the PreHOT mean over days 12-14, whereupon it stabilised (Fig. 17A). Fig. 17B compares the means from each period. The mean plasma bicarbonate concentration during HOT was 15% lower than the PreHOT mean, and the mean in Recovery was 3.2% higher, although not significantly different. The mean plasma bicarbonate concentration in PENs was not different to the PreHOT mean.

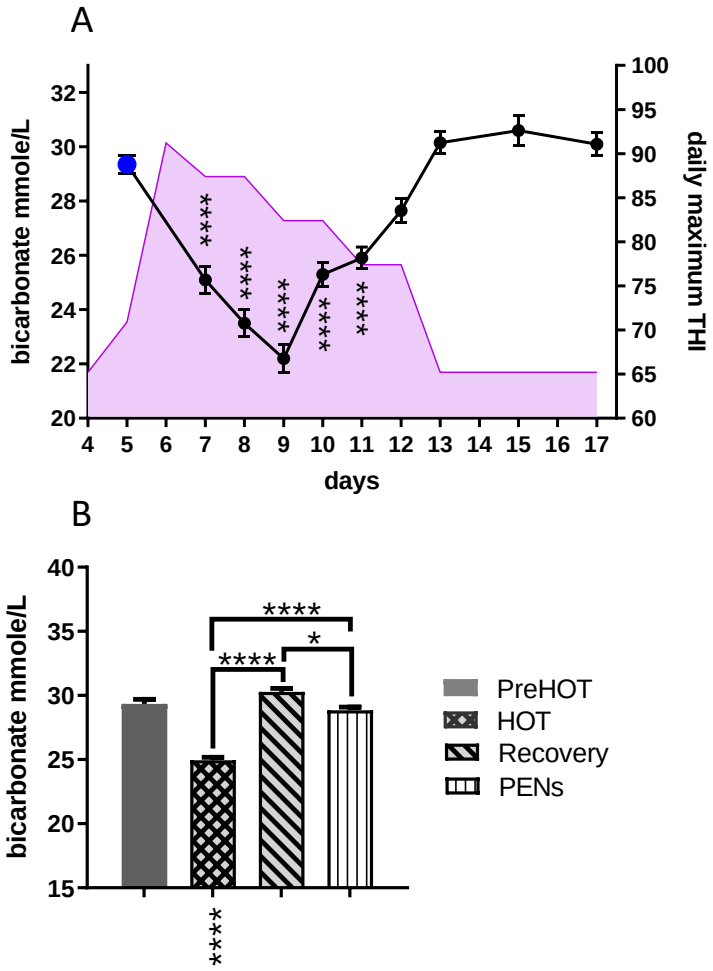

Fig. 17. Changes in plasma bicarbonate concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma bicarbonate concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks.

The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma bicarbonate concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; ****, p <0.0001.

Energy metabolism

The mean plasma glucose concentrations showed a two staged response also (Fig. 18A). There was an initial fall of approximately 14% to day 8, whereupon it stabilised until a second 8% fall on day 11 despite the reduction in thermal load, and resumption of feed intake in Recovery (Fig. 18A). However, plasma glucose concentration had recovered at day 13. Comparison of the means for each period in Fig. 18B, shows that the HOT mean was significantly lower than the means for the other periods, and 16.3% lower than the PreHOT mean.

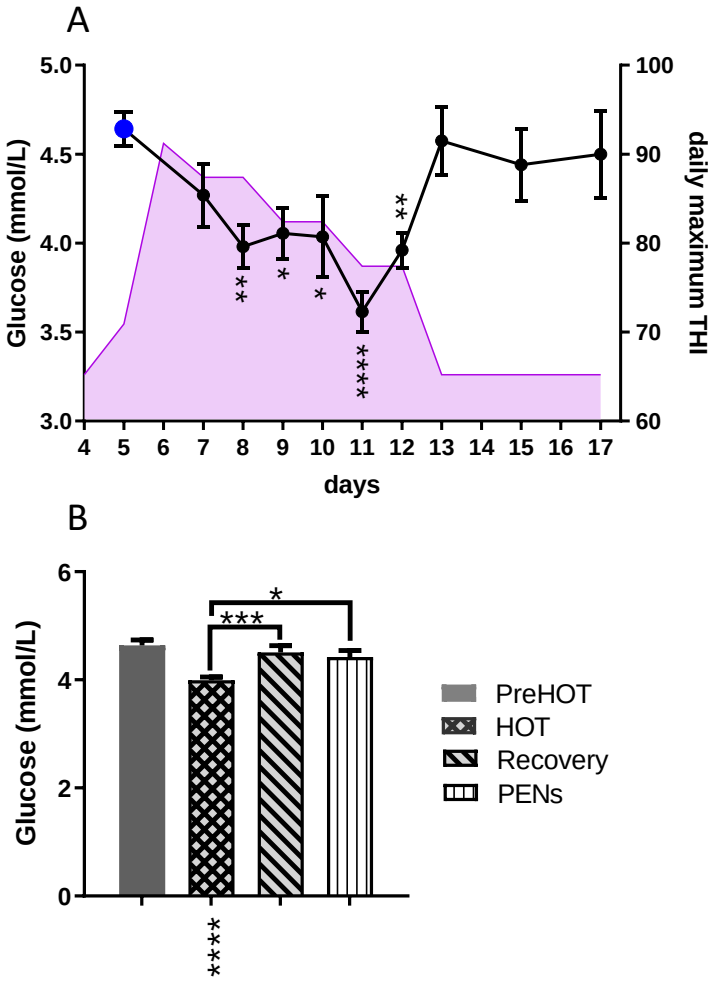

Fig. 18. Changes in plasma glucose concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma glucose concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The

daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma glucose concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; ***, p < 0.001; ****, p <0.0001.

β-hydroxybutyrate is a product of fatty acid oxidation and thus an indicator of the use of fat for energy. The mean plasma β-hydroxybutyrate concentration rose steadily during HOT until day 9 and remained at this plateau until day 12, whereupon it steadily declined (Fig. 19A). The mean plasma β-hydroxybutyrate concentration during HOT was 35% higher than the PreHOT mean, and the mean during Recovery was 17% higher than the PreHOT mean (p = 0.0712, Fig. 19B).

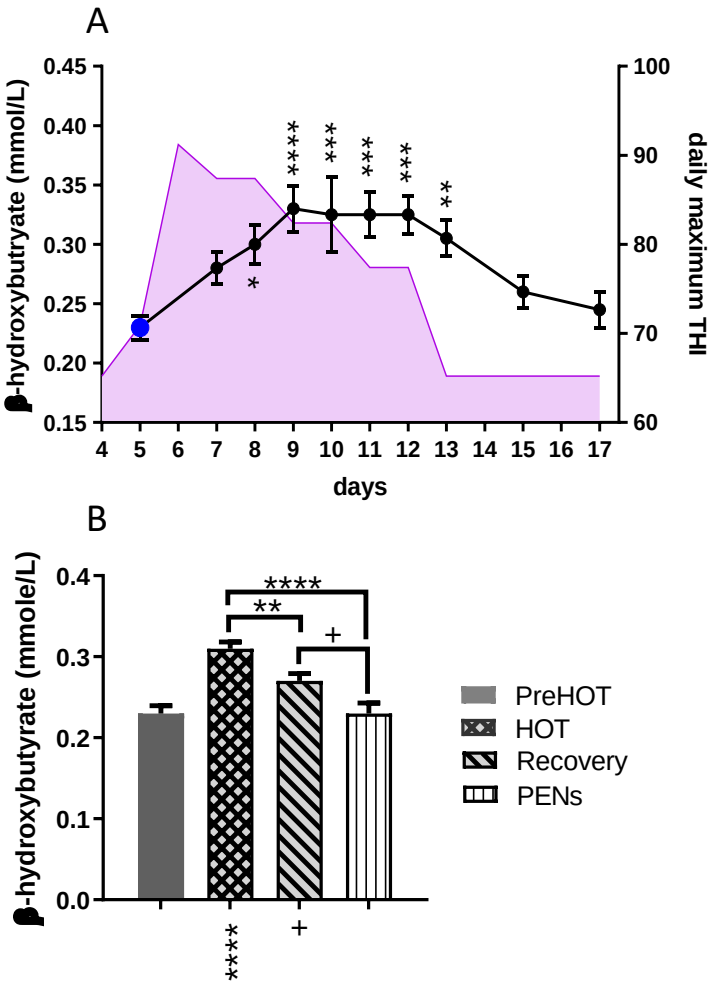

Fig. 19. Changes in plasma β-hydroxybutyrate concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma β-hydroxybutyrate concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are

indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma β-hydroxybutyrate concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p < 0.01; ***, p < 0.001; ****, p <0.0001.

Plasma insulin is the main regulator of both glucose uptake and oxidation of fats by tissues. The plasma insulin concentrations showed much animal variability (Fig. 20A). There was no detectable insulin response to high heat load; only the day 15 mean plasma insulin concentration was significantly lower (~28%) than the PreHOT mean (days 3 and 5). The mean plasma insulin concentration during HOT was 10% lower than that of the PreHOT period although not significantly so. The mean in Recovery tended toward a 20% reduction relative to the PreHOT mean (p = 0.0556, Fig. 20B). The means during HOT and Recovery were significantly lower than the PENs mean.

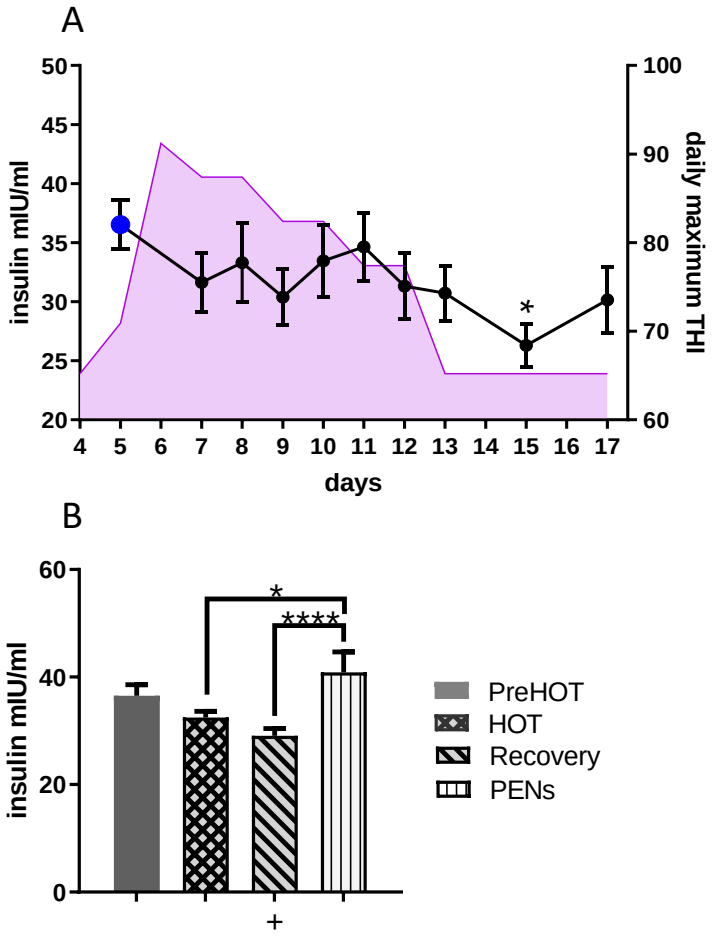

Fig. 20. Changes in plasma insulin concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma insulin concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma insulin concentration (± SEM). The asterisks under the x-axis indicate

statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; ****, p <0.0001.

Use of glutamine as an energy metabolite (glutaminolysis) occurs in mitochondria via partial bypass of the TCA cycle. When glucose is limiting, many cells switch to glutamine for energy. Glutamine has many other roles and functions including contributions to production of urea in the liver and ammonia in the kidneys. The mean plasma glutamine concentration fell rapidly with onset of HOT. During day 9-11 of HOT, the concentration stabilised at ~32% below that of the PreHOT mean despite the falling daily maximum THI (Fig. 21A). The mean glutamine concentration ascended over the following seven days to have fully recovered by day 17. Fig. 21B shows that the mean plasma glutamine concentration for the HOT period was much less than that of all other means, but the means in Recovery and PENs were not different to the PreHOT mean.

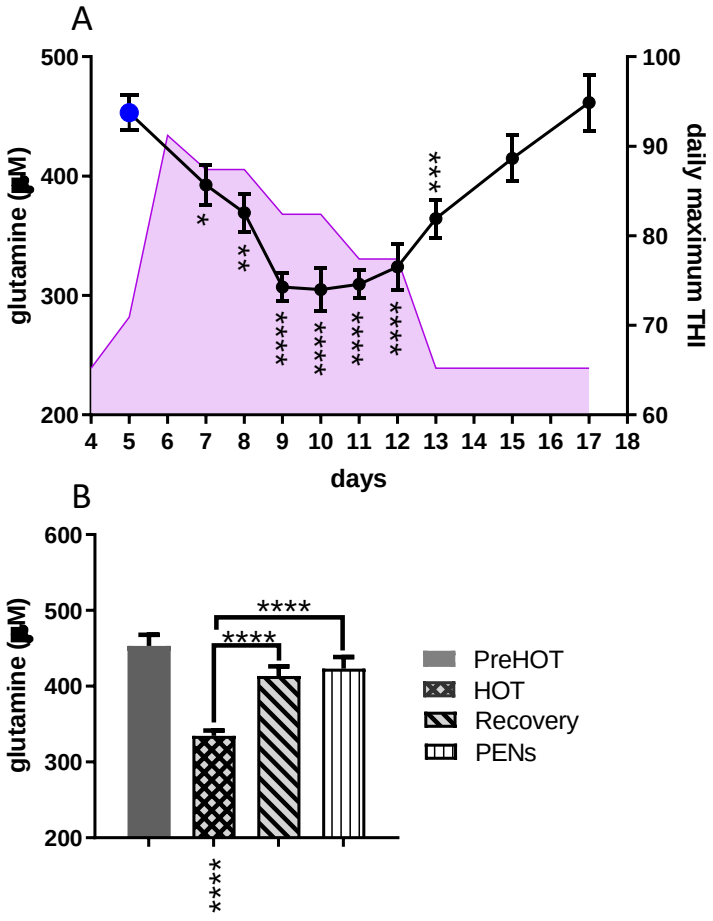

Fig. 21. Changes in plasma glutamine concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma glutamine concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of

the mean plasma glutamine concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Renal Response – Creatinine and Urea

The mean plasma creatinine concentrations rose rapidly in response to HOT (Fig. 22A). The high mean plasma creatinine concentration was sustained for three days at 36-46% over the PreHOT mean. The mean plasma creatinine levels returned to normal levels by day 13. When comparing the means of the four periods, the HOT, Recovery and PENs mean plasma creatinine concentrations were all higher than that of PreHOT (Fig. 22B). The slight increase in mean plasma creatinine concentration in PENs probably reflected increased muscle mass as growth resumed.

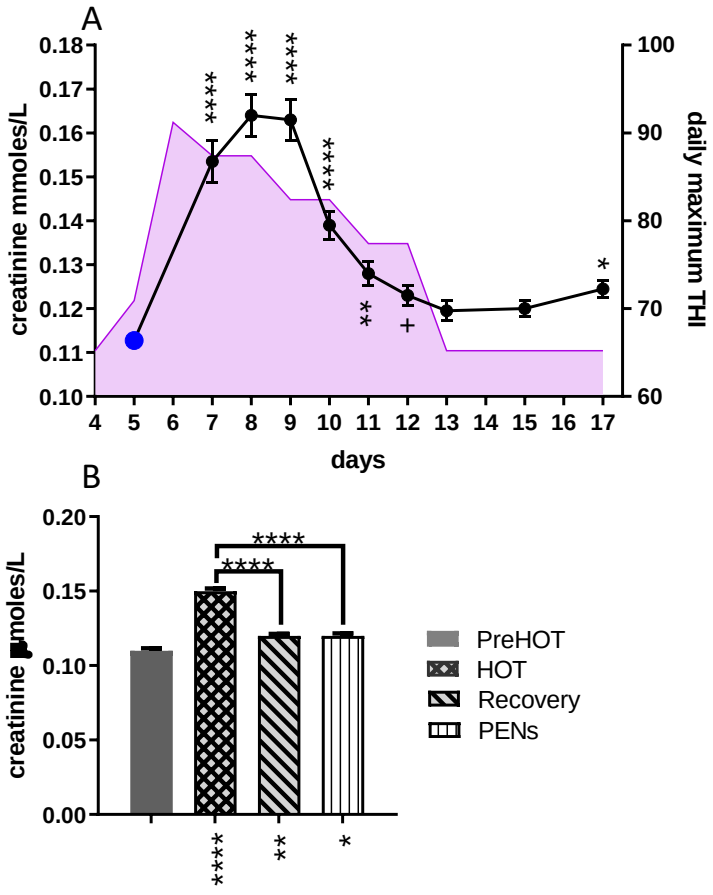

Fig. 22. Changes in plasma creatinine concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma creatinine concentration (± SEM). The statistical significance of the mean at each time relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma creatinine concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with PreHOT. +, p <0.1; *, p <0.05; **, p < 0.01; ****, p <0.0001.

The mean plasma urea concentrations followed a very similar pattern to the creatinine concentrations. There was a significant rise in HOT which decreased as conditions cooled (Fig. 23A). The one major difference in the urea response compared to that of creatinine, is the persistent reduced concentration in Recovery relative to the PreHOT mean (Fig. 23A and B). The HOT mean was 23% higher than the PreHOT mean, while the Recovery mean was 8.5% lower.

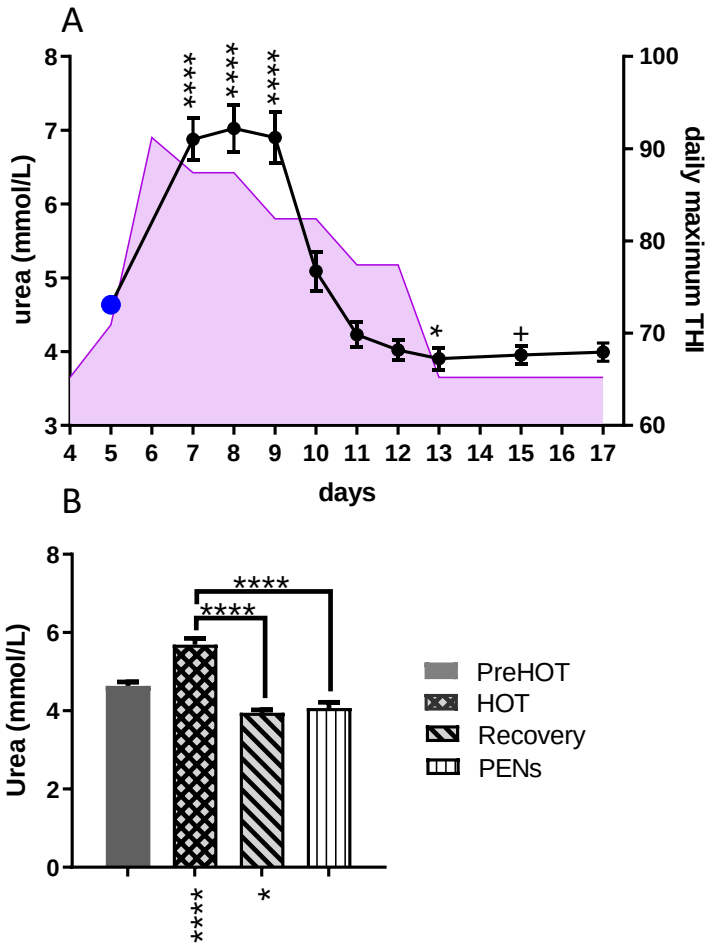

Fig. 23. Changes in plasma urea concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma urea concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma urea concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; ****, p <0.0001.

Liver Function - Liver enzymes

There was a marked reduction in mean plasma ALP activity after 2 days of HOT (Fig. 24A). On day 11, the mean plasma ALP activity was at its lowest, reduced 44% compared to the mean of the PreHOT days. Mean plasma ALP activity only approached normal levels at day 17, well into Recovery. The overall mean ALP activity for the HOT and Recovery periods were 39 and 33% lower than the PREHOT mean respectively (Fig. 24B). The HOT and Recovery mean were not different to each other. Once in PENs, the mean ALP activity fully recovered.

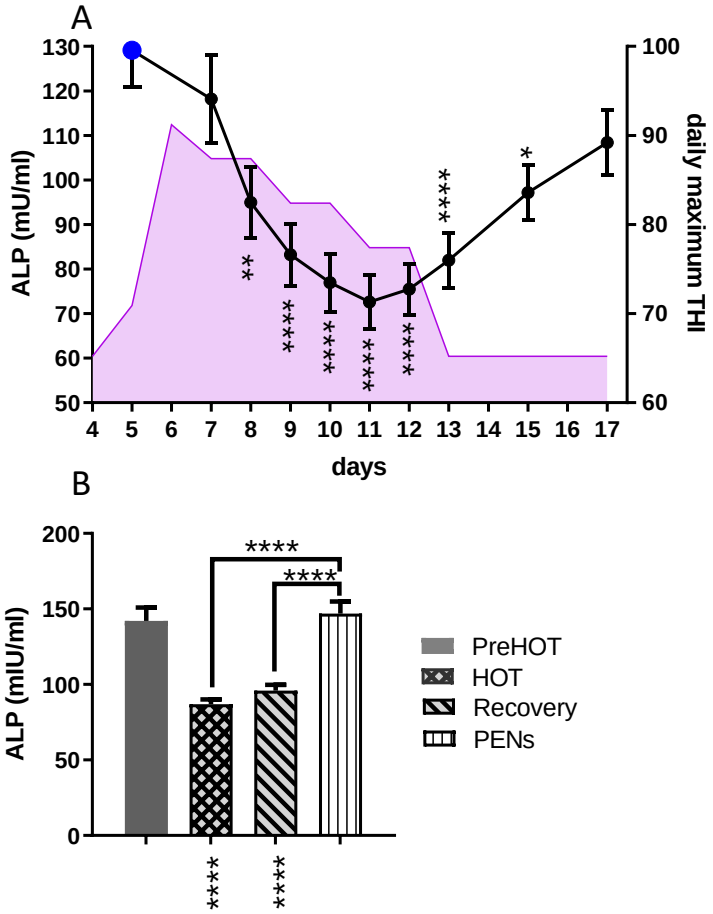

Fig. 24. Changes in plasma ALP activity associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma ALP activity concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma ALP activity (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; ****, p <0.0001.

Plasma AST, GGT and GLDH activities behaved similarly to each other and very differently to ALP activity. There were no overt changes in circulating activity of AST, GGT and GLDH

until the Recovery period (Fig. 25-27). All enzymes displayed high inter-animal variability, however, the elevation in Recovery was clearly detected. Mean plasma AST activity did not alter during day 7-10 of the HOT period; in fact, variability tended to decrease (Fig. 25A). After day 10, as the hot conditions abated, the mean plasma AST activity rose, reaching a plateau on days 13-15, when it was 42% higher than the PreHOT days. The mean for the Recovery period was significantly elevated relative to the means for the three other periods. Once in PENs, mean plasma AST activity returned to normal levels (Fig. 25B).

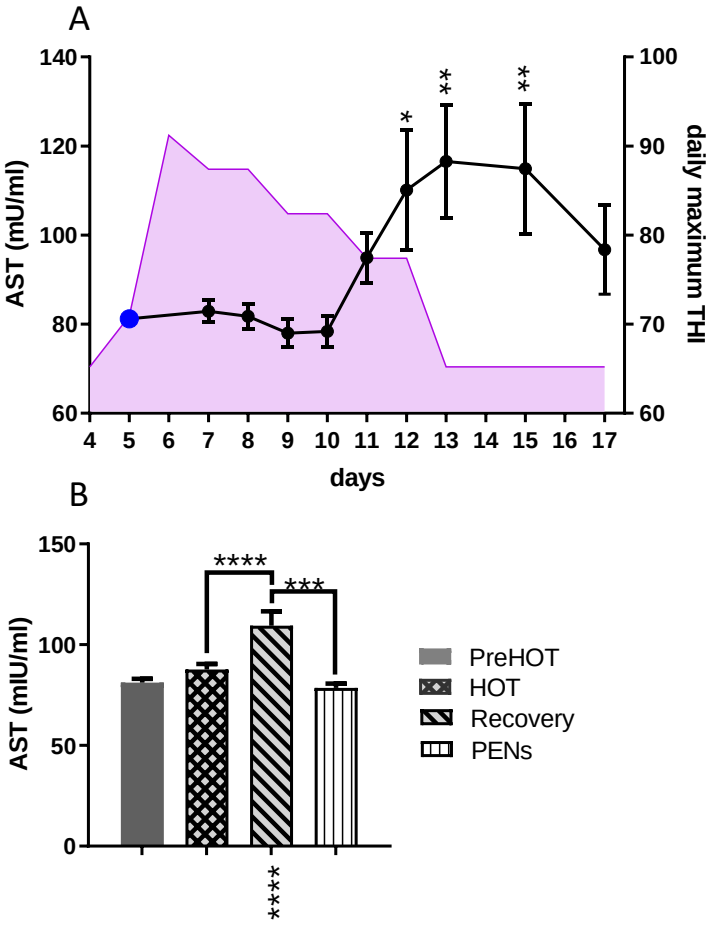

Fig. 25. Changes in plasma AST activity associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma AST activity (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma AST activity (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Due to the high variability in plasma GGT activities, the rise in mean activity in Recovery is not as obvious (Fig. 26A). Only day 17 showed as being significantly elevated relative to the

other daily mean activities. When assessing the overall means for each period, the Recovery mean is significantly higher (~14-15%) than the PreHOT and PENs means (Fig. 26B).

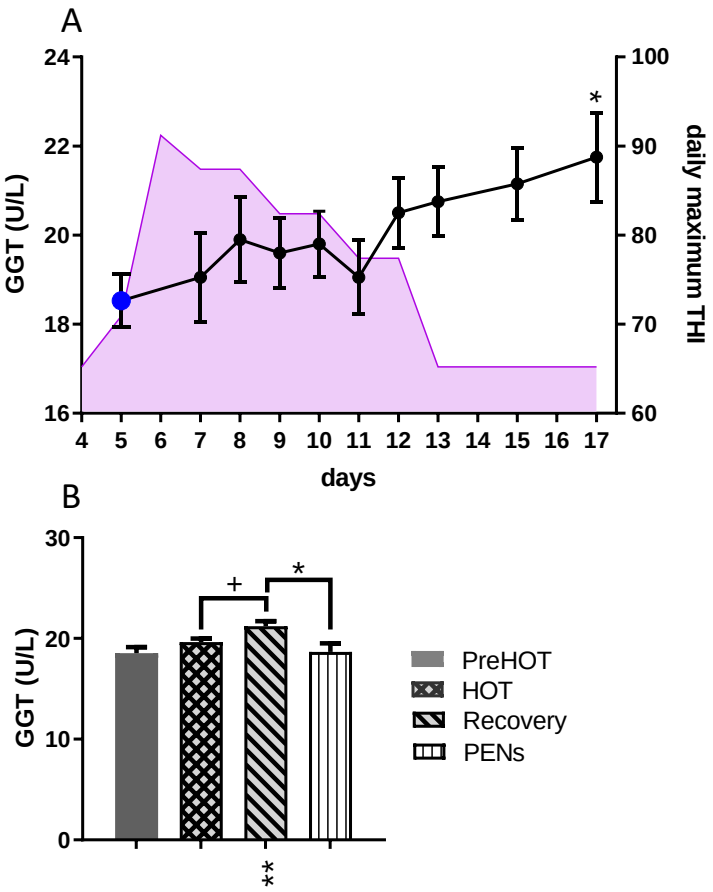

Fig. 26. Changes in plasma GGT activity associated with high heat load and recovery inCC7 and 8. Panel A. The daily mean plasma GGT activity (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma GGT activity (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01.

The elevated plasma GLDH activities during Recovery were very apparent (Fig. 27A and B). As with the daily mean AST activities, the mean GLDH activities rose to a plateau on days 13-15; approximately 85% higher than the mean of the PreHOT days (Fig. 27A). Fig. 27B shows that the Recovery mean GLDH activity was significantly higher than the HOT and PENs means also.

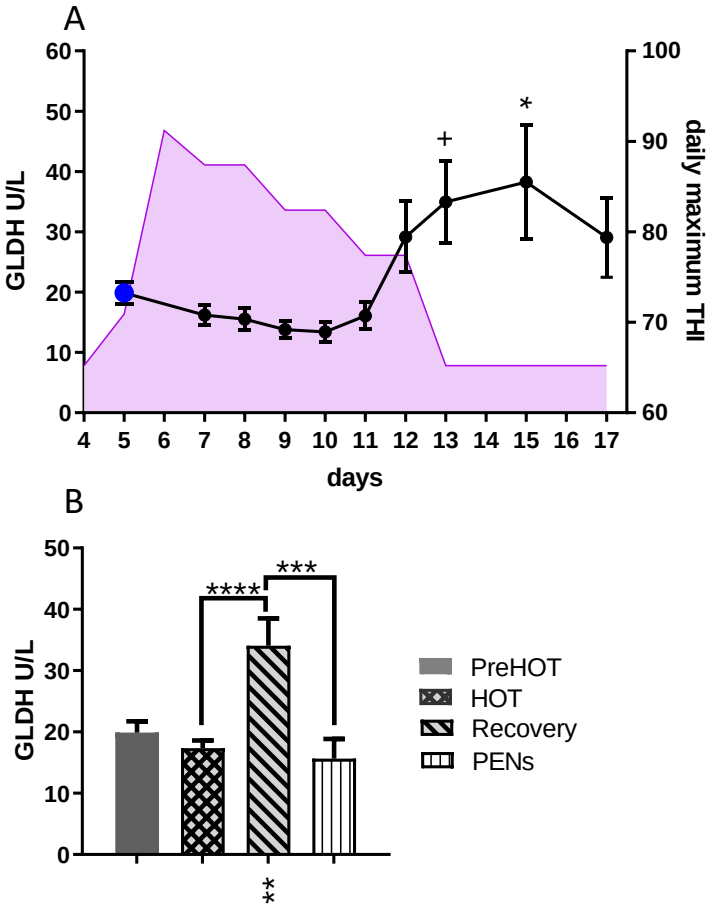

Fig. 27. Changes in plasma GLDH activity associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma GLDH activity (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma GLDH activity (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Liver Function – bilirubin and cholesterol

A rise in plasma bilirubin concentration can be an indicator of red blood cell (RBC) damage and/or cholestasis in the liver when bilirubin and bile acids are ‘backed up” and unable to be released to the gall bladder and intestine. The onset of HOT saw an immediate elevation of mean plasma bilirubin levels which persisted well into Recovery (Fig. 28A). The overall means revealed that the HOT, Recovery and PENs mean bilirubin concentrations were all higher than the PreHOT mean (Fig. 28B). The HOT and Recovery means were 50% higher than the PreHOT mean; and the PENs mean was 30% higher.

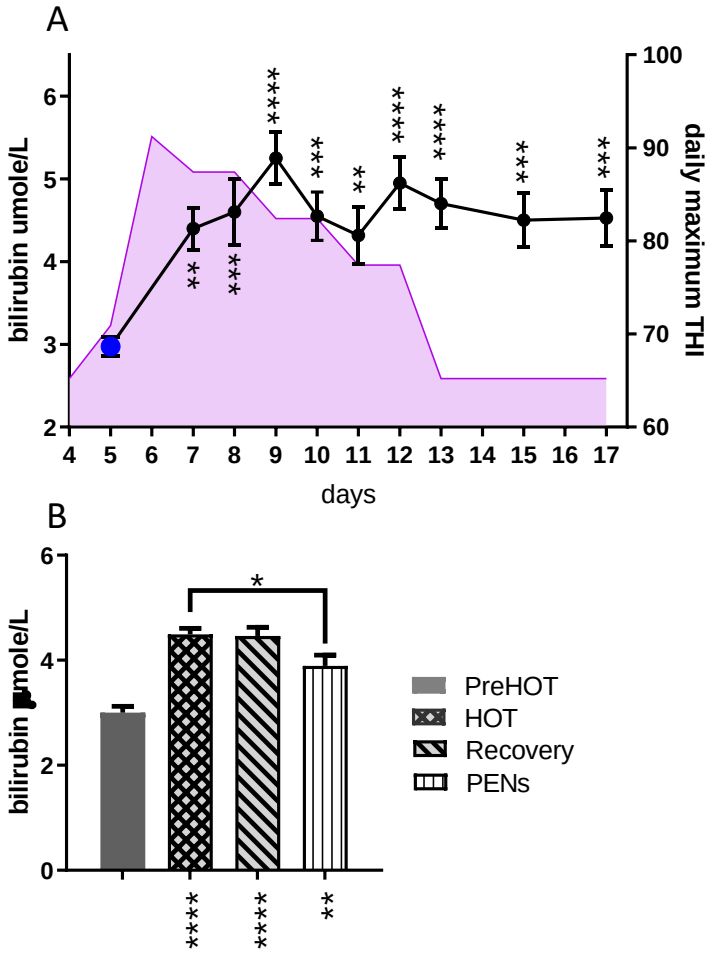

Fig. 28. Changes in plasma bilirubin concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma bilirubin concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma bilirubin concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Cholesterol is synthesised in the liver from acetate. Mean plasma cholesterol concentrations steadily fell with the onset of HOT, reaching its lowest concentration on days 10 and 11 when it was 22% below the PreHOT mean concentration (Fig. 29A). The lower concentrations persisted into Recovery. The overall mean shown in Fig. 29B revealed that both the HOT and Recovery means were 18% lower than the PreHOT mean and the PENs mean was 8% lower than the PreHOT mean.

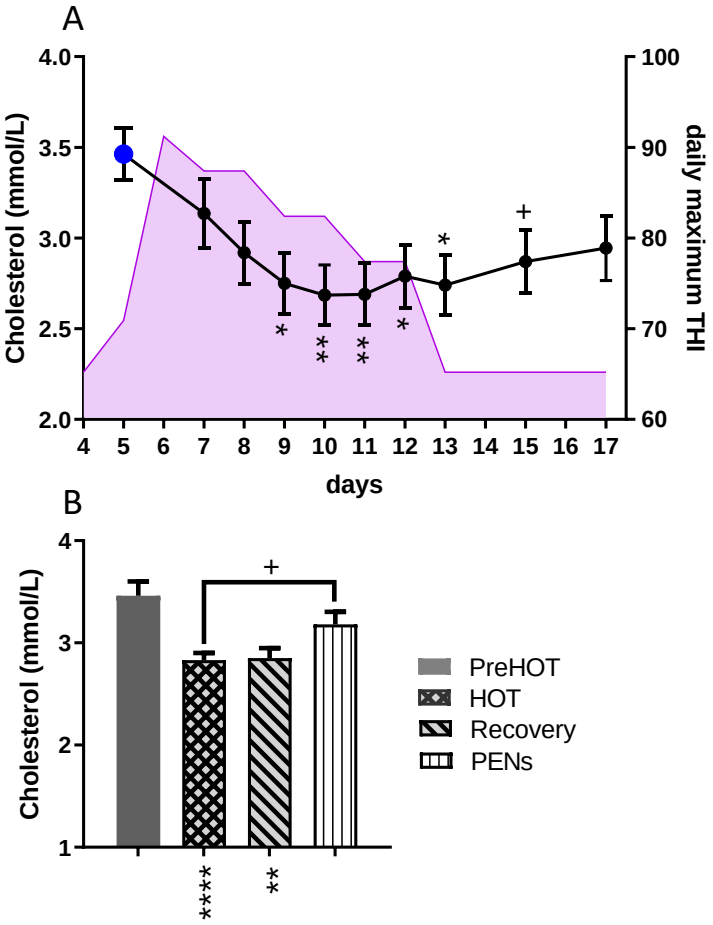

Fig. 29. Changes in plasma cholesterol concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma cholesterol concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma cholesterol concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01; ****, p <0.0001.

Muscle activity or damage – creatine kinase

High levels of creatine kinase (CK) in plasma can indicate muscle damage. On days 9-11, as maximum THI was falling, mean daily plasma CK activity was higher than the mean for the PreHOT days (Fig. 30A). Inter-animal variability was very high over these days also. Due to the high levels of circulating CK activity late in the HOT period, the HOT mean was ~50% higher than the PreHOT mean, and higher for the Recovery and PENs means (Fig. 30B). It should be noted that for most animals the CK activities were below 1000 IU/L throughout the trial. These are relatively low levels compared to pathological conditions.

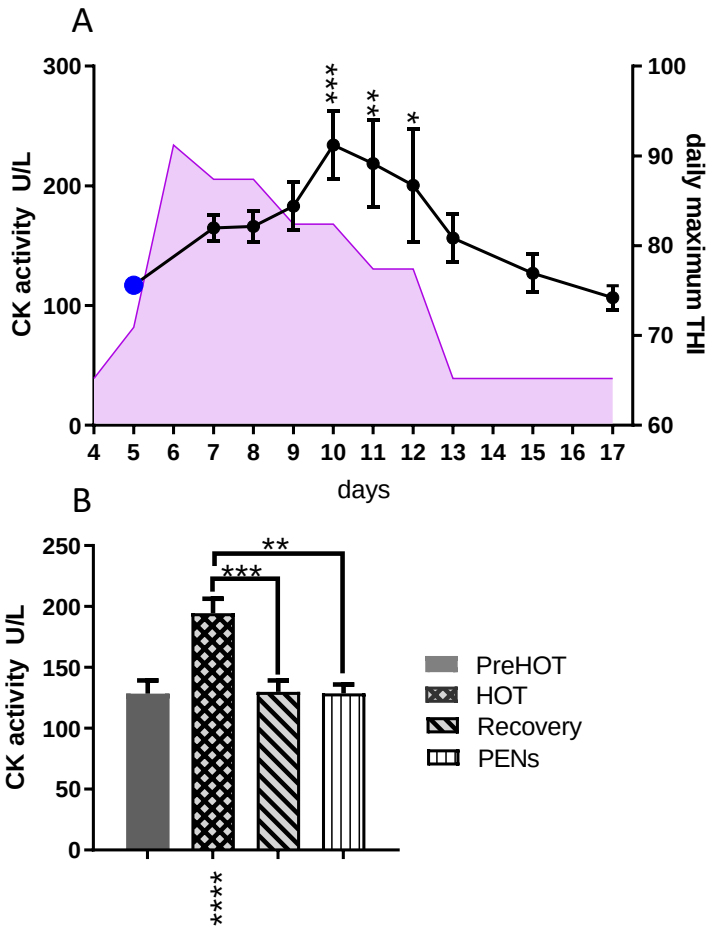

Fig. 30. Changes in plasma CK activity associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma CK activity (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means time relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma CK activity (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Electrolytes

There were rapid but small changes in plasma electrolytes with the onset of HOT. The mean plasma sodium concentration experienced a small but highly significant decrease (2%) over days 7-9 (HOT) and recovered quickly as the daily maximum THI fell below 85 (Fig. 31A). The overall means for each period showed that the HOT, Recovery and PENs means were all lower than the PreHOT mean (Fig. 31B).

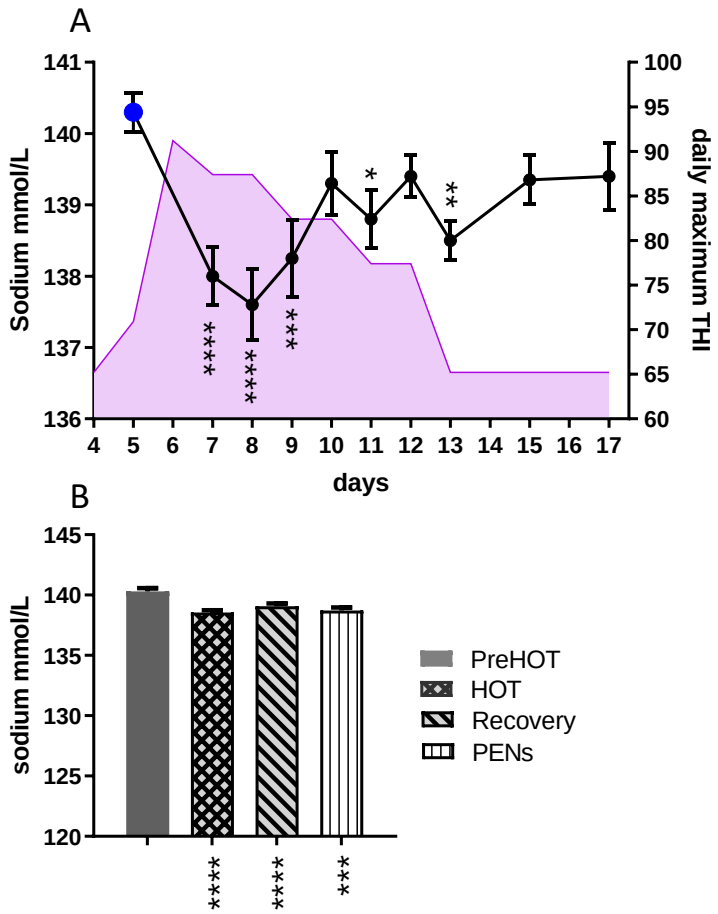

Fig. 31. Changes in plasma sodium concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma sodium concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma sodium concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; *** p <0.001; ****, p <0.0001.

The mean plasma potassium concentration fell (~6%) and was stable during days 8-10 until a sudden spike on day 11 (~14%), from where it recovered to near PreHOT mean concentration by days 15 and 17 (Fig. 32A). Note that this profile with the sharp rise on day 11 was replicated in both cohorts. The overall means for each period shown in Fig. 32B highlight the reduced potassium concentration during HOT.

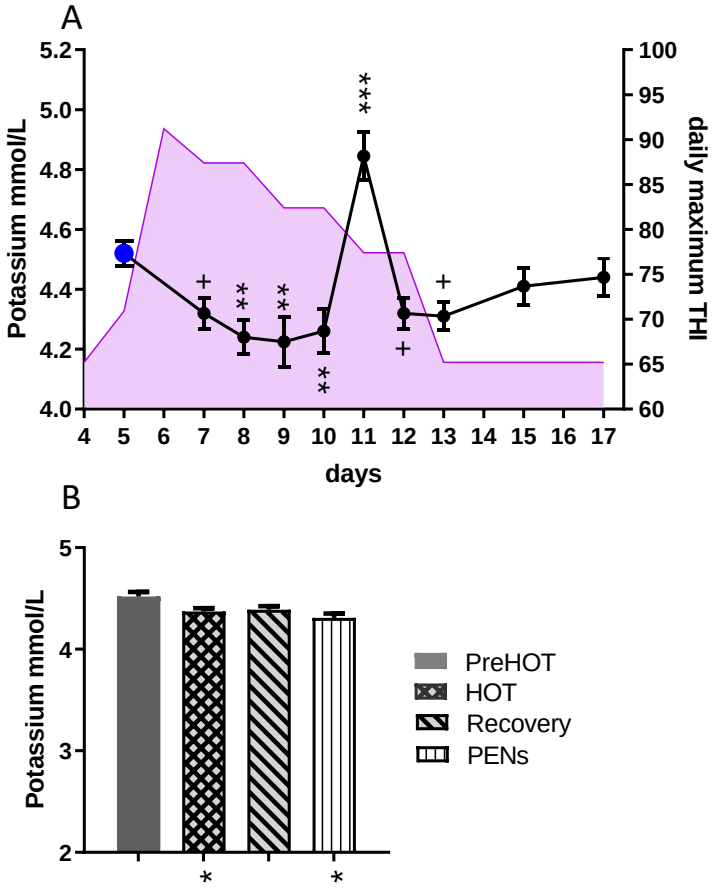

Fig. 32. Changes in plasma potassium concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma potassium concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma potassium concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001.

The mean plasma chloride concentration rose during days 6-8 and remained at a plateau until day 10 at ~3.8% higher than the PreHOT mean (Fig. 33A) and then fell quickly so that by day 13, the chloride concentration had returned to the PreHOT mean. Fig. 33B confirmed that the HOT mean chloride concentration was higher than all the means of all other periods.

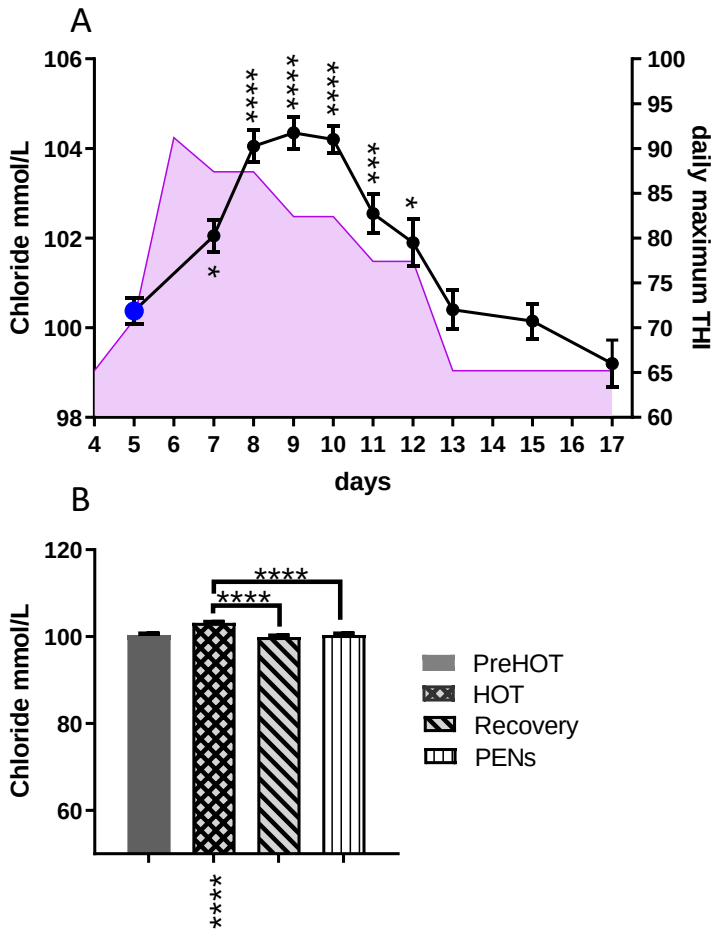

Fig. 33. Changes in plasma chloride concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma chloride concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma chloride concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; ***, p <0.001; ****, p <0.0001.

Calcium-phosphate axis

Mean plasma calcium concentration responded immediately to onset of high heat load with a fall of ~ 7% where it remained until day 10 (Fig. 34A). This was followed by a return to the PreHOT mean by day 13. The overall means for each period showed that the mean calcium concentration in HOT was lower than all other means (Fig. 34B). In contrast, the mean plasma phosphorus concentration was quite changeable over the 17 days (Fig. 35A). There was no immediate response to the onset of HOT, but then it experienced a sudden rise on day 9 (10%) and a fall of 11% below the PreHOT mean. On days 12-13, the mean plasma phosphorus concentration was ~ 12% below the PreHOT mean (Fig. 35A). Recovery was evident by day 17. Fig. 35B shows that the Recovery mean was less than the PreHOT and HOT mean plasma phosphorus concentrations.

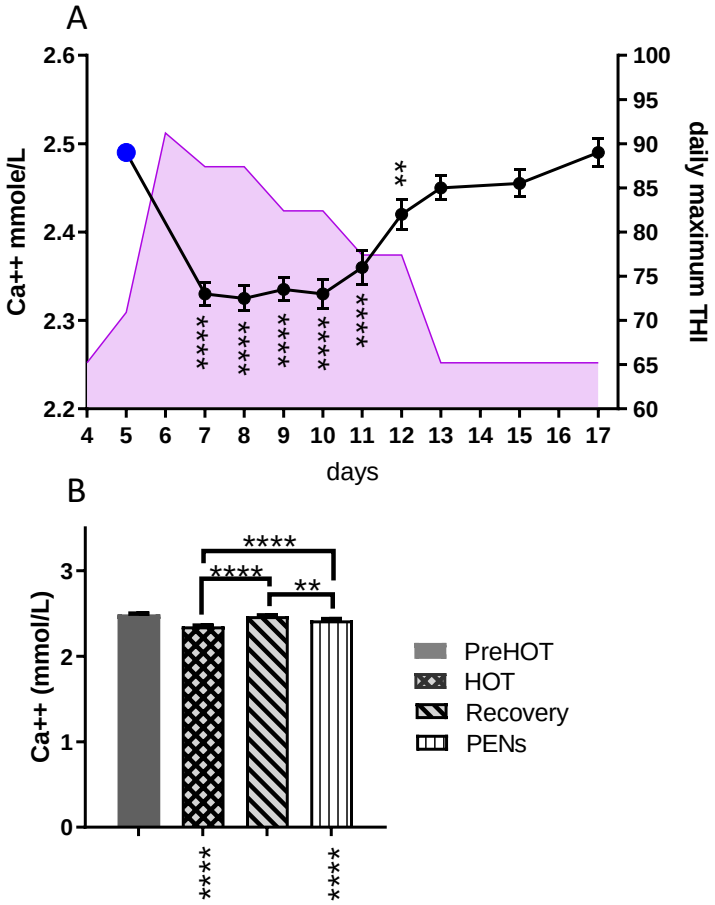

Fig. 34. Changes in plasma calcium concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma calcium concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma calcium concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. **, p <0.01; ****, p <0.0001.

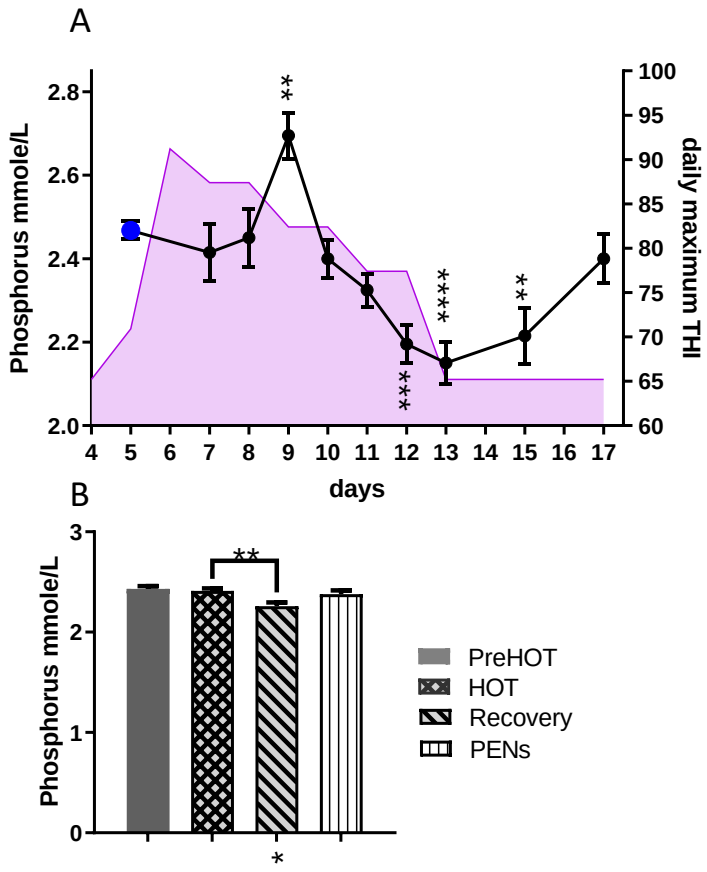

Fig. 35. Changes in plasma phosphorus concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma phosphorus concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma phosphorus concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Total protein concentration

Plasma total protein concentration can act as an indicator of hydration. Blood volume changes can be anticipated during hyperthermia. The mean plasma protein concentrations fell slowly and exhibited a 4.5% decrease on days 10–11 compared to the mean of the PreHOT days, implying a corresponding **increase** in blood volume (Fig. 36A).

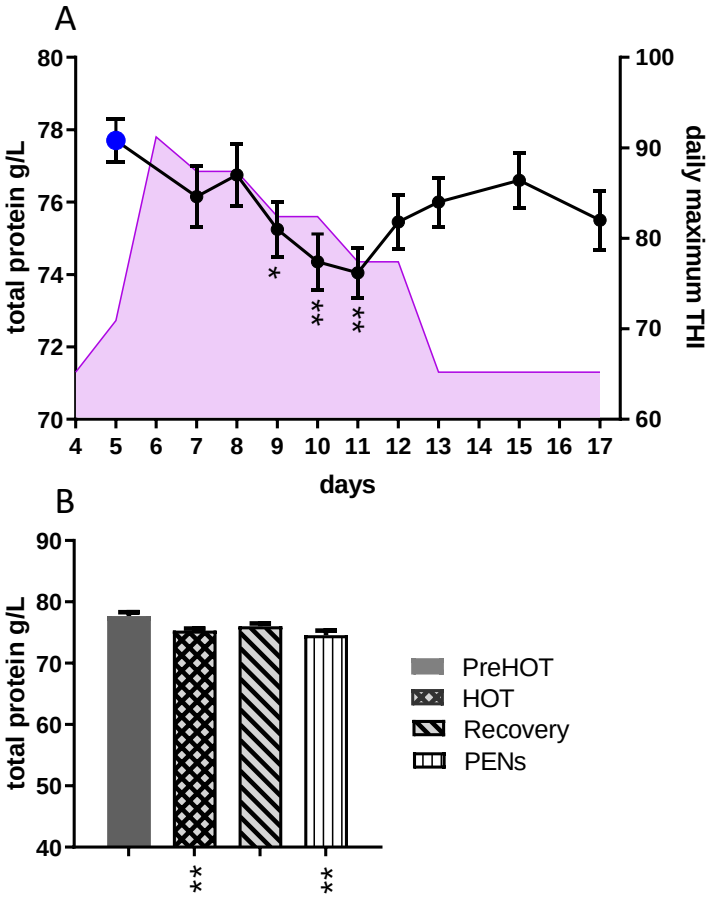

Fig. 36. Changes in plasma total protein concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma total protein concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma total protein concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01.

4.1.1.8 Haematological changes

The white blood cells (WBCs)

The mean daily WBC count experienced an initial rise of 14-15% to day 8-9, followed by a fall to ~8% less than the PreHOT mean by day 12 (Fig. 37A). At the end of the Recovery, the mean WBC count had returned to PreHOT levels. The overall means for HOT, Recovery and PENs period showed no significant difference with the PreHOT mean (Fig. 37B), however, the mean WBC count during HOT was 11-13% higher than those of Recovery and PENs.

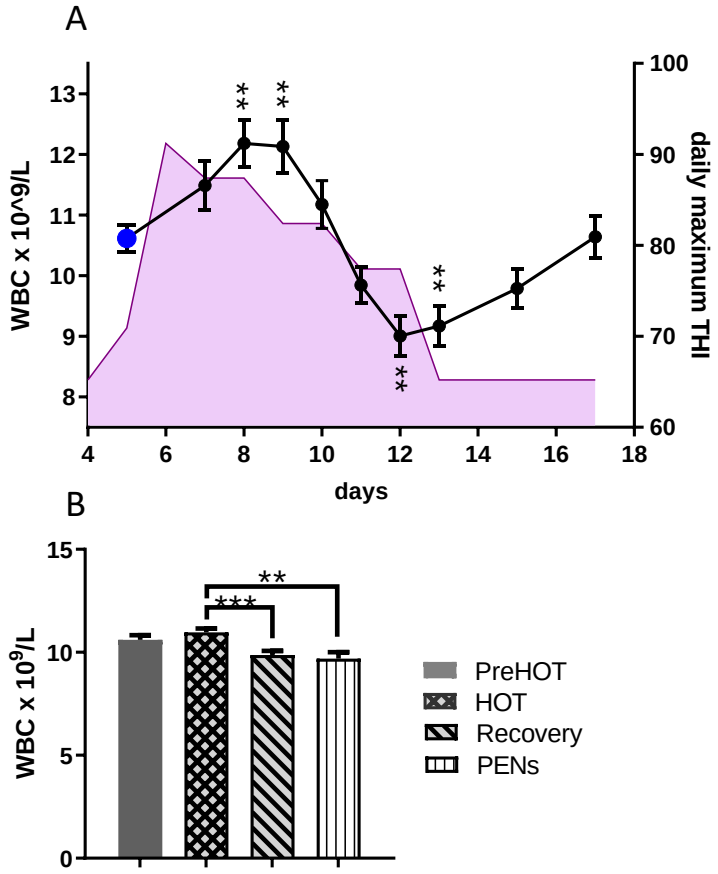

Fig. 37. Changes in white blood cell (WBC) count associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean WBC count (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma WBC count (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. **, p <0.01; ***, p <0.001.

The WBC population is composed of a number of subsets of which the lymphocytes (PBLs) and neutrophils are the most abundant cell types. The early rise in the mean WBC count was not entirely due to increased numbers of PBLs. The ~9.5% increase in mean PBL count on day 8 was not significant (Fig. 38A). The approximately 20% reduction in mean PBL count on day 11 did contribute to the fall in mean WBC count in the latter days of the HOT period. The overall means show that the mean PBL counts remained 9-12% lower in Recovery and PENs relative to the PreHOT mean (Fig. 38B).

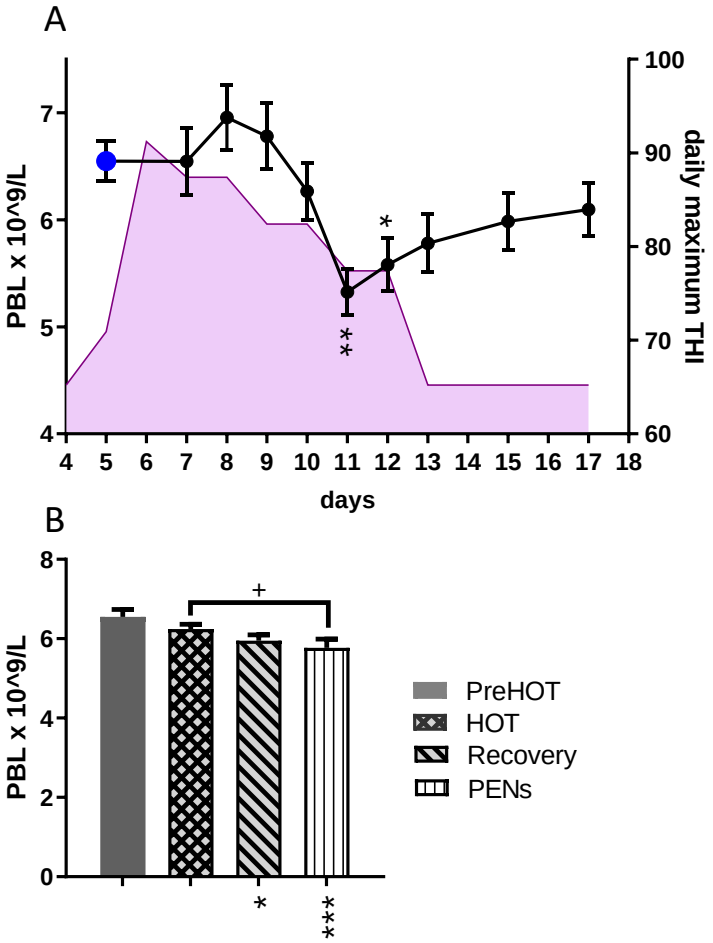

Fig. 38. Changes in peripheral blood lymphocyte (PBL) count associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean PBL count (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma PBL count (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001.

There was a rapid rise in the mean neutrophil count with onset of HOT (Fig. 39A), which reached a plateau on days 8-9 at ~50% higher than the PreHOT mean. The mean neutrophil count returned to normal levels by day 11 as conditions moderated. The mean for the HOT period was 27% higher than the PreHOT mean, and 20% and 28% higher than the Recovery and PENs means respectively (Fig. 39B).

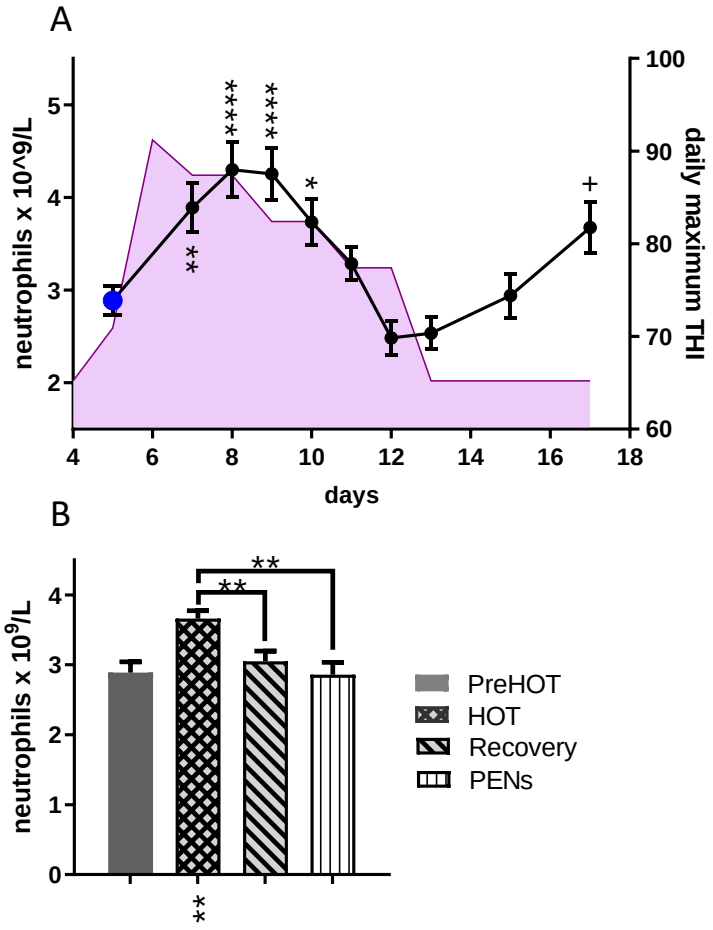

Fig. 39. Changes in neutrophil count associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean neutrophil count (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma neutrophil count (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01; ****, p <0.0001.

The mean eosinophil count responded immediately to HOT with a 56% reduction in circulating numbers on day 7 (Fig. 40A) and then proceeded to rise to 44% above the PreHOT mean by days 10-11, whereupon it fell again in Recovery to below the PreHOT mean. The overall mean for the HOT period was not different to the PreHOT mean; the Recovery mean was 35% lower than the PreHOT and HOT means, whereas in PENs there appeared to be another increase to 32% higher than the PreHOT and HOT means (Fig. 40B).

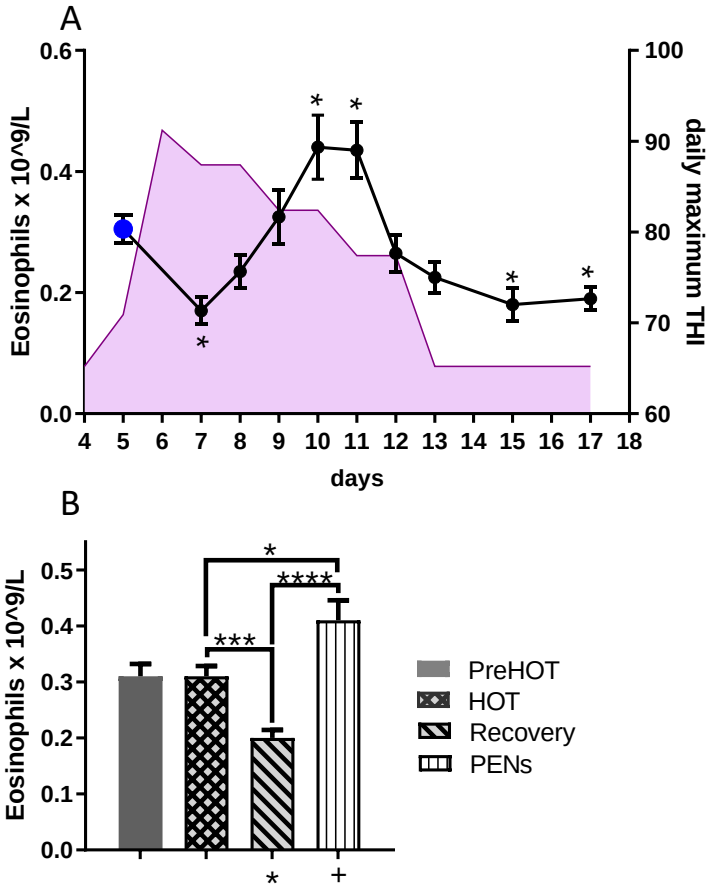

Fig. 40. Changes in eosinophil count associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean eosinophil count (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma eosinophil count (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; ***, p <0.001; ****, p <0.0001.

There were high levels of variability in the daily monocyte count (Fig. 41A). On day 8, the mean monocyte count suffered a fall of ~20% which then recovered somewhat for the remaining HOT days. In Recovery, the daily monocyte counts were stable but less than the PreHOT mean. Fig. 41B shows that the lower mean monocyte counts during Recovery apparently extended into PENs. The mean Recovery and PENs monocyte counts were 26-27% below the PreHOT mean.

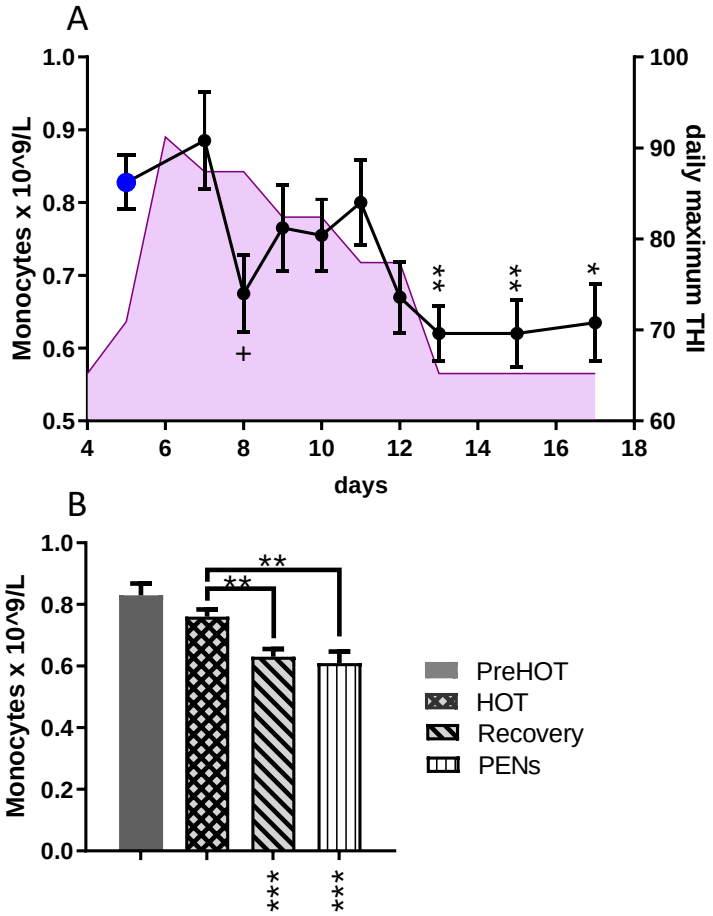

Fig. 41. Changes in monocyte count associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean monocyte count (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma monocyte count (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001.

On a day-to-day basis, there was no significant change in mean RBC count across the 17 days in the chambers and the course of the heat load challenge (Fig. 42A) even though the mean RBC counts for days 10-12 were 4-5% lower than the PreHOT mean. The overall means for each period captured this fall during the HOT period (Fig. 42B); the HOT mean was only 2% less than the PreHOT mean and not significantly different. In PENS, the mean rose about 4% but this was not significantly different to the PreHOT or Recovery means also.

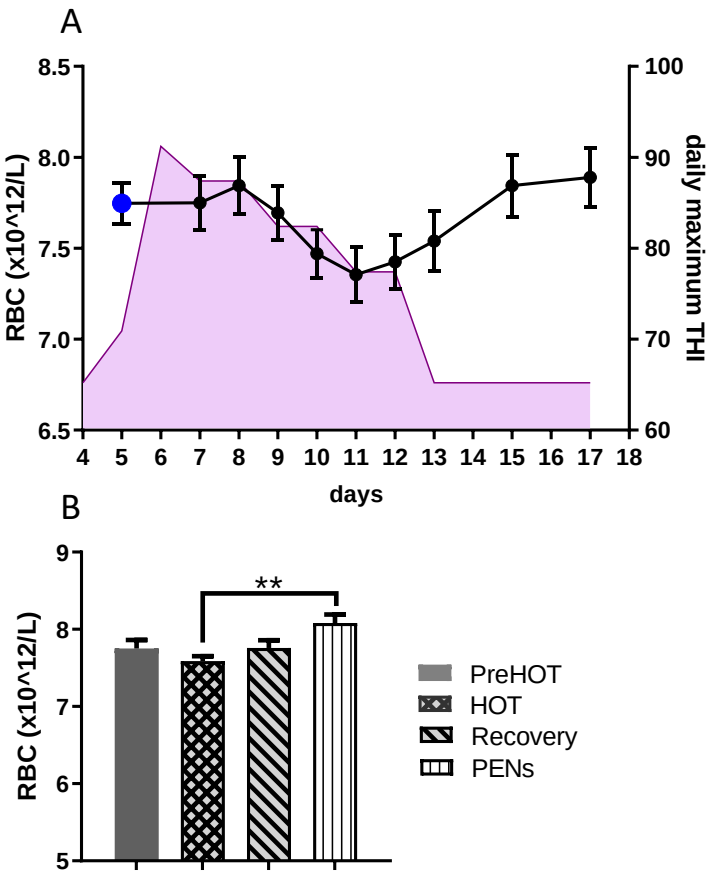

Fig. 42. Changes in red blood cell (RBC) count associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean RBC count (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma RBC count (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. **, p <0.01.

The haematocrit (HCT) which is a similar measure to the packed cell volume (PCV) can act as an indicator of hydration also. Increased HCT suggests a reduced plasma volume. As expected, the HCT trajectory follows the RBC count trajectory (Fig. 43A and B). That is, after the very hot days 6-8, the HCT gradually falls so that mean HCT of days 10 and 11 were 6% lower than the PreHOT mean. When thermoneutral conditions returned, the HCT returned to PreHOT levels by day 15. The overall means shows that the mean HCT during HOT was 4.5% reduced relative to the PreHOT and Recovery means (Fig. 43B).

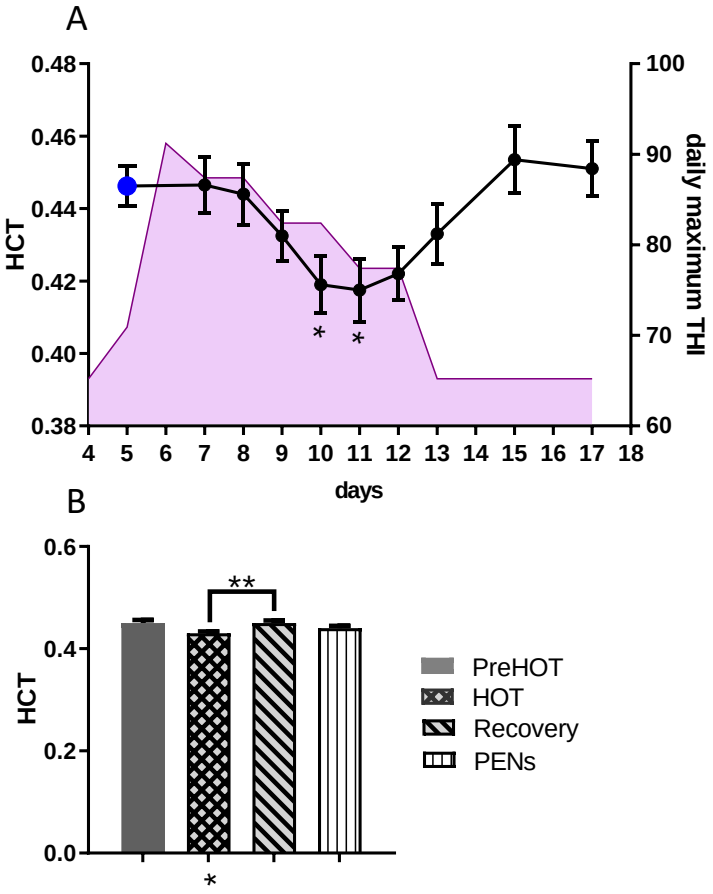

Fig. 43. Changes in haematocrit (HCT) associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean HCT (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma HCT (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01.

Platelets are essential to blood clotting and coagulation. Platelet counts are highly variable between animals. There is no immediate response to high heat load seen from the daily mean platelet counts (Fig. 44A). On day 11 the mean platelet count is tending toward being significantly less (~25%) than the PreHOT mean. As the maximum THI reduced, the mean platelet count rose; on days 15 and 17, it was 35 and 28% higher than the PreHOT mean respectively. The overall means show the distinctive increase in mean platelet count during Recovery, which was higher than the means of all the other periods (Fig. 44B).

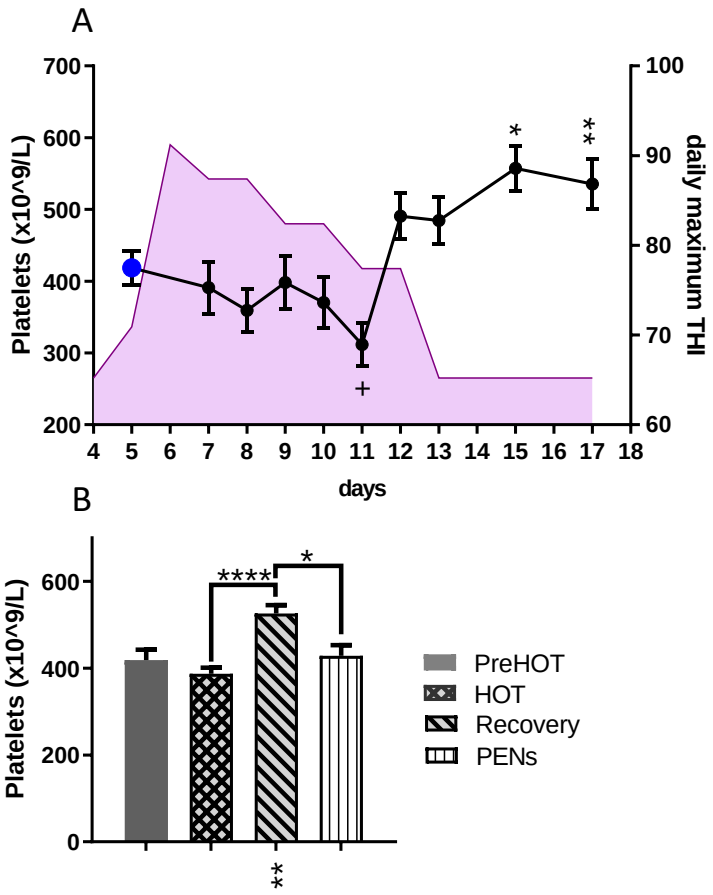

Fig. 44. Changes in platelet count associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean platelet count (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean platelet count (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01; ****, p <0.0001.

4.1.1.9 Endocrine changes

Besides insulin, three other protein hormones, adiponectin, leptin and prolactin, which all participate in the regulation of energy balance and appetite (amongst other functions) were measured in plasma. The thyroid hormones, T3 and T4, and thyroid stimulating hormone (TSH) which is produced by the pituitary, were also assayed.

The mean plasma adiponectin concentration decreased with high heat load. It was at its lowest on day 10 at 43% lower than the PreHOT mean (Fig. 45A). As the heat abated, the adiponectin concentration increased but was highly variable. On day 17, the concentration appeared to fall again. Comparison of the overall means confirmed that the mean adiponectin concentrations were all reduced relative to the PreHOT mean, and the lowest concentration occurred in PENs (Fig. 45B). The HOT and Recovery means which were 35 and 25% lower than the PreHOT mean, were not different to each other.

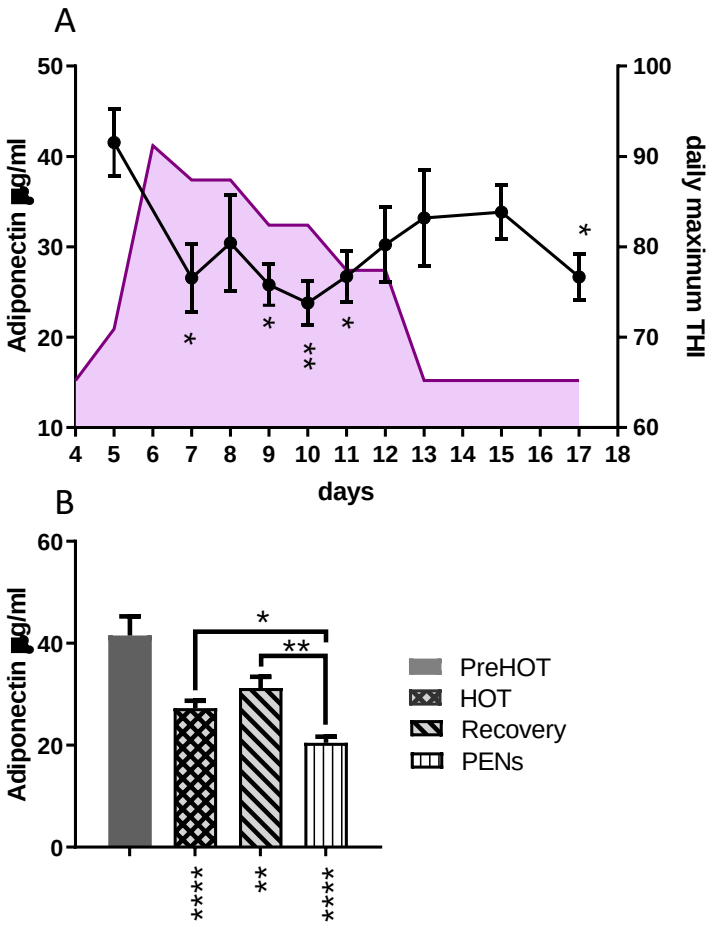

Fig. 45. Changes in plasma adiponectin concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma adiponectin concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma adiponectin concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01; ****, p <0.0001.

The mean leptin concentration appeared to drop by about 40% immediately with the onset of hot conditions and did not recover at any point (Fig. 46A). The means for the HOT, Recovery and PENs periods were all lower than the PreHOT mean and were not different to each other (Fig. 46B).

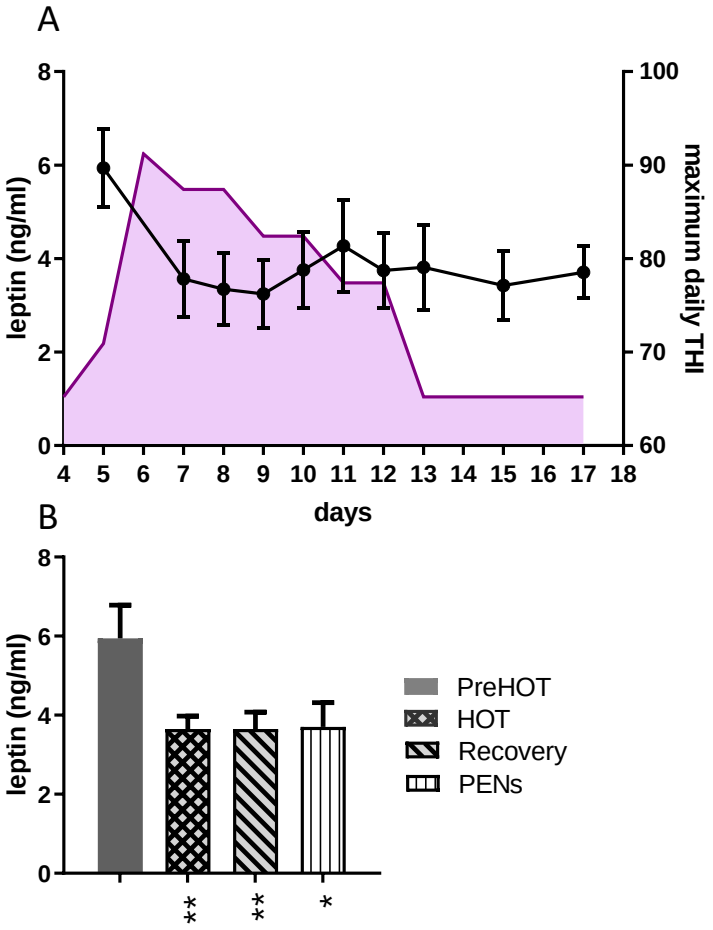

Fig. 46. Changes in plasma leptin concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma leptin concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma leptin concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, $p < 0.05$; **, $p < 0.01$.

Prolactin concentrations were highly variable between animals (Fig. 47A). There appeared to be a decrease in the mean daily prolactin concentrations during the HOT days with little change in Recovery. The overall means as presented in Fig. 47B, indicate that the mean prolactin concentration of the HOT period fell by more than 50% of the PreHOT mean, and was still lower in Recovery.

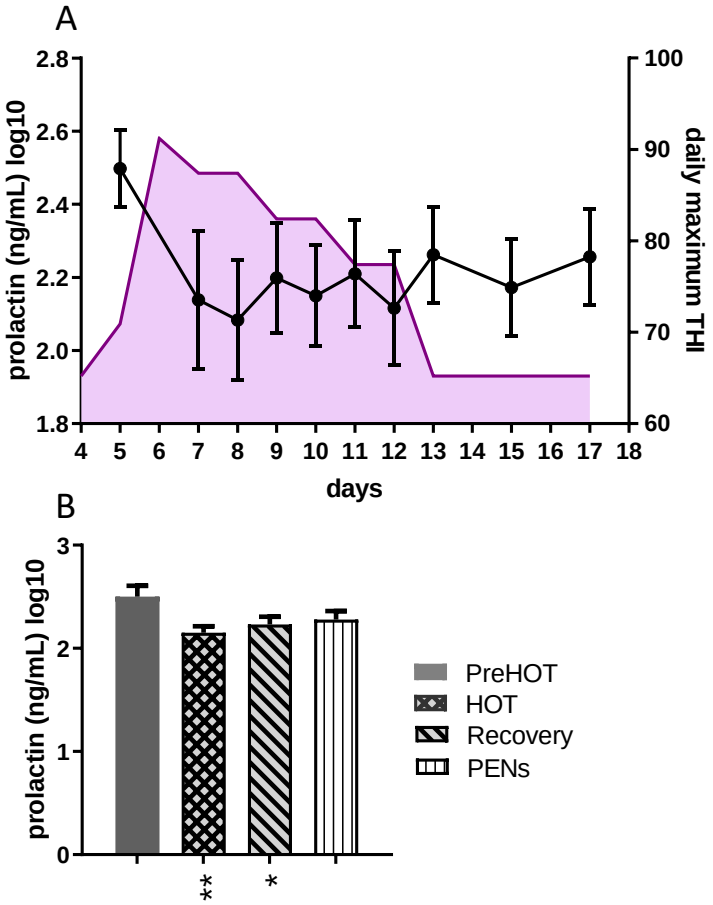

Fig. 47. Changes in plasma prolactin concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean plasma prolactin concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma prolactin concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. *, p <0.05; **, p <0.01.

The thyroid hormones are integral to thermoregulation. The daily mean T4 concentration fell rapidly and was 45% reduced relative to the PreHOT mean by day 9 (Fig. 48A). As conditions cooled the mean T4 concentration increased to reach parity with the PreHOT mean on day 13, the first day of Recovery. The overall mean for the HOT period was 33% lower than the PreHOT mean and lower than Recovery and PENS means (Fig. 48B). The means for the Recovery and PENs periods were all lower than the PreHOT mean also.

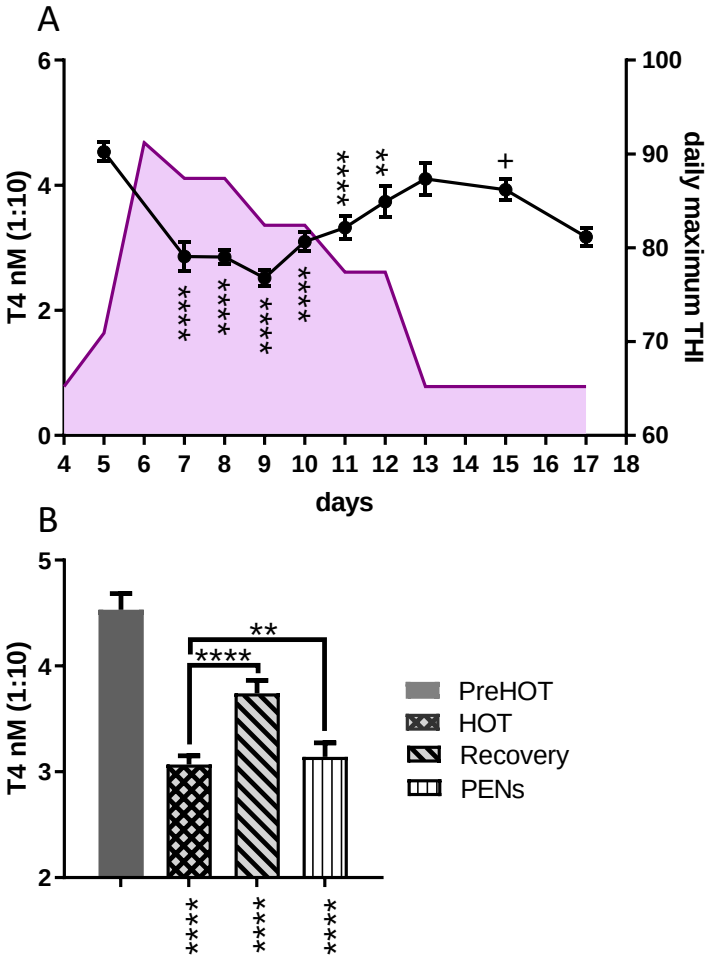

Fig. 48. Changes in T4 concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean T4 concentration (± SEM) in the climate chambers (days 1-17). The statistical significance of the daily means relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean plasma T4 concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with the PreHOT mean. +, p <0.1; **, p <0.01; ****, p <0.0001.

T3 is produced by the removal of one of the four iodine groups from T4. The mean plasma T3 response to high heat load was not as distinctive as that of T4 (Fig. 49A). There was an initial and substantial reduction (~60%) in the mean plasma T3 concentration on day 7, and some level of recovery during the remaining HOT days although still below the PreHOT mean. Concentrations were highly variable during Recovery but approaching the PreHOT mean (Fig. 49A). The overall means simplify the interpretation in that the HOT mean is ~27% decreased compared to the PreHOT mean, and lower than the Recovery and PENs means (Fig. 49B).

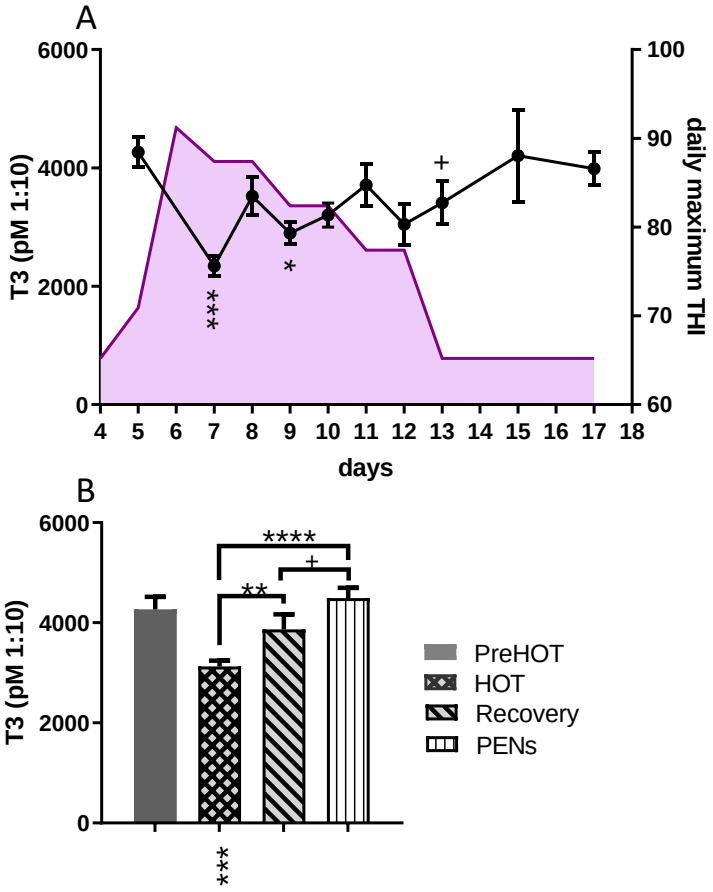

Fig. 49. Changes in T3 concentration associated with high heat load and recovery in CC7 and 8. Panel A. The daily mean T3 concentration (± SEM). The statistical significance of the mean at each time relative to the mean of combined values of days 3 and 5 (PreHOT) are indicated by the asterisks. The daily maximum THI is indicated by the filled area. Panel B. Between periods comparison of the mean T3 concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with PreHOT. +, $p < 0.1$; **, $p < 0.01$; ****, $p < 0.0001$.

The TSH concentration during the HOT days and Recovery days were not different to the PreHOT mean (data not shown). The overall means for each period shows that only the mean PENs concentration was different to the PreHOT mean and to the HOT and Recovery means also (Fig. 50).

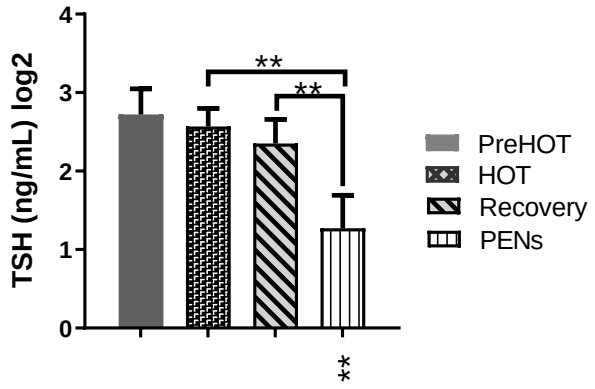

Fig. 50. Changes in TSH concentration associated with high heat load and recovery in CC7 and 8. Between periods comparison of the mean TSH concentration (± SEM). The asterisks under the x-axis indicate statistically significant difference with PreHOT. **, p <0.01.

4.1.1.10 Inflammatory responses

The plasma cytokine and haptoglobin (Hp) concentrations were notable for their individual animal variation. This was somewhat overcome by log transformation of the daily cytokine concentrations. Inspection of Fig. 51 reveals that there was either no change in mean cytokine concentration between PreHOT and HOT, or in fact, the mean concentration decreased in HOT. For TNFα, IFNγ and IL-10, there was no indication of a response to conditions during any period (Fig. 51A, B and E). The mean plasma IL-6 and Hp concentrations remained lower than the PreHOT mean in Recovery (Fig. 51D and F). In PENs, both the mean plasma IL-1β and Hp concentrations were markedly reduced compared to the means of the preceding periods (Fig. 51C and F).

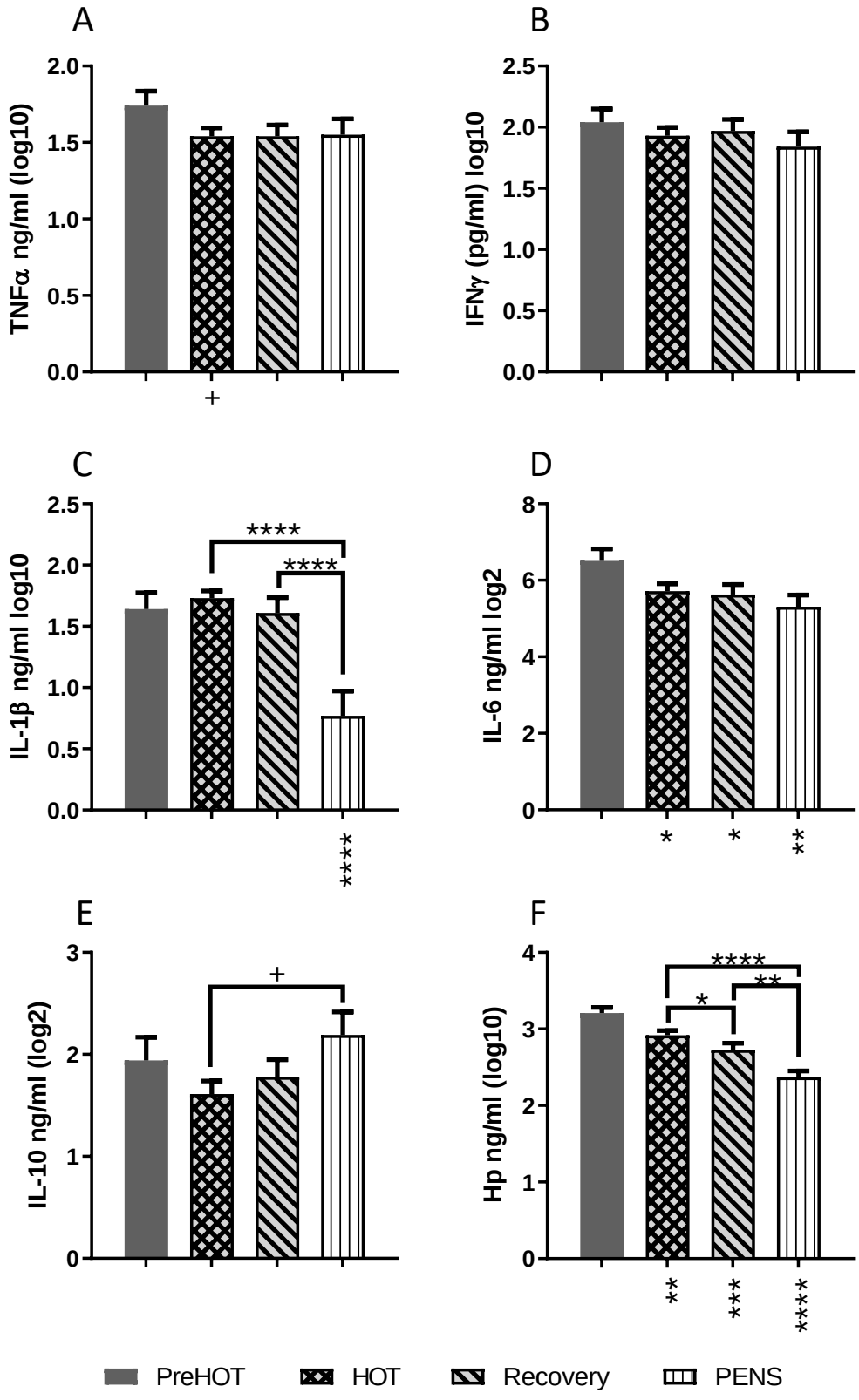

Fig. 51. Changes in plasma cytokine and haptoglobin (Hp) concentrations associated with high heat load and recovery in CC7 and 8. Panels A-F: Between periods comparison of the mean concentration (± SEM) of TNFα, IFNϒ, IL-1β, IL-6, IL-10 and Hp respectively. The asterisks under the x-axis indicate statistically significant difference with PreHOT. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

4.1.1.11 Discussion and Conclusions

This was the pivotal experiment in this research program. The HOT conditions in CC7 and 8 trials were able to induce marked physiological, metabolic and immune cell responses to high heat load. During the HOT period the animals presented with elevated respiration rate, surface temperatures, panting score and rumen temperature. Feed intake reduced to approximately 65% of PreHOT amounts during the HOT period. Recovery can be impacted by changes in feed intake (increases) and overcompensating in terms of heat dissipation despite the abatement of hot conditions. Indications of this "overcompensation" are hinted at in the reduced respiration rate, rectal temperature, and maximum and minimum rumen temperatures during Recovery. However, only the mean rectal temperatures in Recovery were significantly different to the PreHOT mean.

This experiment has allowed for the first time a description of the metabolic phenotype during heat stress in feedlot cattle. Plasma glucose is tightly regulated, however, during high heat load with reductions of over 10 and 20%, these cattle were hypoglycaemic for at least five days. Even though feed intake resumed on day 9, plasma glucose did not rise until day 12, and did not recover until day 13. There was a four day delay until homeostasis was achieved. A two staged recovery in plasma glucose was observed. During the first phase, plasma glucose may have been defended by utilising hepatic glycogen and/or supplemented by conversion of lower amounts of propionate coming from the rumen. In the second phase, plasma glucose may have been supported by hepatic glycogen and/or gluconeogenesis using muscle derived amino acids. There was no rise in insulin to inhibit glycogenolysis or fatty acid oxidation, but no significant fall either despite the large fall in glucose.

As plasma glucose fell, the animals resorted to oxidation of fatty acids as evidenced by the rise in plasma β-hydroxybutyrate. This is a new finding. Previous work by Baumgard et al., had suggested, due to low plasma fatty acid levels, that fatty acid oxidation was not occurring in heat stress. It is possible that heat stressed cattle oxidise hepatic fat and thus do not mobilise subcutaneous fat reserves. We have not conducted plasma NEFA assays on the samples from this experiment. Early studies in Rockhampton demonstrated depletion of hepatic fat during heat stress. Plasma glutamine can act indirectly as an energy metabolite in many tissues. The concentration of this amino acid was reduced in HOT implying rapid depletion from circulation without adequate release from tissues such as muscle to replace it.

Creatinine flows freely from the kidney into the urine. Its retention in plasma as evidenced by the marked rise in plasma creatinine concentration during HOT suggests reduced blood flow to kidney and filtration from the kidney. The retention of urea is likely to be due to reduced renal filtration also. Interesting that there is no increase in plasma urea with increased feed intake; plasma urea was actually low during Recovery suggesting rapid uptake of N for resumption of tissue turnover and for compensatory gain after heat stress.

The kidneys are intimately involved in the regulation of plasma electrolytes and bicarbonate also. Notwithstanding an initial fall in the plasma cations, there tended to be a retention of both sodium and potassium as the high heat load persisted. Clearly, plasma chloride was under different regulation with immediate retention of this anion, as this may be an attempt to compensate for the loss of the other major plasma anion, bicarbonate, which fell dramatically with onset of HOT.

There is clear evidence to support a hepatic ‘slowdown’. Two indicators, plasma ALP activity and cholesterol concentration, both decreased during HOT with no immediate recovery with increased feed intake and cooler conditions. The fall in cholesterol is probably due to less acetate reaching the liver from the rumen for conversion to cholesterol, and decreased cholesterol synthesis if hepatic ‘slowdown’ was initiated. ALP, an intracellular hepatic enzyme, followed the same course as plasma cholesterol concentration.

Further indication of a hepatic slow down might be surmised by the high plasma bilirubin concentrations that occurred during HOT and persisted into Recovery. Bilirubin is mostly produced in the liver and is derived from heme, a breakdown product of red cell haemoglobin. Most of the bilirubin is passed out of the liver to the gall bladder and intestine along with bile acids. High plasma bilirubin can occur with increased red blood cell (RBC) damage (e.g. a haemolytic anaemia) or a ‘back up” of bilirubin if there is a problem with secretion in to the bile and gall bladder. The haematology data excludes loss or altered RBC shape, size or haemoglobin content.

There is no evidence of actual liver damage during HOT. Three of the classic diagnostic liver function enzymes, AST, GGT and GLDH, did not change activity during the HOT days but rose during Recovery. This type of response is reminiscent of reperfusion following hepatic ischemia.

As alluded above, ALP activity decreased significantly in HOT, and only slowly recovered. It did not behave like the other hepatic enzymes. Plasma ALP is derived almost equally from bone and liver. We know that both isoforms are equally depressed in this study (Stephen Anderson, unpublished data). We also have evidence for active bone resorption in these animals during HOT; unusual in a growing animal. It might be expected that bone resorption would lead to increased plasma calcium concentrations. However, a substantial fall in calcium concentration was seen during HOT. We have no explanation for this yet.

Plasma creatine kinase activity can inform of muscle damage. In the first days of HOT there was no change in plasma CK activity indicating no major release of enzyme from muscle. Toward the end of HOT, as conditions cooled, plasma CK activity rose, but not to levels indicating pathology (which reaches 1000s IU/L). This rise in plasma CK activity may indicate increased blood flow to the muscle after the very hot conditions.

One common expectation in heat stress is haemoconcentration due to water loss to perspiration and respiration. The mean RBC count, haematocrit and total protein concentration during HOT were all slightly decreased, and not increased. This indicates that all animals maintain hydration throughout, and the appropriately increased water intake also supports this. Blood volume may have increased.

The behaviour of three protein hormones associated with feeding behaviours and appetite and energy balance were investigated also. Amongst insulin’s many functions, the most relevant here are its roles in reducing plasma glucose concentrations by increasing cellular uptake of glucose and reducing the production of free fatty acids by adipose cells. In HOT and Recovery, a small but insignificant decrease in plasma insulin levels was seen. Given the marked fall in plasma glucose concentration, a larger response in plasma insulin levels could be expected. Furthermore, during a stress that induces increased noradrenaline, plasma insulin should fall.

Adiponectin, a relatively abundant hormone in plasma, is produced mostly by adipose tissue. In humans, and rodent models is known to increase in plasma concentration during weight loss and reduced feed intake. During HOT, despite the large drop in mean DMI, mean plasma adiponectin fell substantially and only recovered well into the Recovery period. The fall in adiponectin concentration suggests that it did not have a major role in the low levels of plasma glucose concentration and likely limited gluconeogenic response.

Leptin, like adiponectin, is mainly synthesised in the adipose tissues and also in the small intestine. Leptin has wide ranging functions and acts in the brain as well as peripheral tissues and organs. It is mostly known for inhibiting hunger. During HOT, plasma leptin concentration decreased and remained low during Recovery. This might seem an appropriate response to encourage feeding as soon as conditions allowed. Prolactin, while renowned for its role in lactation, is now recognised to influence metabolism and body weight, and water balance in adults. As with the other protein hormones, plasma prolactin concentration was reduced during HOT and Recovery.

While there were muted responses by the protein hormones, the thyroid hormone, T4, was highly sensitive to increased heat load. Mean plasma T4 concentrations fell sharply with onset of HOT, rose as THI fell, but not to PreHOT levels. The lower T4 concentration in PENs may reflect increased age and maturity of the animals. T3, which is produced from T4, and vastly the more active hormone, also fell in HOT as might be expected if there is less T4 available. It also rose in Recovery and achieved PreHOT levels in Recovery and PENs. The reduced level of circulating T4, which is produced by the thyroid, implicates regulation of its production in heat stress. Since higher levels of thyroid hormones increase metabolic rate, their reduction during high heat load is appropriate. It appears that the liver function of converting T4 to T3 was not affected by high heat load.

While the major metabolically active organs might have shifted into a ‘quiet’ state, there is much flux in the circulating immune cells. The WBC count was increased during HOT but fell as the high heat load persisted. With the onset of HOT, the number of circulating neutrophils rose dramatically, and fell as dramatically, corresponding with daily maximum THI. These cells are normally involved in fighting bacterial infection. It is possible that tissue based neutrophils may have migrated into circulation, but there is no change in the number of circulating lymphocytes until the later phase of HOT. The reduced total WBC and PBL counts in Recovery are a concern.

The speciality WBC subclasses, the monocytes and eosinophils, experienced flux also. In both cases there was an early transient fall in numbers with the onset of HOT. This may indicate a retreat into tissues or actual loss of cells. It is a window of vulnerability to viruses and parasites. The eosinophil numbers rise late into HOT, but both cell types have lower cell numbers during Recovery compared to PreHOT, implying vulnerability to infection or exacerbating current viral and parasitic infections.

These changes in circulating WBC populations are occurring while most of the major cytokines are ‘missing-in-action’. With the onset of HOT and in Recovery, four of five major cytokines showed no change in plasma concentration. IL-6 and Hp concentrations fell in HOT and Recovery. It would appear that generalised or systemic inflammation is not involved in the heat stress response or recovery from it.

In summary

The overall picture of the high heat load steer is of behavioural and metabolic ‘quietening’ to reduce endogenous heat production and to increase heat loss. We know from the work of researchers in the 1960’s that blood flow is diverted from the major organs including the liver, rumen and intestine, kidneys and skeletal muscle in high heat load ruminants. Furthermore, metabolic rate as regulated by the thyroid hormones must decrease as the plasma concentration of these hormones fall. The protein metabolic hormones give an indifferent response possibly due to decreased synthesis in the organs and tissues that produce them.

Under this level of heat load, the cattle are hypoglycaemic as the decreased feed intake reduces supply of acetate and propionate. Adding to this, gluconeogenesis from amino acids may become limited as the amino acid levels fall in the blood. We do not yet fully understand if skeletal muscle is or can supply amino acids under these conditions. There is no indication of muscle damage. We do not know if the supply of fatty acids from adipose is limiting although these animals are oxidising fatty acids. We know there is further hepatic involvement by the build-up of bilirubin, and the reduced production of cholesterol.

Production of urea by the liver may be the result of an increased use of amino acids and/or decreased release of urea into the rumen due to decreased blood supply to that organ. There appears to be a reduction in glomerular filtration as seen by the reduced excretion of creatinine and urea. The kidney is working to retain chloride ions to balance the loss of bicarbonate from the increased respiration rate. But these animals are well hydrated. Bone growth and deposition are likely to be stalled.

There is a surge of neutrophils into the blood. These are the main bacteria fighting blood cells. In late HOT, as the worst of the heat subsides, there is a surge of eosinophils whose main target are parasites. There is no indication of a systemic inflammatory response. This would seem appropriate as many of the cytokines are pyrogenic and high levels will only increase endogenous heat load.

As the heat load reduces, rumen temperature and respiration rate normalise. There are indications of an ‘overcompensation’ with lowered respiration rates and body temperatures than prior to high heat load. Feed intake gradually returns, and plasma glucose levels increase. The rumen and liver cooperate to get this ‘essential service” re-established quickly. However, these animal shows signs of a “generalised reperfusion syndrome” as blood supply increases to allow normal metabolic function and rate, although that may be simplistic. This is more evident in the next section. There is increased release of liver enzymes into the blood presumably as full blood returns to flush out the liver, removing cell debris that may have accumulated, and encouraging tissue repair. This is likely to be occurring in all organs that have suffered some reduction of blood flow. There is a transient increase of CK activity in plasma in late HOT and early Recovery also. Interestingly, the systemic inflammatory system is not provoked. There is no rise in the concentrations of any cytokine.

Nevertheless, in Recovery, full liver function has not returned yet, as plasma bilirubin is still higher than normal, and ALP and cholesterol levels are still low. Of concern, is the low

number of lymphocytes in later HOT days and low counts of monocytes and eosinophils in Recovery. As mentioned earlier, this is a window of vulnerability.

A testament to the resilience of these animals, is that by twelve days after the HOT period, most physiological and blood parameters have returned to normal and the animals have gained weight.

4.1.2 High load diet climate chamber study - CC9-12

4.1.2.1 Introduction

This experiment investigated the efficacy of a heat load diet for feedlot cattle on finisher ration, and the timing of its inclusion in the diet, in preparation for, or in response to a sudden high heat load challenge. Diet 1 proceeded with finisher ration throughout the four treatment periods, PreHOT, HOT, Recovery and PENs. Diet 2 introduced the heat load ration at the onset of HOT (day 9). Diet 3 introduced the heat load ration two days prior to the onset of HOT (day 7). See Table 3 below. Four cohorts of twelve steers, CC9-12, proceeded sequentially through the chambers and returned to feedlot pens for 21 days after exit from the climate chambers. Each cohort was split into three groups of four animals so that the three diets were tested during each cohort.

Table 3. Climatic and dietary schedules for day 1-20 in climate chambers. Application of the heat load (HL) ration is denoted by the blue shaded cells.

Days	Period	Diet 1	Diet 2	Diet 3
0	Acclimation	Finisher	Finisher	Finisher
1	Acclimation	Finisher	Finisher	Finisher
2	PreHOT	Finisher	Finisher	Finisher
3	PreHOT	Finisher	Finisher	Finisher
4	PreHOT	Finisher	Finisher	Finisher
5	PreHOT	Finisher	Finisher	Finisher
6	PreHOT	Finisher	Finisher	Finisher
7	PreHOT	Finisher	Finisher	*HL ration
8	PreHOT	Finisher	Finisher	HL ration
9	HOT	Finisher	HL ration	HL ration
10	HOT	Finisher	HL ration	HL ration
11	HOT	Finisher	HL ration	HL ration
12	HOT	Finisher	HL ration	HL ration
13	HOT	Finisher	HL ration	HL ration
14	Recovery	Finisher - hold	HL ration - hold	HL ration - hold

15	Recovery	Finisher - Increase 1/3 (30%)	Increase 1/3 (30%) – HL ration	Increase 1/3 (30%) – HL ration
16	Recovery	Finisher - hold	Hold – HL 50%; ^F 50%	Hold – HL 50%; F 50%
17	Recovery	Finisher - Increase 1/3 (60%)	Increase 1/3 (60%) – HL 50%; F 50%	Increase 1/3 (60%) – HL 50%; F 50%
18	Recovery	Finisher - hold	Hold – 100% Finisher	Hold – 100% Finisher
19	Recovery	Finisher – 90%	Increase 1/3 (90%) – 100% Finisher	Increase 1/3 (90%) – 100% Finisher
20	Recovery	Finisher	Finisher	Finisher

*, HL, heat load; ^, F, finisher

The conditions for this trial are based on the high heat load trial (CC7 and 8), except for the conditions during PreHOT and Recovery. For the CC7 and 8 trials discussed in the previous section, the PreHOT and Recovery conditions were conducted in thermoneutral for beef cattle i.e. ambient temperature ranged from 19.0 - 21.0°C and the THI ranged over 65-69. The climatic conditions in this high heat load diet trial were adjusted in PreHOT and Recovery to reflect average Dalby summer conditions. The December and January average daily maximum and minimum temperatures and THI were calculated from hourly data collected by the BoM weather station at Dalby airport over 2012-17. The maximum and minimum temperatures were 30.8 and 19.0°C respectively, and the maximum and minimum THI were 77.2 and 65.5 respectively. These conditions were used in the PreHOT and Recovery periods in the climate controlled chambers (Fig. 52).

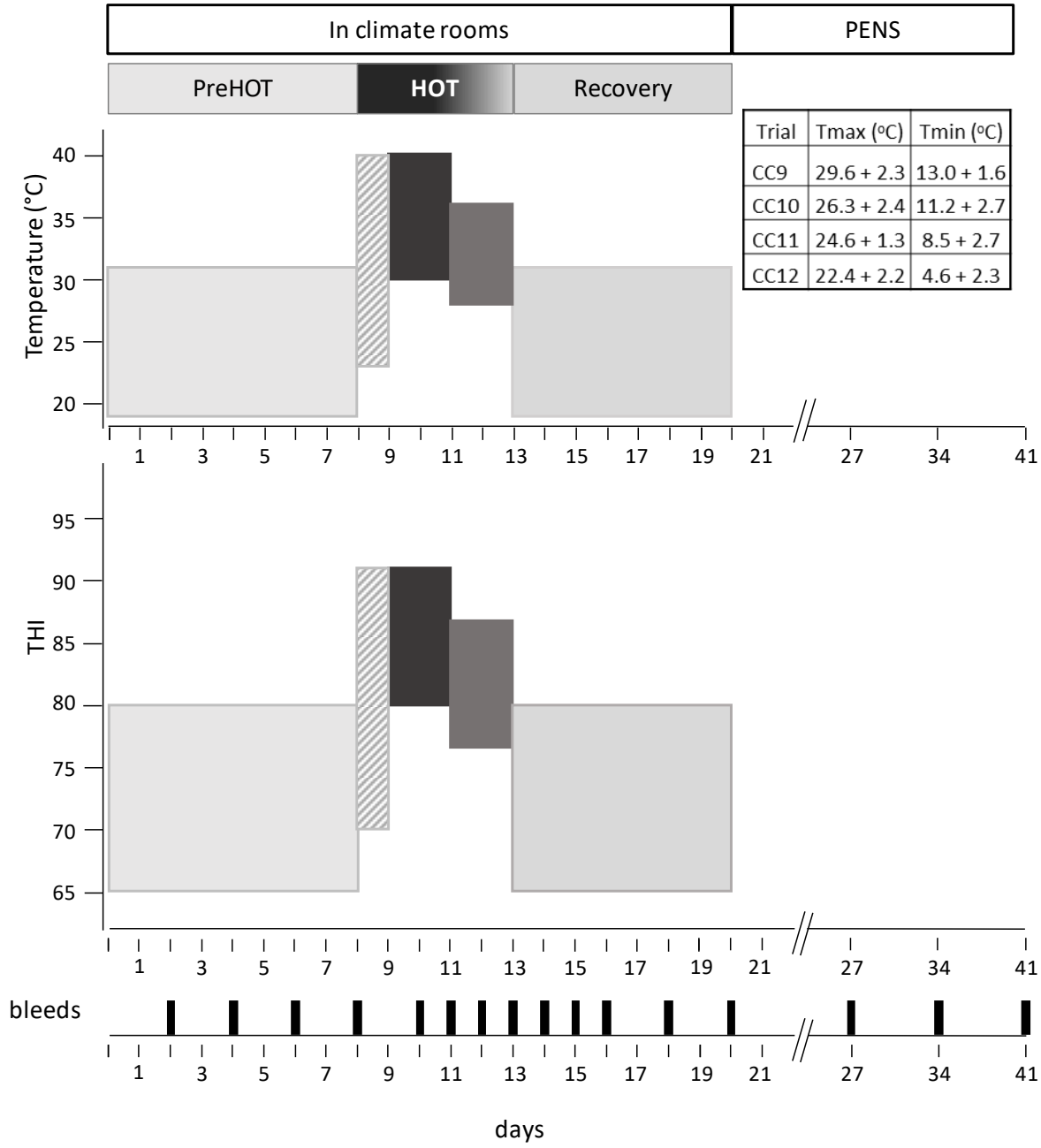

Fig. 52. The overall climatic regime of the high heat load challenge imposed during the CC9-12 trials. The range of the ambient temperature and Temperature-Humidity Index (THI), and duration of each period is depicted for the 41 days of the experiment. The PreHOT, HOT and Recovery periods were conducted in climate chambers. The PENs interval occurred in outdoor feedlot pens; the climatic conditions (mean ± SD) for each replicate is presented in the inserted tables. The PENs mean maximum and minimum temperatures for each cohort are based on Gatton BoM weather station data. Daily maximum and minimum THI data was not available. Blood sampling days are indicated also.

4.1.2.2 Performance measurements

Animals withdrawn from the trial

Based on previously developed physiological indicators for heat stress in these subjects, six animals were withdrawn from the trial over days 10-13 in the chambers. Three of the animals were from the Diet 1 group and three animals were from the Diet 2 group. All Diet 3 animals proceeded through the whole trial (see Fig. 53). The data collected at any time during the experiment from the withdrawn animals has been excluded from the analyses of the physiological, biochemical and haematological data presented hereafter.

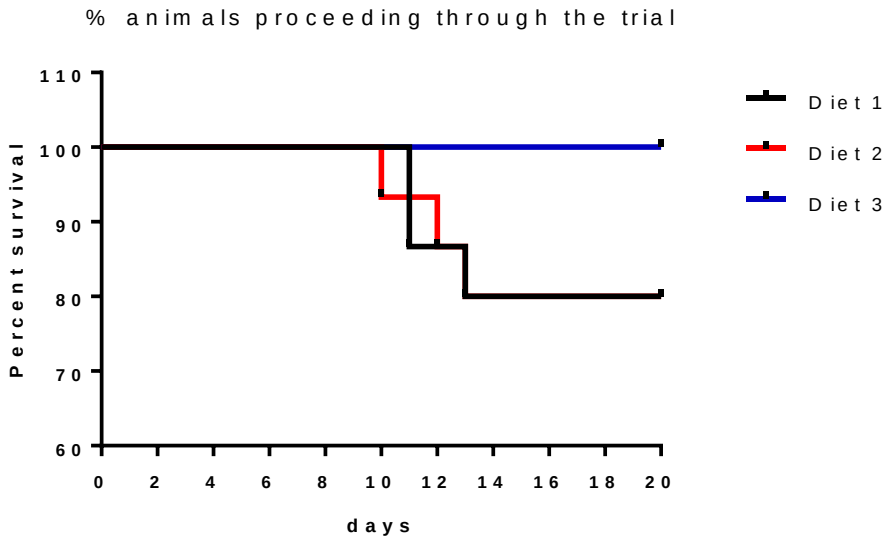

Fig. 53. Percent of animals in each diet that proceeded through the trial during and after the thermal challenge.

Dry matter intake

During PreHOT the daily mean DMI across the three diet groups were not different, with an overall mean of 9.73 kg/head/day. Changes in mean DMI of the diet groups were as expected with a notable decrease in intake as HOT was imposed on day 9 (Fig. 54, Table 4). DMI of all diet groups decreased from day 8 as heat load increased leading into HOT days. The overnight conditions on day 8 were slightly warmer (THI 69-71) compared with days 2 -7 (THI 64), this had an impact on the reduced DMI of all animals seen on day 8.

The lowest mean DMI occurred on days 10 and 11 when HOT conditions were maximised. Diet 2 animals presented the lowest mean DMI at 1.46 ± 0.41 and 0.85 ± 0.36 kg/head/day on days 10 and 11 respectively. These were significantly lower than the Diet 1 animals which had the highest mean DMI on days 10 and 11: 2.70 ± 0.41 kg/head/day ($p = 0.04$) and 2.37 ± 0.36 kg/head/day ($p = 0.006$).

On day 12, the mean DMI for all diet groups increased as heat load decreased. Diet 2 presented the lowest mean DMI on days 12 (2.53 ± 0.38 kg/head/day), 13 (3.46 ± 0.41 kg/head/day) and 16 (6.26 ± 0.28 kg/head/day). The markedly reduced DMI during HOT may have impeded some of the effects of the diets, particularly the very low intakes of Diet 2 in HOT days. All diet groups reached a similar intake level by day 19 on exit from the chambers.

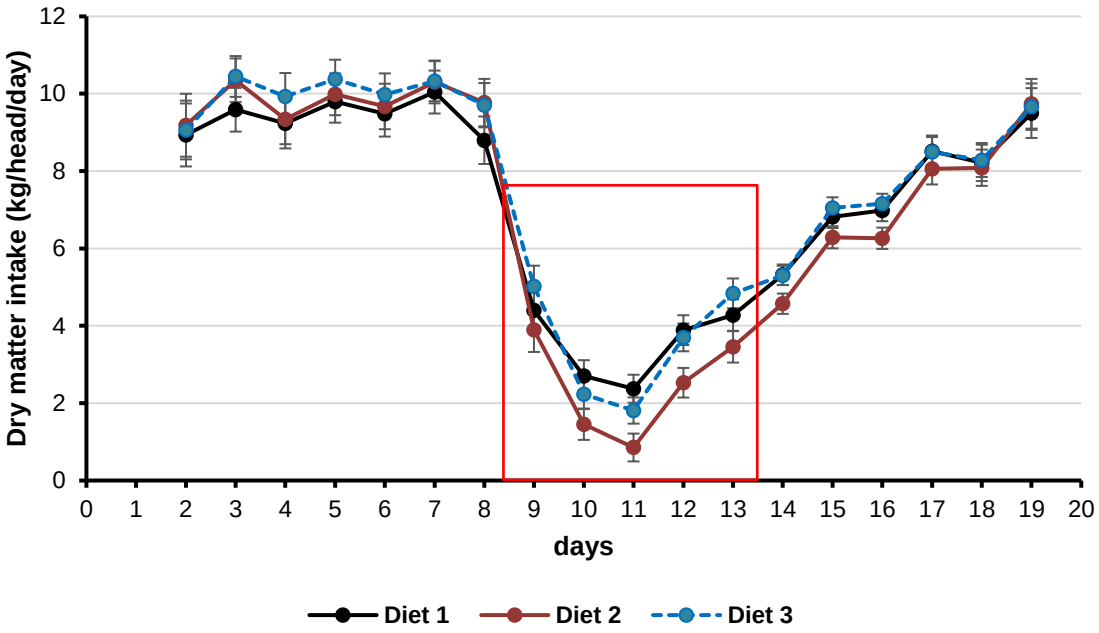

Fig. 54. Mean daily dry matter intake (kg/head/day) (± SEM) for all diet groups across all days in climate chambers. (HOT days highlighted in red box).

Table 4. Mean dry matter intake (kg/head/day) (± SEM) for all diet groups across all days in climate chambers.

Day	Climate	Diet 1	Diet 2	Diet 3	P value
2	PreHOT	8.93 ± 0.81	9.19 ± 0.81	9.06 ± 0.76	≥ 0.05
3	PreHOT	9.59 ± 0.57	10.35 ± 0.56	10.45 ± 0.53	≥ 0.05
4	PreHOT	9.23 ± 0.65	9.34 ± 0.64	9.93 ± 0.60	≥ 0.05
5	PreHOT	9.79 ± 0.54	9.99 ± 0.54	10.38 ± 0.51	≥ 0.05
6	PreHOT	9.48 ± 0.59	9.67 ± 0.59	9.98 ± 0.55	≥ 0.05
7	PreHOT	10.04 ± 0.56	10.31 ± 0.55	10.33 ± 0.52	≥ 0.05
8	PreHOT	8.80 ± 0.61	9.77 ± 0.61	9.70 ± 0.57	≥ 0.05
9	HOT	4.40 ± 0.58	3.90 ± 0.58	5.02 ± 0.54	≥ 0.05
10	HOT	2.70 ± 0.4^{a}	1.46 ± 0.41^{b}	2.23 ± 0.38^{ab}	**0.04**
11	HOT	2.37 ± 0.36^{a}	0.85 ± 0.36^{b}	1.81 ± 0.34^{ab}	**0.006**
12	HOT	3.89 ± 0.38^{ac}	2.53 ± 0.38^{bd}	3.70 ± 0.36^{ac}	**1 vs 2 - 0.02** **2 vs 3 - 0.03**
13	HOT	4.27 ± 0.41^{ab}	3.46 ± 0.41^{b}	4.84 ± 0.38^{a}	**0.03**
14	Recovery	5.32 ± 0.27	4.57 ± 0.26	5.30 ± 0.25	≥ 0.05
15	Recovery	6.82 ± 0.29	6.29 ± 0.29	7.05 ± 0.27	≥ 0.05
16	Recovery	6.98 ± 0.28^{ab}	6.26 ± 0.28^{b}	7.15 ± 0.26^{a}	**0.03**
17	Recovery	8.51 ± 0.41	8.06 ± 0.41	8.50 ± 0.38	≥ 0.05
18	Recovery	8.21 ± 0.47	8.09 ± 0.47	8.29 ± 0.44	≥ 0.05
19	Recovery	9.50 ± 0.64	9.74 ± 0.64	9.67 ± 0.60	≥ 0.05

Means with different superscripts are significantly different; means with no superscripts are not significantly different p ≥0.05.

During the PreHOT there was little difference between the DMI % of LW (live weight; p ≥ 0.05) of the diet groups. As HOT days commenced on day 9 there was a notable reduction in DMI % of LW for all treatment groups and this continued to decline until day 11 when the DMI % of LW was at its minimum with Diet 2 having the lowest value at 0.17 ± 0.09. This value was significantly lower than Diet 1 (0.42 ± 0.09; p = 0.04) but not Diet 3 (0.35 ± 0.08). On day 13 the Diet 3 animals presented the highest DMI % of LW at 0.91 ± 0.08. This value was significantly higher than Diet 2 (0.66 ± 0.09; p = 0.04) but not Diet 1 (0.78 ± 0.09). On day 12 the DMI % of LW began to increase for all treatment groups with the values returning to levels similar to PreHOT by day 19.

Live weights and carcass characteristics

The mean LWs of the three diet groups were similar at entry to feedlot, individual pens and CCR (Table 5). Diets 1 and 3 gained the least weight in the 19 days in the climate chambers. All treatments exhibited similar carcass characteristics (HCW, pH, EMA, P8 fat depth and dressing percentage) at slaughter (Table 5). Diet 3 animals received the highest carcass value price. This price is based on overall carcass grading, including fat (depth and colour), carcass weight and class and meat colour.

Table 5. Mean live weights (LW) and carcass characteristics (± SEM) across the experimental period.

	Diet 1	Diet 2	Diet 3
LW Feedlot entry (DOF 0) (kg)	433.77 ± 7.84	429.68 ± 7.90	429.33 ± 7.29
LW Individual pens entry (DOF 51) (kg)	522.17 ± 9.62	527.04 ± 9.69	530.11 ± 8.95
LW CCR entry (DOF 61) (kg)	537.13 ± 10.03	538.91 ± 10.11	542.55 ± 9.33
LW CCR exit (DOF 81) (kg)	540.72 ± 9.87	537.04 ± 9.74	549.01 ± 9.32
LW Feedlot exit (DOF 104) (kg)	607.73 ± 14.21	607.27 ± 14.32	611.37 ± 13.22
Hot carcass weight (kg)	327.99 ± 7.26	327.72 ± 7.32	331.11 ± 6.75
Dressing percentage (%)	54.04 ± 0.41	53.97 ± 0.41	54.22 ± 0.38
P8 fat depth (mm)	14.54 ± 1.20	17.00 ± 1.21	16.87 ± 1.12
Carcass pH	5.53 ± 0.01	5.53 ± 0.01	5.51 ± 0.01
Eye muscle area (cm^2)	79.63 ± 1.20	76.80 ± 1.21	79.69 ± 1.16
Carcass Value ($)	1686.71 ± 43.46	1689.36 ± 43.81	1716.35 ± 40.44

DOF – days on feed; CCR – climate chambers; means are not significantly different when p ≥0.05.

4.1.2.3 Physiological responses

Rumen pH

In PreHOT, rumen pH ranged from 6.15 – 6.64 for all diet groups. The rumen pH exhibited a clear diurnal rhythm with the daily maximum pH occurring at 0900 h and the minimum at 2300 h. The daily maximum coincides with morning feeding time. In PreHOT and Recovery, the animals consumed most of the feed offered at the specified feeding times (0900 and 1300 h). The change to the heat load ration for Diet 3 on day 7 did not alter rumen pH for this group (Fig. 55).

During days 11 and 12 (HOT) loss of the diurnal rhythm was observed for all diets. Diet 2 presented the highest mean pH during HOT (day 11) (Fig. 55). The increases in rumen pH seen in HOT days may reflect the increase in saliva production through drooling and panting under the HOT conditions, and/or the reduced fermentative activity in the rumen due to low feed intake.

In Recovery, the rumen pH ranged from 5.71 – 6.62. Also, pH returned to a diurnal rhythm albeit with an increased amplitude compared with PreHOT. The pH maxima recovered more quickly than the pH minima which remained at least 0.1 unit lower than the very stable

PreHOT rumen pH minima. This may indicate that it takes some days after the thermal challenge for rumen pH to stabilise. It may be related to the changes in DMI seen during Recovery and the steady return to PreHOT intakes (Fig. 54, Table 4). Based on the mean rumen pH values obtained no steers were not acidotic at any point during the trial and did not approach ruminal acidosis (≤ pH 5.5).

Rumen temperature

Overall, there were no differences ($p > 0.05$) in rumen temperature between the diet groups when in the climate chambers. The diet groups had similar daily diurnal patterns for rumen temperature during PreHOT. Mean rumen temperature reaching a maximum at 2100 h and a minimum at 1200 h (Fig. 56). The occurrence of the daily minimum rumen temperature at 1200 h is an unexpected outcome, as in previous experiments conducted under similar climatic conditions and heat load, the daily minimum has occurred prior to 0900 h. This may be the result of the mild heat load conditions experienced in PreHOT and Recovery. The transition to the heat load ration for Diet 3 on day 7 did not result in a change in rumen temperature for this group (Fig. 56).

At the onset of HOT, the diurnal rhythm was altered (Fig. 56) and did not return to the PreHOT rhythm in Recovery. This indicates that the animals may be readjusting their thermoregulation mechanisms during this period. When exposed to HOT, rumen temperature increased for all diet groups, with the Diet 3 animals having the greatest mean rumen temperature across HOT days (days 9 – 13) (Fig. 56). The Diet 1 animals reached their maximum rumen temperature on day 9 (41.71 ± 0.14°C), whereas Diet 2 and 3 animals reached their maximum rumen temperature on day 11 (41.65 ± 0.14°C and 41.92 ± 0.13°C respectively).

When conditions transitioned to Recovery on day 16, all diet groups presented a lower mean rumen temperature compared with PreHOT and HOT. This may be related to the reduced DMI in Recovery. The transition back to full finisher Diet on day 19 induced a slight increase in rumen temperature of all animals (Fig. 56).

Faecal scores

The faecal scores of the animals across the three periods in the climate chambers are presented in Fig. 57. There was little difference in the faecal scores of the diet groups. The faecal score of all treatments deteriorated in HOT days compared with PreHOT, with the minimum for all treatments reached on day 13. On day 13, the faecal score of the Diet 3 animals (2.94 ± 0.14) was lower than Diet 1 (3.37 ± 0.15; $p = 0.03$) but not Diet 2 (3.15 ± 0.15). When conditions cooled in Recovery, the faecal score of all treatments returned to scores similar to PreHOT. The deterioration in faecal scores of all diet groups on day 13 may reflect the slight increase in DMI on day 12. This may indicate that despite an increased appetite under cooler climatic conditions the rumen and gut may still be recovering from HOT conditions.

Water consumption

During a heat load event, there is generally an increase in water usage. This includes an increase in drinking behaviour and an increase in head dunking and splashing from water troughs. Overall, there was little difference between the diet groups in water consumption

(WC; $p \geq 0.05$) and across the 19 days in the climate chamber (Fig. 58). Diet 2 animals had the highest overall WC, with a notable increase in WC on day 9, coinciding with the onset of HOT conditions and the transition to the HL ration diet. There was a clear decrease in WC for Diet 3 when the HL ration was initially fed to this treatment group. The WC of Diet 3 continued to decline until day 11 when WC began to increase to quantities similar to that during PreHOT. The WC of Diet 1 remained relatively consistent with a slight increase in Recovery. In PreHOT, both Diets 2 and 3 displayed a distinct cyclic pattern in WC between days 2 - 7, on day 8 this pattern ceased to occur and did not re-occur.

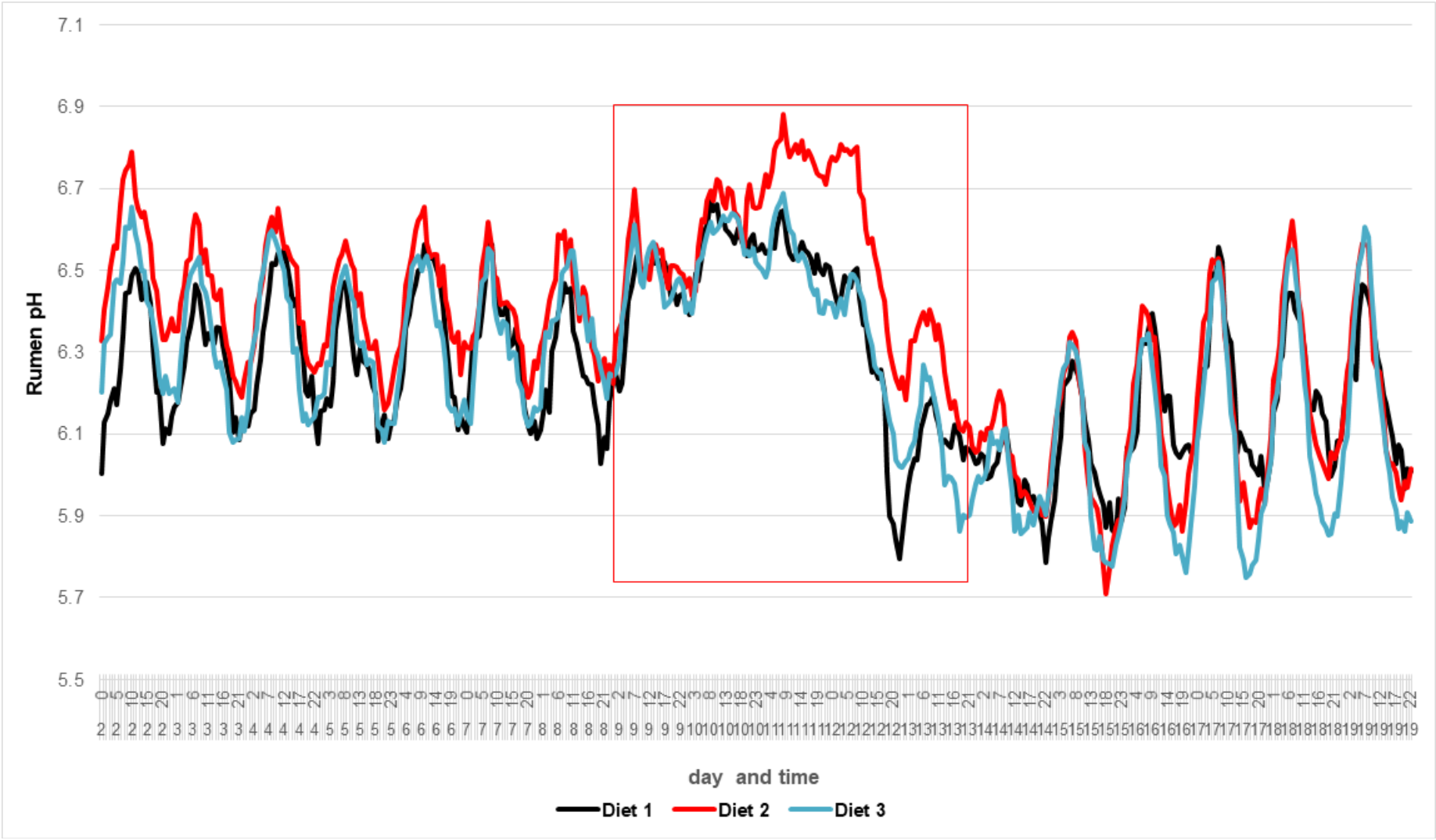

Fig. 55. Mean rumen pH for all diet groups across all days in climate chambers (HOT days highlighted in red box).

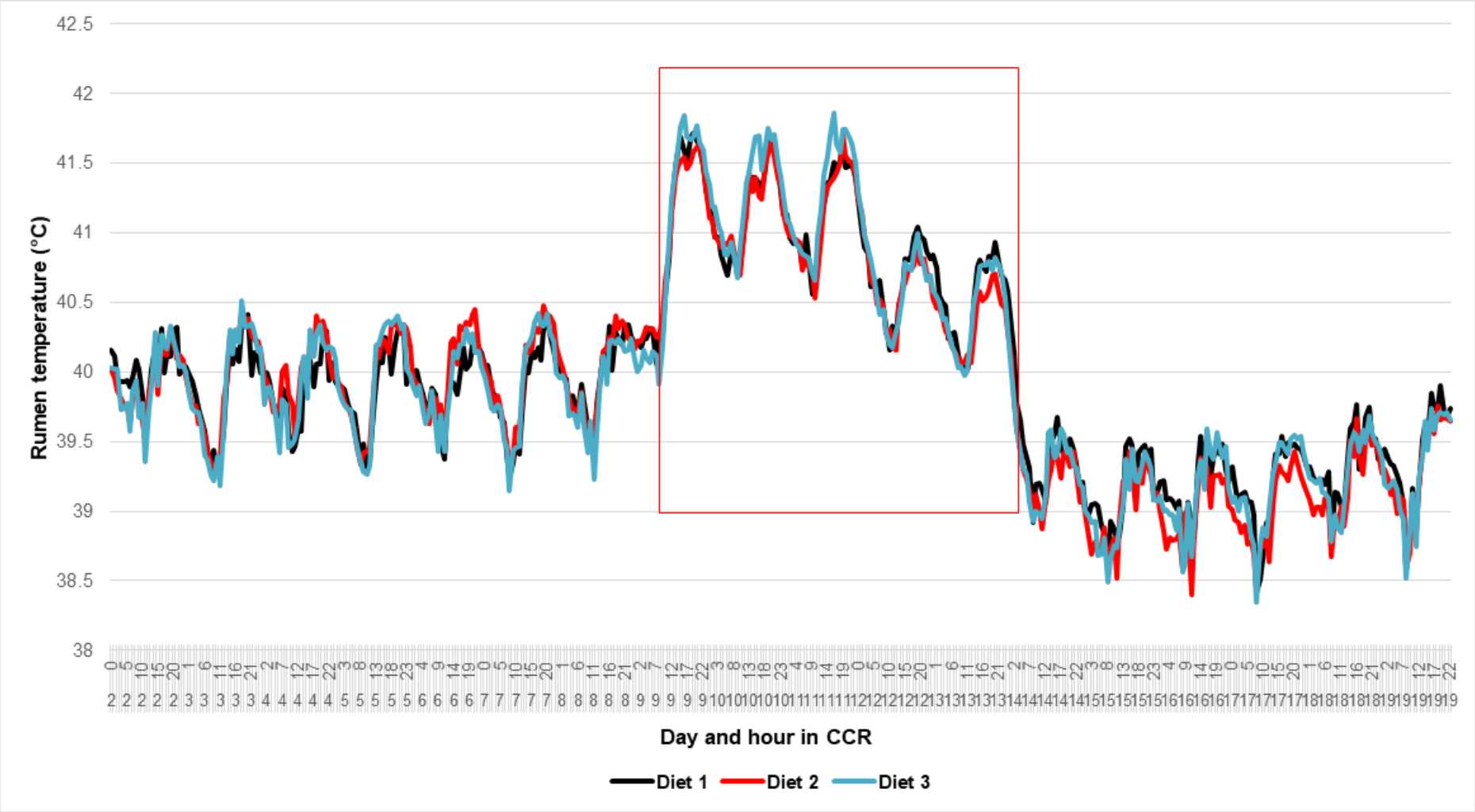

Fig. 56. Mean rumen temperature for all diet groups across all days in climate chambers (HOT days highlighted in red box*).*

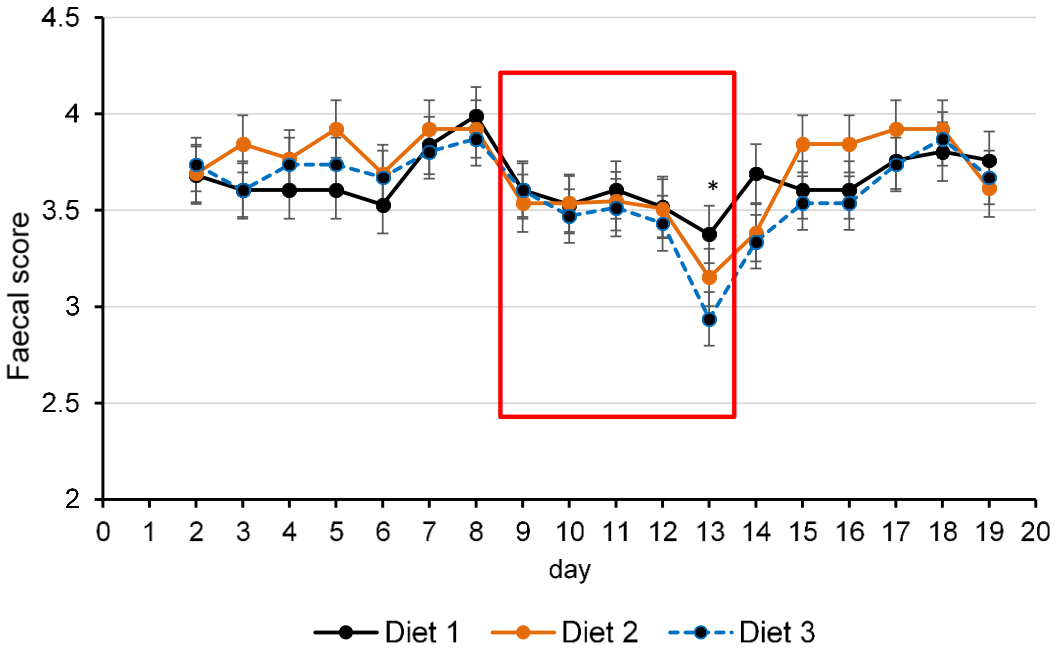

Fig. 57. Mean faecal score (± SEM) for all diet groups across all days in climate chambers (HOT days highlighted in red box) * indicates significant difference between diets.

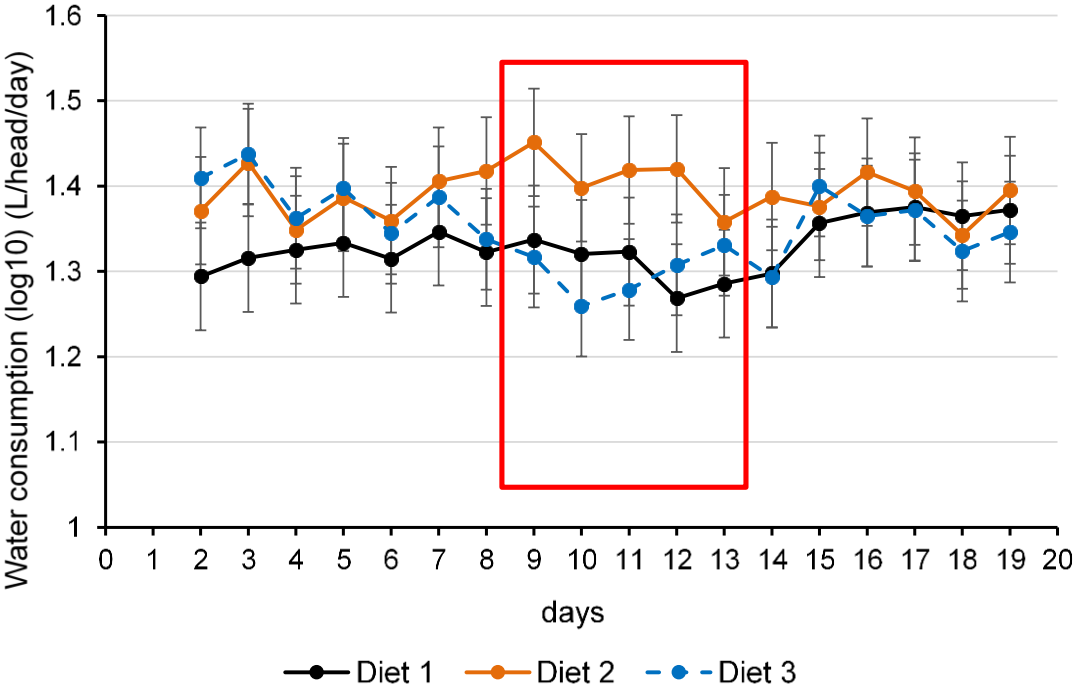

Fig. 58. Mean daily water consumption (WC) (log10) (L/head/day) of all diet groups across all days in climate chambers (HOT days highlighted in red box).

Over the 19 days in the chambers, Diet 1 animals had the lowest WC % of LW, with this WC rate remaining relatively consistent across this period (Table 6). Diets 2 and 3 had greater variability in their WC rate with Diet 2 animals increasing in WC % LW in HOT days and Diet 3 animals decreasing in WC % of LW in the same period. On day 11, the WC % of LW of Diet 3 was significantly lower than Diet 2 but not Diet 1. On day 12, WC % of LW of Diet 3 was significantly lower than Diet 2 but not Diet 1. As conditions cooled in Recovery, the Diet 3 animals began to increase WC % of LW and Diet 2 began to decrease their WC to return to levels similar to PreHOT.

Table 6. Mean daily water consumption (L/head/day) expressed as a percentage (%) of live weight (LW) (kg) of all diet groups across all days in climate chambers.

Day	Diet 1	Diet 2	Diet 3	P value
2	4.09 ± 0.88	4.66 ± 0.88	5.41 ± 0.83	≥ 0.05
3	4.31 ± 0.88	5.30 ± 0.88	5.63 ± 0.83	≥ 0.05
4	4.39 ± 0.88	4.62 ± 0.88	4.99 ± 0.83	≥ 0.05
5	4.52 ± 0.88	4.98 ± 0.88	5.43 ± 0.83	≥ 0.05
6	4.29 ± 0.88	4.86 ± 0.88	4.76 ± 0.83	≥ 0.05
7	4.62 ± 0.88	5.20 ± 0.88	5.09 ± 0.83	≥ 0.05
8	4.46 ± 0.88	5.44 ± 0.88	4.91 ± 0.83	≥ 0.05
9	4.76 ± 0.88	6.61 ± 0.88	4.44 ± 0.83	≥ 0.05
10	4.58 ± 0.88	5.87 ± 0.88	3.88 ± 0.83	≥ 0.05
11	4.59 ± 0.88^{ab}	6.76 ± 0.88^{a}	4.00 ± 0.83^{b}	2 vs 3 – 0.02
12	4.65 ± 0.88^{ab}	6.69 ± 0.88^{a}	4.11 ± 0.83^{b}	2 vs 3 – 0.04
13	4.74 ± 0.88	6.77 ± 0.88	4.56 ± 0.83	≥ 0.05
14	4.76 ± 0.88	5.51 ± 0.88	4.57 ± 0.83	≥ 0.05
15	5.22 ± 0.88	4.96 ± 0.88	5.59 ± 0.83	≥ 0.05
16	5.29 ± 0.88	5.61 ± 0.88	5.29 ± 0.83	≥ 0.05
17	5.33 ± 0.88	5.21 ± 0.88	5.25 ± 0.83	≥ 0.05
18	4.92 ± 0.88	4.60 ± 0.88	4.89 ± 0.83	≥ 0.05
19	4.95 ± 0.88	5.28 ± 0.88	4.62 ± 0.83	≥ 0.05

Means with different superscripts within a row are significantly different; means with no superscripts are not significantly different p ≥0.05.

A more informative measure on water consumption might be the water consumption:DMI ratio. This was similar for all treatment groups in PreHOT and Recovery periods (Fig. 59). When conditions changed to HOT on day 9 the WC:DMI ratio increased for all diet groups. This indicates that more water was consumed per kilogram of DMI. The peak in water consumption occurred on day 11 for all diet groups coincided with the decrease in DMI on this day (Fig. 54; Table 4). During the HOT period Diet 2 has a higher ratio than Diet 1 on

days 10 - 12 ($P \leq 0.001$) and Diet 3 on days 9 - 13 ($P \leq 0.03$). There was little difference between Diets 1 and 3 across HOT days (days 9 -13; $p > 0.05$).

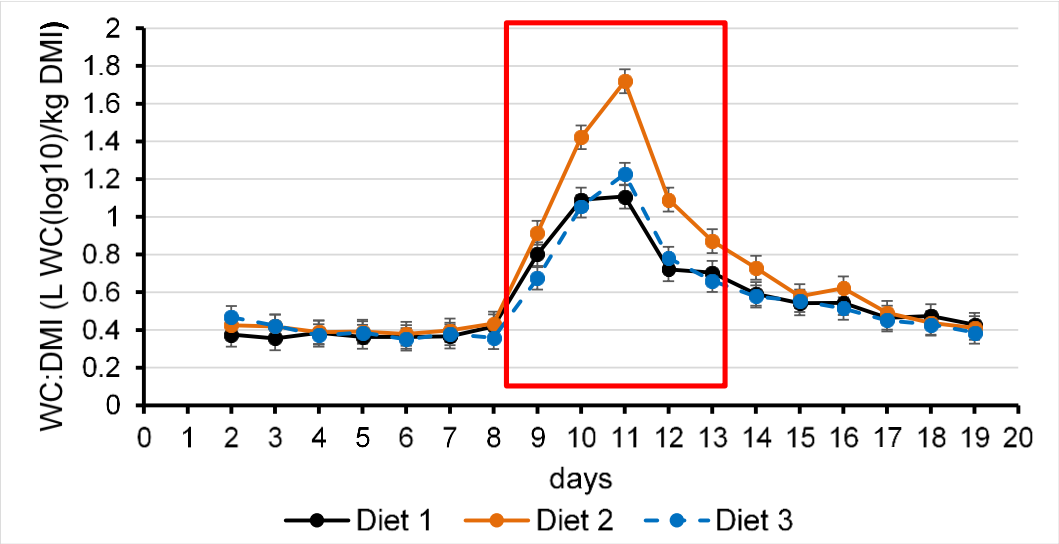

Fig. 59. Mean daily water consumption (WC) (log_{10}) (L/head/day) to dry matter intake (DMI) (kg/head/day) ratio of all diet groups across all days in climate chambers. *(*HOT days highlighted in red box).

Respiration rate

The respiration rate (RR) for all treatments increased during HOT when compared with PreHOT and Recovery (Fig. 60). When exposed to HOT, mean RR reached a peak on day 9 (Diet 1: 135.11 ± 1.81 bpm; Diet 2: 138.71 ± 1.79 bpm; Diet 3: 139.29 ± 1.71 bpm). From day 10, mean RR decreased indicating that the animals had begun to acclimatise to the ongoing conditions HOT conditions. There were small differences in RR between diet groups as indicated in Table 7. On days 5, 10, 11, 12 animals fed Diet 1 had the lowest mean RR.

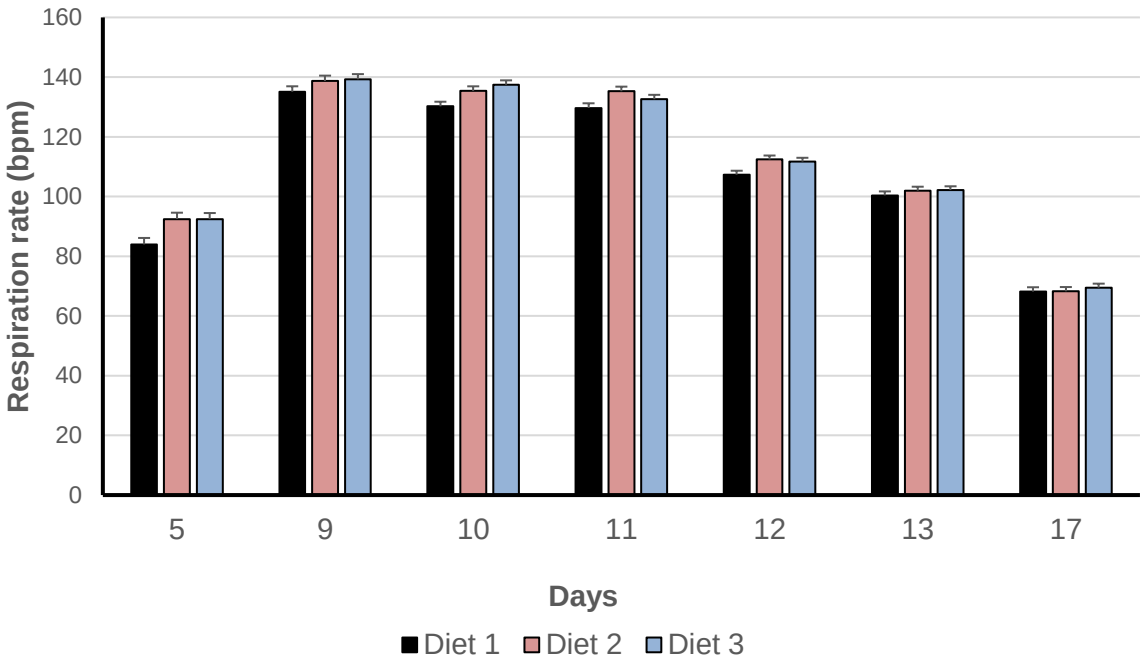

Fig. 60. Mean respiration rate (bpm) (± SEM) for all diet groups across HOT days and day 5 in PreHOT and day 17 in Recovery in climate chambers.
Note: Day 5 (PreHOT) and 17 (Recovery) and HOT days (day 9 - 13) had 24 hour observations.

Table 7. Mean respiration rate (bpm) (± SEM) for all diet groups across HOT days and day 5 in PreHOT and day 17 in Recovery in climate chambers.

Day	Respiration rate (bpm)			
	Diet 1	Diet 2	Diet 3	P value
5	83.97 ± 2.15^{ad}	92.46 ± 2.13^{bc}	92.44 ± 2.03^{bc}	1 vs 2 – 0.01 1 vs 3 – 0.01
9	135.11 ± 1.81	138.71 ± 1.79	139.29 ± 1.71	≥ 0.05
10	130.22 ± 1.55^{ad}	135.39 ± 1.53^{bc}	137.46 ± 1.50^{bc}	1 vs 2 – 0.02 1 vs 3 – 0.003
11	129.67 ± 1.57^{a}	135.26 ± 1.55^{b}	132.59 ± 1.48^{ab}	0.02
12	107.32 ± 1.32^{ad}	112.43 ± 1.3^{bc}	111.73 ± 1.25^{bc}	1 vs 2 – 0.01 1 vs 3 – 0.03
13	100.39 ± 1.33	101.98 ± 1.31	102.18 ± 1.25	≥ 0.05
17	68.18 ± 1.43	68.27 ± 1.41	69.47 ± 1.35	≥ 0.05

Means with different superscripts are significantly different; means with no superscripts are not significantly different (p ≥0.05). Note: Day 5 (PreHOT) and 17 (Recovery) and HOT days (day 9 - 13) had 24 hour observations.

Body Surface Temperatures

Generally, there was limited differences in surface temperatures between diet groups. Slight differences between diet groups were evident in lower leg surface temperature with Diet 3 having the highest daily mean lower leg temperature across five out of the six days ($p \leq 0.04$). The lack of differences in surface temperature between the diet groups concurs with the lack of differences seen in rumen temperature measurements.

Activity level measurements

Activity level as measured by the SmaXtec bolus is a direct reflection of the physical activity level of the animals. Overall, Diet 1 exhibited the lowest activity level with Diets 2 and 3 having a similar activity level across the 19 days in the climate chambers (Fig. 61). All diet groups increased in activity on day 9 at the onset of HOT conditions. This may reflect the animals adjusting their behaviour to cope with conditions and restlessness under the altered climatic conditions. From day 10 the activity level of all diet groups began to decline to reach activity levels slightly lower than PreHOT. A clear pattern in activity level can be seen in PreHOT and HOT with the daily minimum activity level occurring at 0600 – 0700 h and the daily maximum activity level occurring at 1500 – 1700 h in both of these periods. This pattern does not occur in Recovery indicating that the animals had not readjusted their behaviour to the PreHOT pattern of activity while in Recovery.

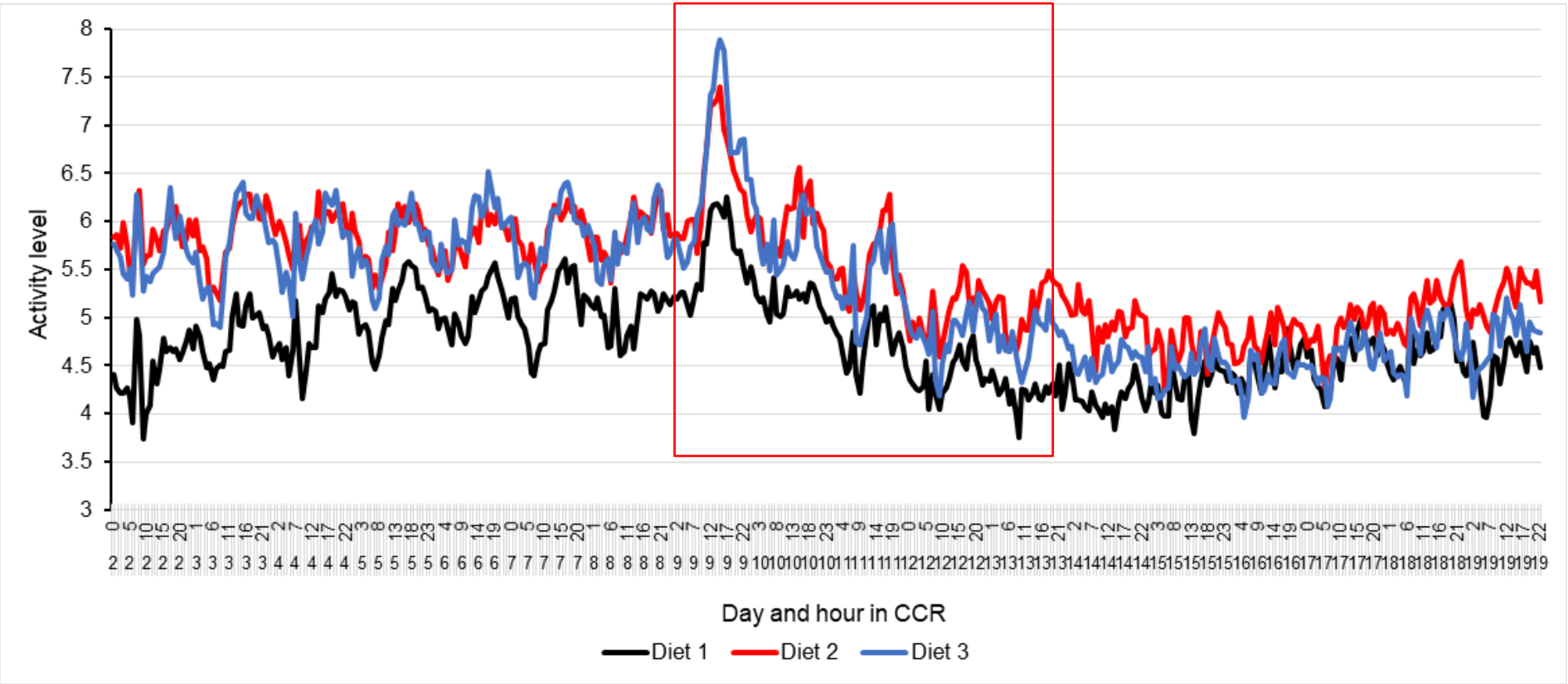

Fig. 61. Mean activity level (square root) for all diet groups across all days in climate chambers (HOT days highlighted in red box).

4.1.2.4 Discriminating blood plasma variables

Several plasma biochemical markers indicated that Diet 2 was the least favourable to metabolism during the thermal challenge and recovery.

The plasma biochemical measure that best discriminated between the Diets 1 and 3 and Diet 2 during Recovery was bilirubin. Over the course of the trial i.e. from PreHOT to PENs, Diet 2 bilirubin concentrations were very significantly different to Diets 1 ($p = 0.0020$) and Diet 3 ($p < 0.0001$). While all animals experienced a rise in mean plasma bilirubin concentrations during HOT, Diet 2 animals had significantly higher levels during HOT and Recovery (Fig. 62 A and B). During these periods, more Diet 2 animals had pathological levels of plasma bilirubin (i.e. > 8 µmol/L bilirubin) (Fig. 63). Bilirubin levels increase in plasma when there is increased red blood cell damage or liver dysfunction.

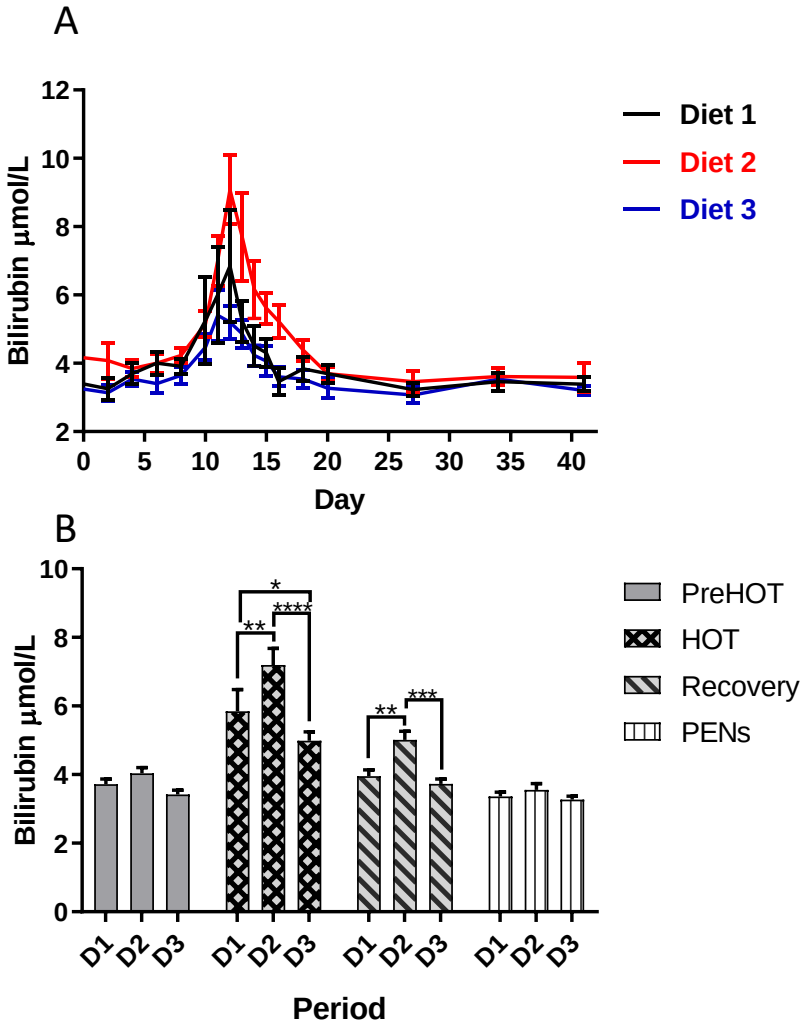

Fig. 62. Mean plasma bilirubin concentrations for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily plasma bilirubin concentrations for the Diets 1-3 groups over days 2-40. Panel B. Mean plasma bilirubin concentrations for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

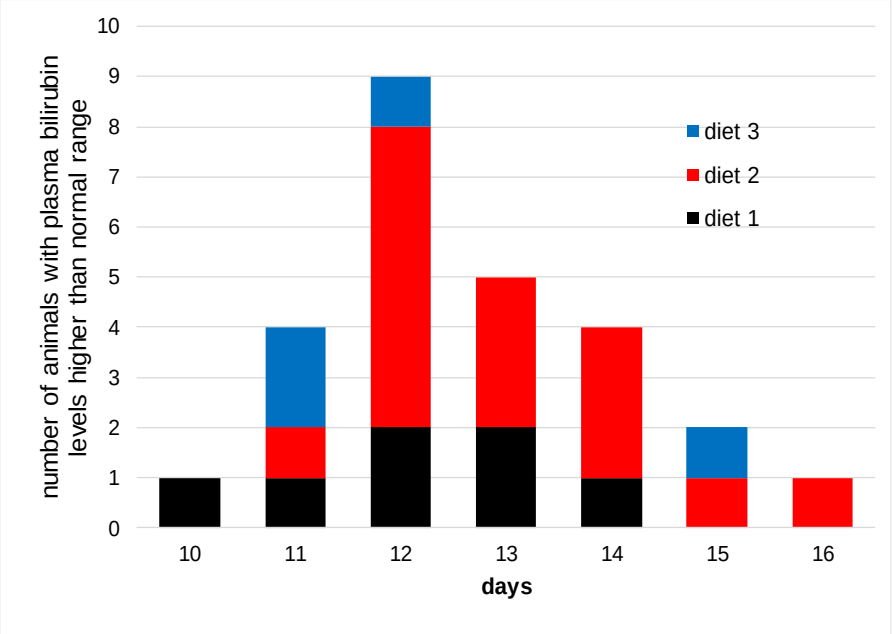

Fig. 63. Number of animals in each diet group with pathologically high plasma bilirubin concentrations (i.e. > 8 µM) during the HOT and Recovery periods.

There were differences in blood osmolality and plasma electrolyte concentrations between the diet groups during HOT and Recovery. These tended to be more subtle changes than that of the bilirubin concentrations. Mean plasma chloride concentrations rose during HOT for all diet groups reaching maximum concentrations over days 11-12 (Fig. 64A). For Diets 1 and 3, the maximum chloride concentration was ~4 mM higher than the PreHOT mean, whereas for Diet 2, the rise was only 2 mM (Fig. 64A and B). For Diets 1 and 2, the mean concentrations in Recovery were not different to the HOT means; Diet 3 saw a reduced mean chloride concentration in Recovery relative to its HOT mean. In summary, Diet 2 animals had a muted chloride response; Diet 3 animals saw a more rapid reduction of chloride concentration in Recovery. There were no differences between the groups in PENs.

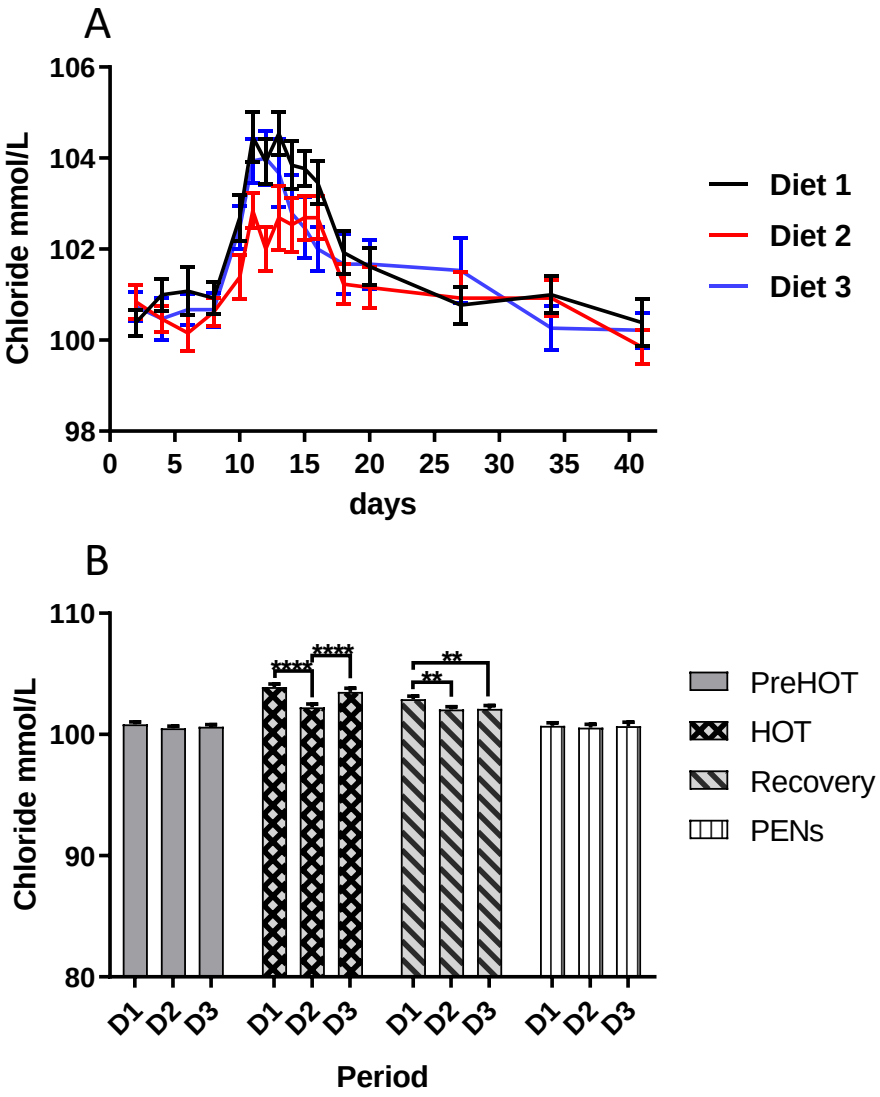

Fig. 64. Mean plasma chloride concentrations (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily plasma chloride concentrations for the Diets 1-3 groups over days 2-40. Panel B. Mean plasma chloride concentrations for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. **, p <0.01; ****, p <0.0001.

Mean osmolality was reduced in all diet groups during HOT, Recovery and PENs relative to their PreHOT means (Fig. 65A and B). After PreHOT, Diet 1 showed the least change in blood osmolality and was significantly different to the other diets over the course of the experiment, particularly during HOT and Recovery. Diets 2 and 3 saw the largest fall (~2.2%) in HOT and recovered somewhat in Recovery. There were no differences between the groups in PENs. Since the main contributor to blood osmolality is sodium concentration, differences between diets and periods in the mean plasma sodium concentration will be similar to mean osmolality.

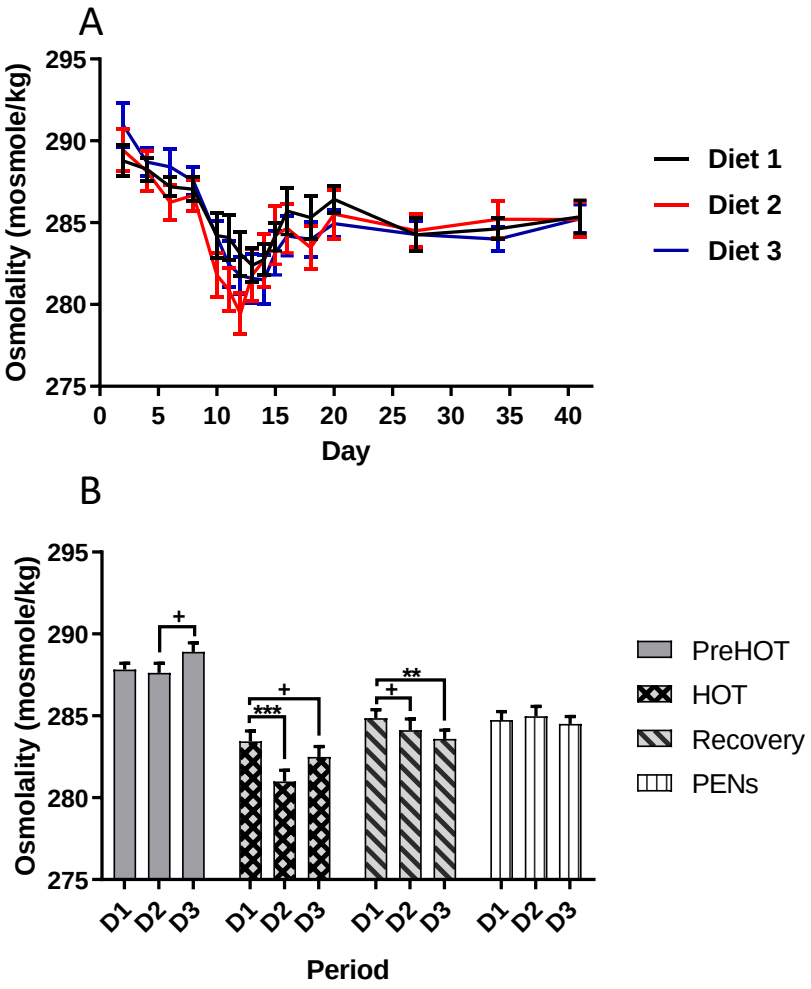

Fig. 65. Mean blood osmolalities (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily blood osmolalities for the Diets 1-3 groups over days 2-40. Panel B. Mean blood osmolalities for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. +, $p < 0.1$; **, $p < 0.01$; ***, $p < 0.001$.

While Diet 2 had the least increase in plasma chloride concentration during HOT, it had the greatest fall in plasma sodium concentration in this period at day 12 (Fig. 66A and B). The Diet 2 animals also arrived at their lowest sodium concentrations 24 h ahead of the Diet 1 and 3 minima (Fig. 66A). Diet 3 had the lowest sodium concentration during Recovery (Fig. 66B). When in PENs, there were no differences between the groups.

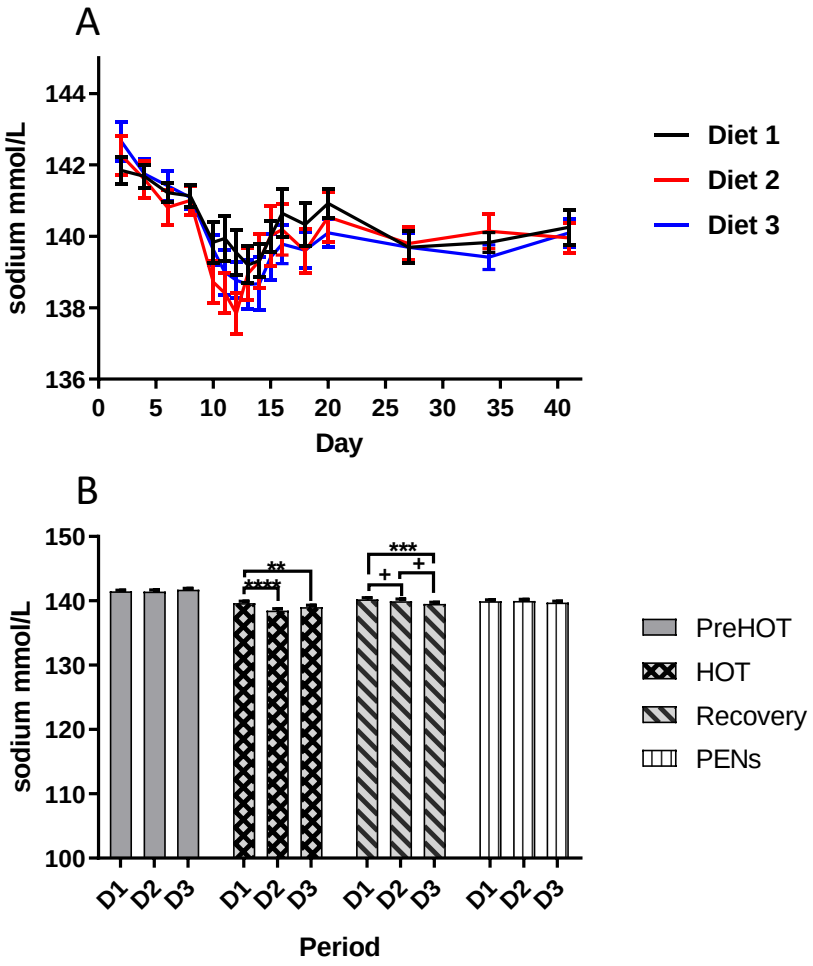

Fig. 66. Mean plasma sodium concentrations (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily plasma sodium concentrations for the Diets 1-3 groups over days 2-40. Panel B. Mean plasma sodium concentrations for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. +, p <0.1; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Consistent with CC7 and 8, the HOT period saw a marked rise in plasma creatinine concentrations. Peak creatinine was achieved on day 12 with the daily mean concentrations of 0.168-0.181 mM: right at the upper limit of the reference range for cattle. During HOT all of the nine animals in the Diet 2 group that completed the trial had pathologically high levels of plasma creatinine, whereas about half of the Diet 1 and 3 animals had such high levels of plasma creatinine. Diet 3 produced the lowest mean concentration during HOT and was significantly different to Diet 2 ($p = 0.022$) which produced the highest mean plasma creatinine concentration (Fig. 67A and B).

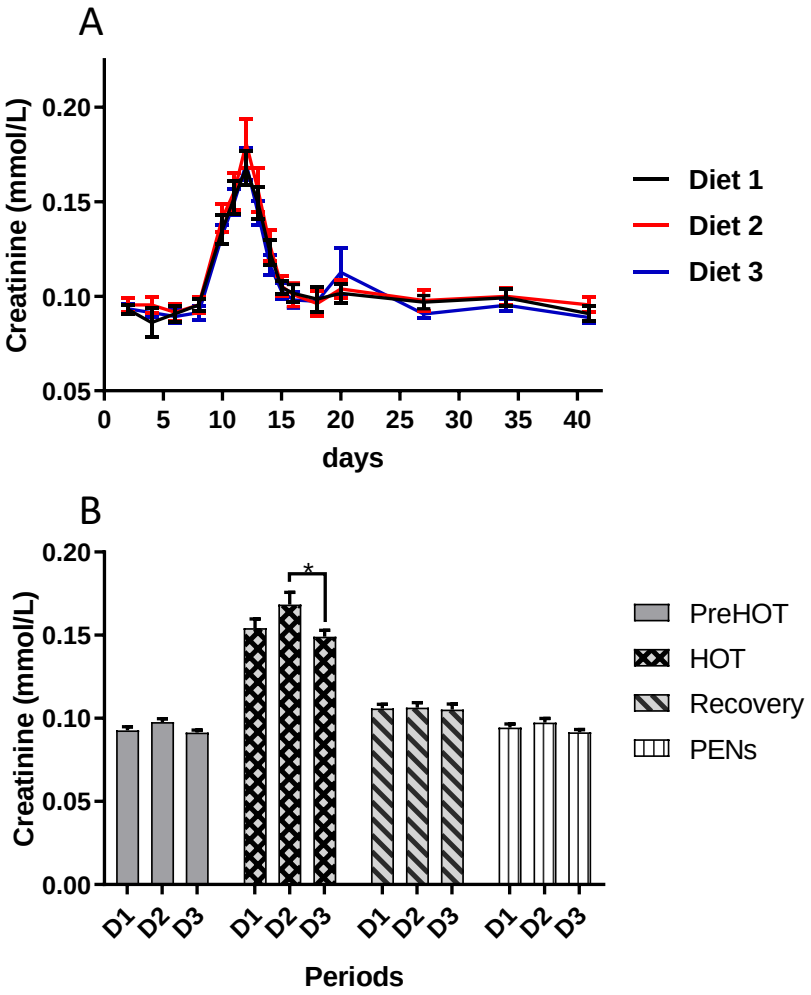

Fig. 67. Mean plasma creatinine concentrations (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily plasma creatinine concentrations for the Diets 1-3 groups over days 2-40. Panel B. Mean plasma creatinine concentrations for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. *, $p < 0.05$.

Similarly, the plasma urea levels rose with onset of HOT, but were followed by a dramatic fall in Recovery to below the PreHOT and PENs means (Fig. 68A and B). Peak urea was reached on day 12. Seven of the nine animals that proceeded through Diet 2 had pathologically high plasma urea concentration during HOT, while less than half of the animals in Diets 1 and 3 reached such levels. Diet 2 experienced the largest rise in mean urea concentration during HOT but also the largest fall in Recovery. Diet 2 was significantly different to Diet 1 during HOT. During Recovery, Diet 2 was significantly different to Diet 3.

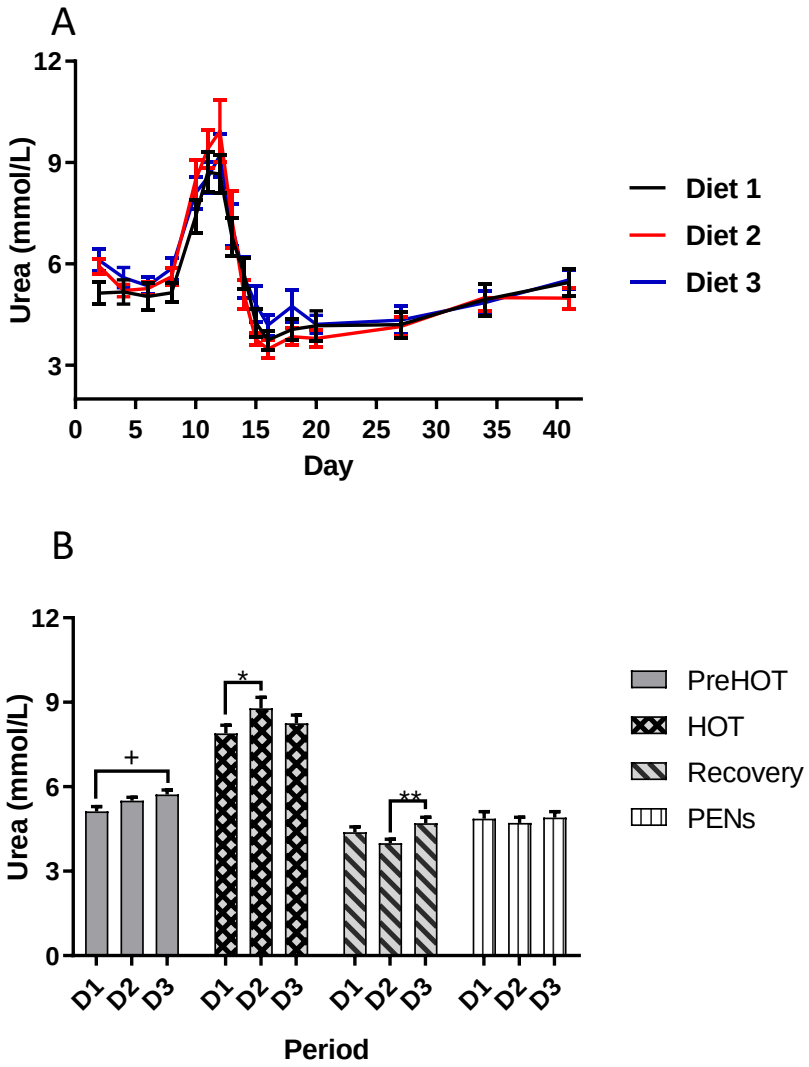

Fig. 68. Mean plasma urea concentrations (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily plasma urea concentrations for the Diets 1-3 groups over days 2-40. Panel B. Mean plasma urea concentrations for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. +, p <0.1; *, p <0.05; **, p <0.01.

The liver enzyme activities, particularly AST and GLDH, indicated a greater level of hepatic disturbance in Diet 2 animals during Recovery. Daily mean AST activity maxima for Diets 1 and 3 occurred on day 16 at ~200-210 IU/L; the maximum for Diet 2 was detected on day 18 (Fig. 69A), recorded at 313 IU/L. Clearly, the Diet 2 AST mean plasma activity during Recovery was very significantly increased relative to the Diet 1 and 3 means (Fig. 69A and B). The Diet 2 GLDH mean plasma activity during Recovery has a similar relationship with the Diet 1 and 3 means (Fig. 70). The maximum activities for Diets 1 and 3 occurred on day 16. The maximum activity for Diet 2 occurred on day 18 with 159 IU/L.

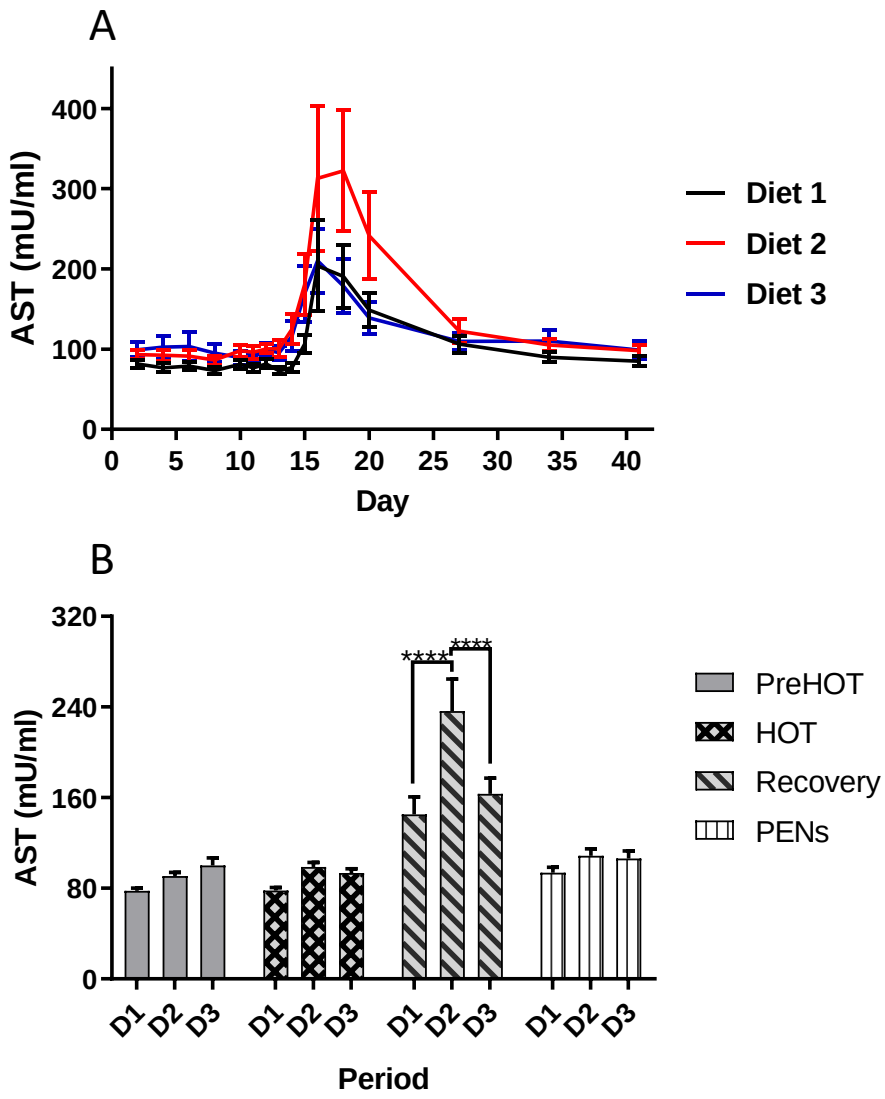

Fig. 69. Mean plasma AST activity (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily plasma AST activities for the Diets 1-3 groups over days 2-40. Panel B. Mean plasma AST activities for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. ****, p <0.0001.

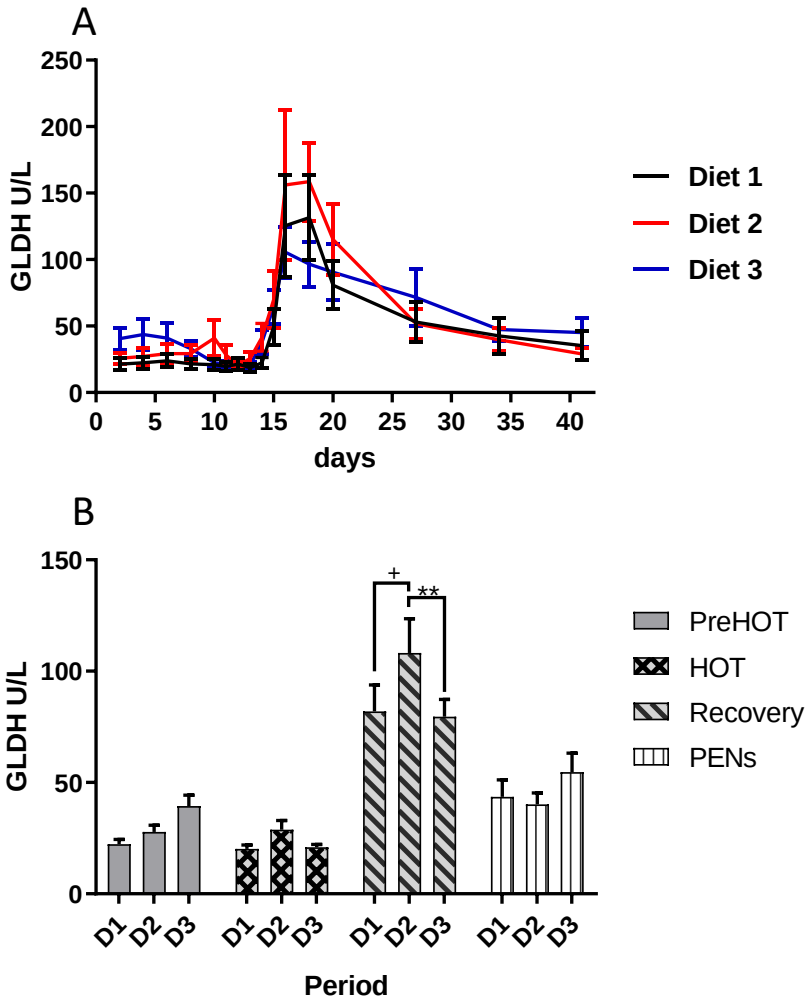

Fig. 70. Mean plasma GLDH activity (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily plasma GLDH activities for the Diets 1-3 groups over days 2-40. Panel B. Mean plasma GLDH activities for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. +, $p < 0.1$; **, $p < 0.01$.

A rise in plasma GGT activity was evident for all diet groups in Recovery which persisted into PENs (Fig. 71A). Each diet group reached a maximum on day 20, the last day of Recovery. Diet 1 saw the smallest rise compared to the other groups, and Diets 2 and 3 had higher mean GGT activity in Recovery and PENs compared to Diet 1 (Fig. 71B). However, both Diets 2 and 3 showed higher mean GGT activity in PreHOT. When looking within the Diet groups, Diets 2 and 3 experienced the more pronounced increases in mean plasma GGT activities relative to their PreHOT and HOT means (Fig. 71C). It is likely that these two diets were associated with greater release of GGT into the circulation during Recovery and PENs.

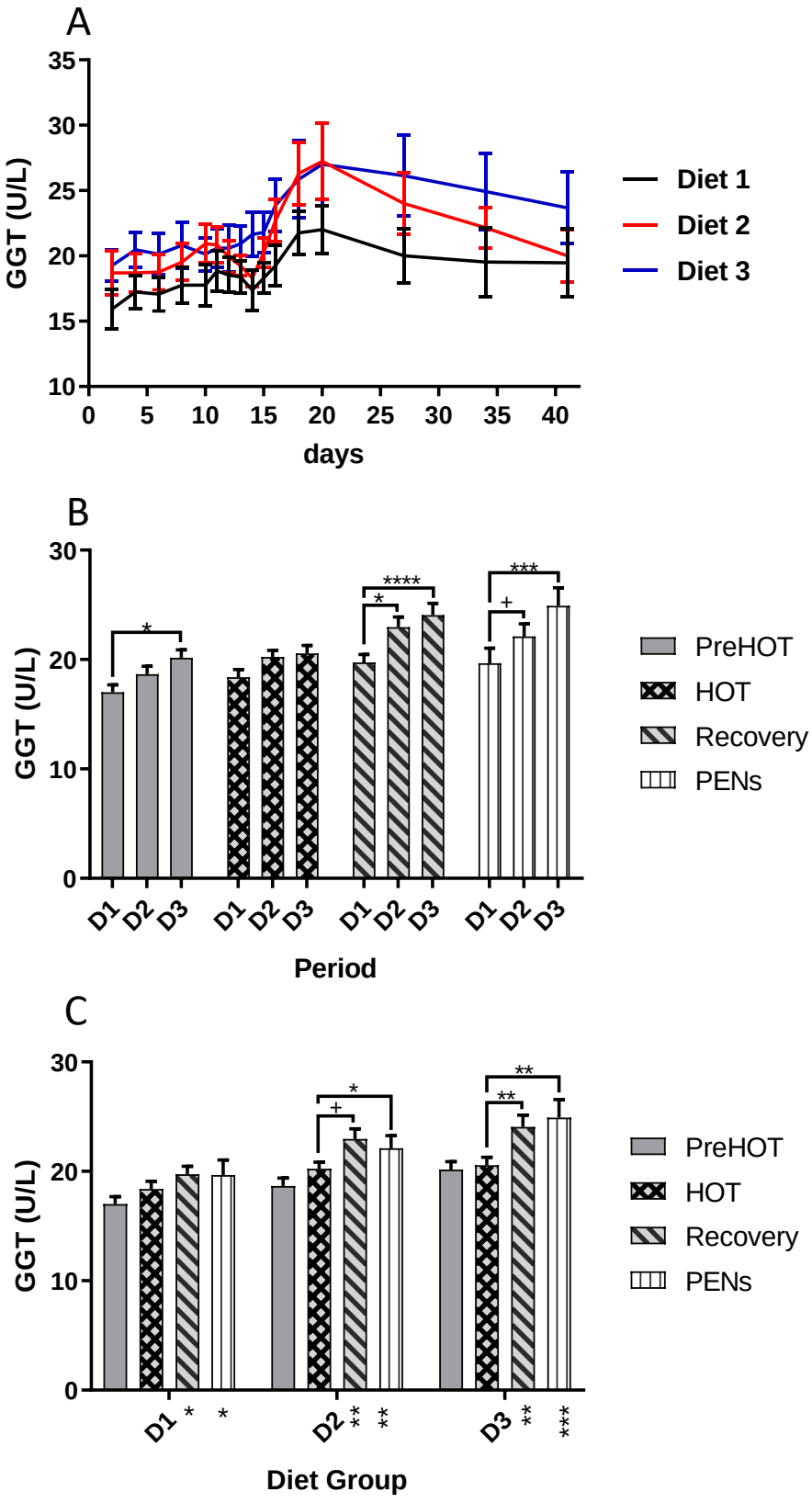

Fig. 71. Mean plasma GGT activity (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily plasma AST activities for the Diets 1-3 groups over days 2-40. Panel B. Mean plasma

GGT activities for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. Panel C. Mean plasma GGT activities for each diet group (D) over the four periods (PreHOT, HOT, Recovery, PENs). The asterisks below the x-axis denotes the level of significant difference with the PreHOT mean. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Some of the leucocyte counts were discriminant between diet group during HOT and Recovery. The total white blood cell count rose during HOT and fell in Recovery (Fig. 72A). The highest counts for all groups occurred on day 12 and the lowest on day 16. The means of HOT and Recovery periods revealed that Diet 2 was associated with lowest counts compared to Diets 1 and 3 (Fig. 72B). The differences in the white blood cell response between the diets is more obvious when inspecting the neutrophil counts (Fig. 73A and B). The highest neutrophil counts for all groups occurred on day 12 and the lowest on day 16 for Diets 1 and 2, and over days 15 and 16 for Diet 3. The Diet 2 animals had the lowest count during HOT and Recovery when compared to Diets 1 and 3.

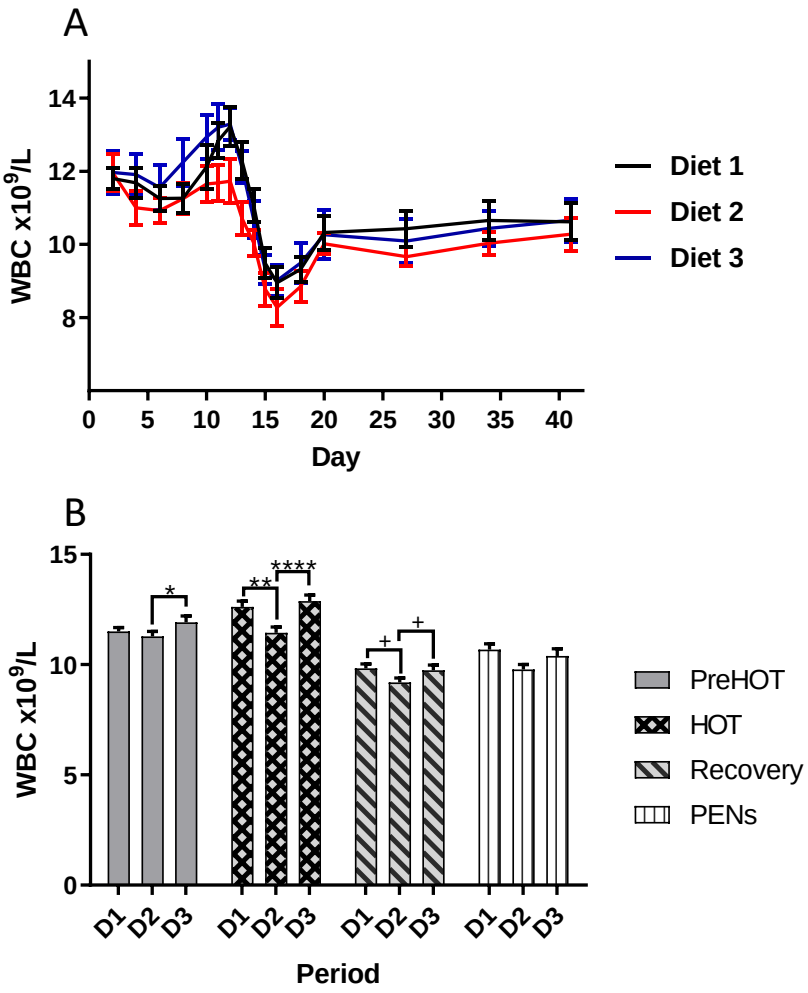

Fig. 72. Mean white blood cell (WBC) count (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily WBC count for the Diets 1-3 groups over days 2-40. Panel B. Mean WBC count

for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. +, p <0.1; *, p <0.05; **, p <0.01; ****, p <0.0001.

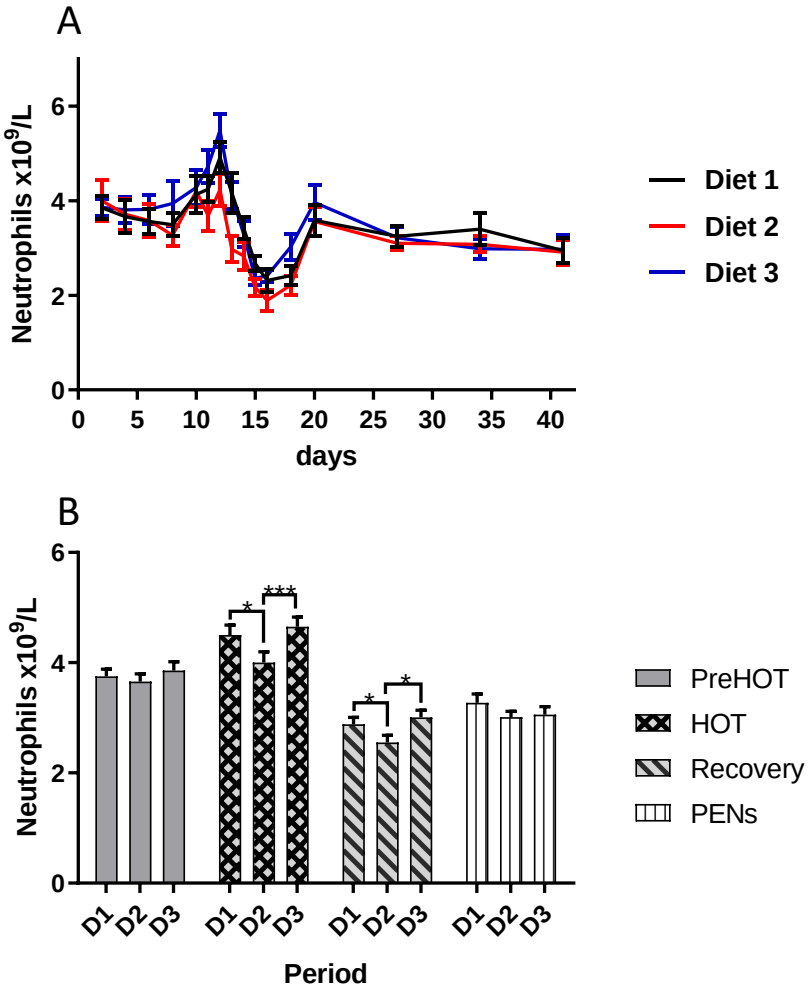

Fig. 73. Mean neutrophil count (± SEM) for Diets 1-3 during the three periods in climate chambers and in feedlot pens (PENs) following the 21 days in chambers. Panel A. Mean daily neutrophil count for the Diets 1-3 groups over days 2-40. Panel B. Mean neutrophil count for each period (PreHOT, HOT, Recovery, PENs) and for each diet (D) group. *, p <0.05; ***, p <0.001.

4.1.2.5 Discussion and Conclusions

Overall, Diet 2 has the least potential as a means of ameliorating the effects of high heat load on feedlot cattle. Diet 2 animals had the lowest DMI and DMI % of live weight, and the highest water consumption for feed intake during the HOT period. The rumen pH was higher during the last days of the HOT period relative to their Diet 1 and 3 counterparts. Diet 2 induced high plasma bilirubin concentrations during HOT. Some animals of this group exhibited pathologically high levels implicating liver dysfunction and/or increased RBC damage. The Diet 2 cohort showed altered plasma electrolyte responses relative to other

diet groups, suggesting that renal compensation was affected. Altered kidney function may be the reason for the higher plasma creatinine and urea in the Diet 2 animals during HOT and/or Recovery. The high plasma levels in the Diet 2 animals of two 'liver' enzymes during Recovery are further evidence of liver dysfunction and/or damage. Diet 2 animals also showed an altered leucocyte response with reduced circulating lymphocytes and neutrophils during HOT and possibly Recovery.

Diets 1 and 3 are difficult to separate by either physiological or metabolic responses. The clearest differences are that Diet 3 animals had the lowest mean plasma bilirubin concentrations during HOT implying the least hepatic perturbation. Moreover, Diet 3 was the only group where all animals completed the trial.

4.1.3 Acid-base balance in high heat load climate chamber study (CC9-12)

4.1.3.1 Introduction

This trial saw the introduction of blood gas parameters to ascertain changes in blood pH, lactate and oxygenation during high heat load. These parameters can assist in identifying acid-base balance changes during the treatment periods.

Blood pH: Blood pH is maintained in the very narrow range of 7.35-7.45. Fig. 74 presents the mean blood pH changes observed during the trial, and includes the data from the two bleeds prior to entry into the climate chambers (days -19 and -5). Overall, mean blood pH was lower in the climate chambers than prior to the chambers (PreCCR) and in PENs. The PreCCR and PENs mean pH approached an alkalotic pH.

The low mean blood pH recorded on day 2 (pH 7.36) was the first bleed in the climate chambers but the mean returned to pH 7.40 by day 8 (Fig.74); the mean pH for the PreHOT period was 7.39 ± 0.003 (Table 8). The HOT mean pH was 7.40 ± 0.003, but four animals displayed consistent pH >7.45. The Recovery period saw the mean pH veer toward acidotic (pH 7.37 ± 0.003); the daily means for days 15 and 16 was 7.36. Eight animals experienced persistent acidosis (pH<7.35) in this period. On return to PENs, the mean blood pH returned to similar values as PreCCR (Fig. 74). On days 34 and 42, the mean pH was 7.436. Nine animals had registered at least two readings of pH >7.45.

Blood pCO_2: During PreHOT, the pCO_2 levels were stable with a mean of 42.1 ± 0.29 mm Hg (Fig. 74). The onset of HOT induced a rapid fall to approximately 31.9 mm Hg by day 12. The mean for the HOT period was 34.1 mm Hg (Table 8), thus the mean change in pCO_2 level was -8.0 mm Hg. After day 12, levels rose rapidly to produce a mean of 42.3 mm Hg. Whilst in PENs, the pCO_2 levels steadied at 40.0 mm Hg.

Blood bicarbonate (HCO_3^-): Blood bicarbonate concentration generally follows that of pCO_2 as CO_2 and HCO_3^- are constantly interconverted in the blood. The PreHOT mean HCO_3^- concentration was 24.9 ± 0.19 mM, although the day 2 mean (the first bleed in the chambers) was notably lower (23.8 mM). The concentration fell by 4.5 mM to 20.4 mM during HOT; the lowest concentration, 19.0 mM, was achieved on day 12. The Recovery period saw a return to 24.5 mM on day 20 (Fig. 74). The blood bicarbonate mean concentrations were stable from day 20 except for a rise to 26.9 mM by day 43 (last bleed in PENs).

Blood lactate: Fig. 74 displays the mean blood lactate concentrations from day -19 to day 41. The high daily mean blood lactate concentration observed on day 2 (4.35 mM) coincided with the first bleed in the climate chambers (Fig. 74). 13 animals experienced persistent lactic acidosis (< 4.0 mM) over the PreHOT period, contributing to the PreHOT mean of 3.54 mM. Overall, mean blood lactate concentration was significantly lower from the onset of hot conditions and thereafter, relative to PreCCR and PreHOT. The daily mean blood lactate concentrations fell the first two days of HOT and remained at 1.8 – 2.0 mM over days 11-15, that is, well into the Recovery period. The low blood lactate means were achieved despite seven animals continuing to experience higher lactic acid levels during most of the HOT period, and five animals with high levels during Recovery. The range of the median values over days 11-15 was 1.2 - 1.3 mM. There was a small increase on days 16 and 18, however, there was no significant differences in mean blood lactate concentrations between the HOT, Recovery and PENs periods (Table 8).

Blood Anion Gap (AG): The AG is calculated from the plasma sodium, potassium, bicarbonate and chloride concentrations. Increased AG indicates the presence of other, usually organic, anions. During PreCCR and PENs, the mean AG were 17.5 and 17.2 mM respectively. The day 2 bleed witnessed a substantial rise in mean AG which then recovered to 18.8 mM by day 8. Day 12 during HOT saw a small rise also, subsequent to a fall during Recovery (Fig. 74). The mean AG during PreHOT and HOT were significantly different to those of the Recovery and PENs periods (Table 8).

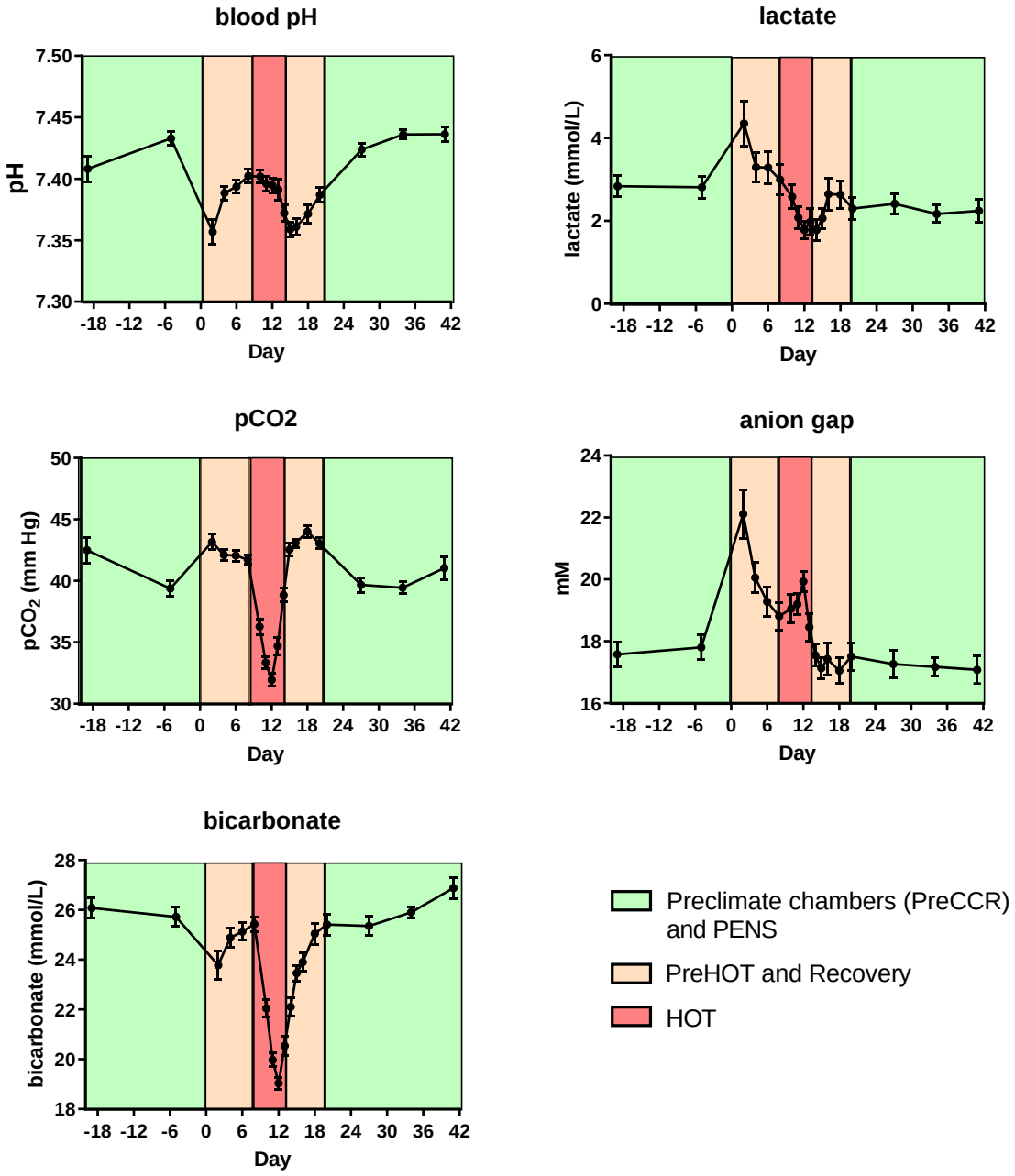

Fig. 74. The course of blood gas analytes (mean ± SEM), pH, lactate, pCO_2 and bicarbonate, and the anion gap (AG) during the sequential Pre-climate chamber (PreCCR), PreHOT, HOT, Recovery and PENs periods. The animals were housed in individual outdoor pens during the PreCCR and feedlot pens during PENs periods. Data from all cohorts and diets are pooled.

Table 8. Mean blood gas parameters (± SEM) for each period of the experiment. The data is calculated from only those animals that completed the 42 days of the experiment.

Blood pH		p-values			
period	Mean ± SEM	PreHOT	HOT	Recovery	PENs
PreHOT	7.388 ± 0.003		0.0765	<0.0001	<0.0001
HOT	7.396 ± 0.003	0.0765		<0.0001	<0.0001
Recovery	7.370 ± 0.003	<0.0001	<0.0001		<0.0001
PENs	7.432 ± 0.004	<0.0001	<0.0001	<0.0001	
pCO_2	mm Hg				
PreHOT	42.1 ± 0.29		<0.0001	n.s.	<0.0001
HOT	34.1 ± 0.28	<0.0001		<0.0001	<0.0001
Recovery	42.3 ± 0.31	n.s.	<0.0001		<0.0001
PENs	40.0 ± 0.32	<0.0001	<0.0001	<0.0001	
HCO_3^-	mM				
PreHOT	24.9 ± 0.19		<0.0001	0.0008	0.0001
HOT	20.4 ± 0.18	<0.0001		<0.0001	<0.0001
Recovery	24.0 ± 0.17	0.0008	<0.0001		<0.0001
PENs	26.0 ± 0.22	0.0001	<0.0001	<0.0001	
Anion Gap	mM				
PreHOT	20.0 ± 0.22		0.0105	<0.0001	<0.0001
HOT	19.2 ± 0.21	0.0105		<0.0001	<0.0001
Recovery	17.4 ± 0.19	<0.0001	<0.0001		n.s.
PENs	17.2 ± 0.24	<0.0001	<0.0001	n.s.	
Base Excess	mEq/L				
PreHOT	-0.22 ± 0.20		<0.0001	<0.0001	<0.0001
HOT	-3.56 ± 0.20	<0.0001		<0.0001	<0.0001
Recovery	-1.31 ± 0.18	<0.0001	<0.0001		<0.0001
PENs	1.68 ± 0.23	<0.0001	<0.0001	<0.0001	
lactate	mM				
PreHOT	3.54 ± 0.15		<0.0001	<0.0001	<0.0001
HOT	2.15 ± 0.17	<0.0001		n.s.	n.s.
Recovery	2.35 ± 0.13	<0.0001	n.s.		n.s.
PENs	2.30 ± 0.17	<0.0001	n.s.	n.s.	
Na^+	mM				
PreHOT	141.6 ± 0.14		<0.0001	<0.0001	<0.0001
HOT	139.1 ± 0.14	<0.0001		<0.0001	0.0003
Recovery	139.9 ± 0.12	<0.0001	<0.0001		n.s.
PENs	139.9 ± 0.16	<0.0001	0.0003	n.s.	
Cl^-	mM				
PreHOT	100.7 ± 0.14		<0.0001	<0.0001	n.s.
HOT	103.2 ± 0.14	<0.0001		<0.0001	<0.0001
Recovery	102.3 ± 0.13	<0.0001	<0.0001		<0.0001
PENs	100.6 ± 0.16	n.s.	<0.0001	<0.0001	

n.s. – not significant

Blood Base Excess (BE): This is a calculated index that attempts to estimate the acid-base balance based on the bicarbonate concentration. The normal range is between +2 and -2; an acidotic condition will yield a negative BE. The mean BE prior to entry to the climate chambers (PreCCR) was 1.5 mEq/L, and fell to -1.8 mEq/L by day 2 in the chambers (Fig. 75). The BE recovered by day 8 (~0.6 mEq/L). The PreHOT mean was -0.22 mEq/L (Table 12). The onset of HOT induced a rapid fall to -4.7 mEq/L by day 12, which was followed by a steady rise during Recovery to ~0.2 mEq/L by day 20. The HOT and Recovery mean BE were -3.6 and -1.3 respectively. Return to the PreCCR mean only occurred on day 34 (in PENs) (Fig. 75). The means of each period were highly significantly different to each other.

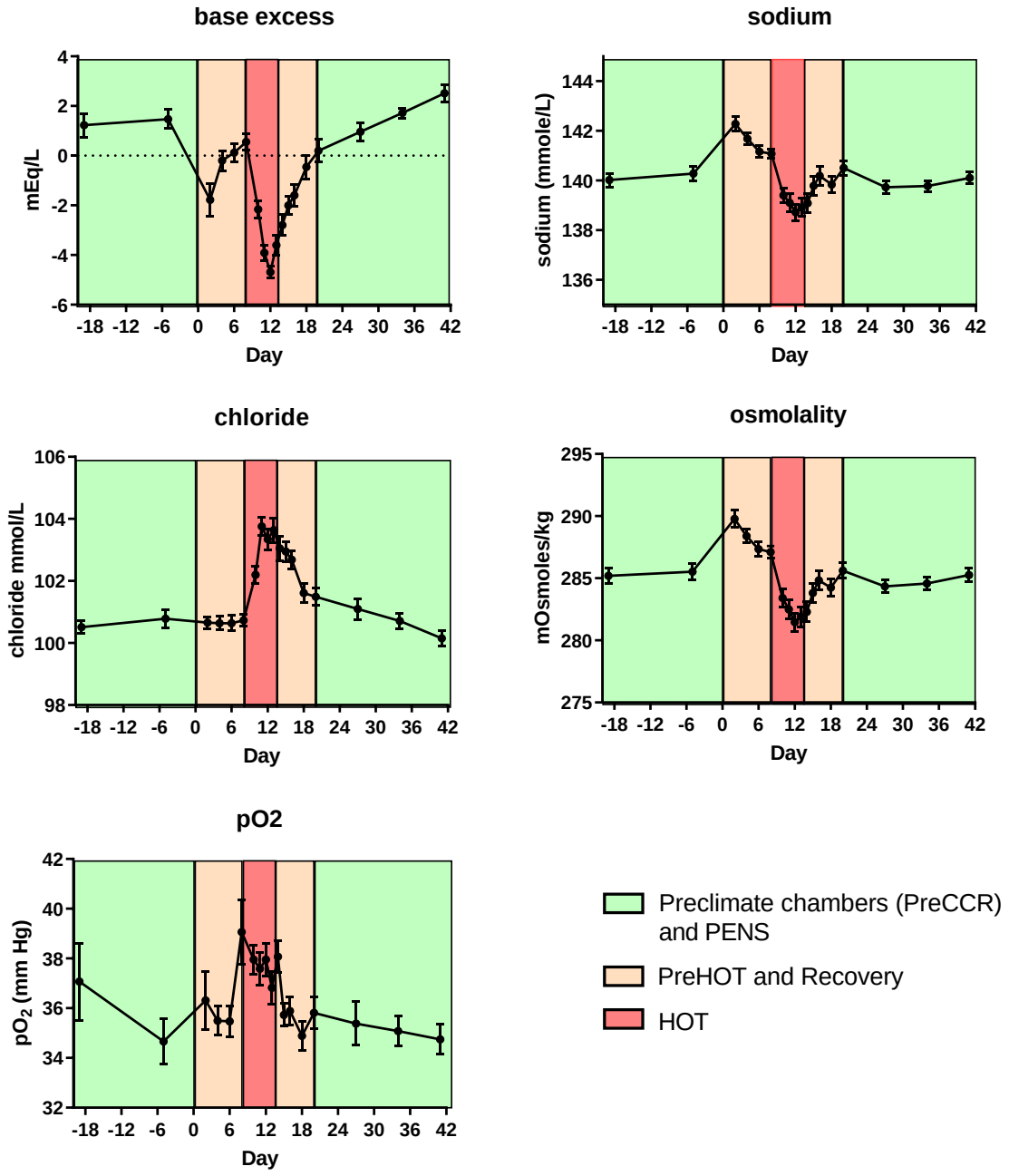

Fig. 75. The course of blood gas analytes (mean ± SEM), Base Excess, sodium, chloride, osmolality, and pO_2 during the sequential Pre-climate chamber (PreCCR), PreHOT, HOT,

Recovery and PENs periods. The animals were housed in individual outdoor pens during the PreCCR and feedlot pens during PENs periods. Data from all replicates and diets are pooled.

4.1.3.2 Discussion

The blood gas data collected during the course of the experiment has produced evidence of the very powerful homeostatic mechanisms in place to maintain blood pH and electrolyte balance. During the course of the experiment, the animals are subject to two different acid-base changes bordering on disorders (summarised in Table 9).

PreHOT: When compared to the PreCCR data, there is a clear effect of the change of housing and handling on many blood parameters. This was very apparent on day 2 (the first bleed in chambers) which was characterised by reduced blood pH, increased pCO_2 levels, but reduced bicarbonate concentrations accompanied by increased lactic acid concentrations, a high AG and negative BE. The animals are likely to have experienced a transient combined metabolic and respiratory acidosis, and then required some days to return to a metabolically stable state. This seems to have been achieved by day 8, the last day of the PreHOT period.

HOT: During high heat load, the animals experienced normal blood pH but markedly reduced blood pCO_2 levels and HCO_3^- concentrations indicating a respiratory alkalosis. However, the mean HCO_3^- concentration (20.4 mM) indicated that the mean pCO_2 level should be 38 mm Hg, not the low 34 mm Hg observed. This difference points to the involvement of a metabolic acidosis where the protons from organic acids are buffered by HCO_3^-, thus removing more HCO_3^- from the blood. To maintain charge neutrality, Cl^- ions are retained by the kidneys, leading to increased plasma chloride concentration. The mean BE was -3.6 mEq/L during HOT indicating the depth of the loss of HCO_3^- ions. The high AG despite the increased plasma chloride indicates the presence of organic acids. Just less than 50% of animals presented with persistently high AG indicating buffering by unmeasured anions. A third of these may be explained by high plasma lactic acid concentrations. Oxidation of lipids to β-hydroxybutyrate and acetoacetate during HOT will have contributed to the high AG also. The two percent reduction in osmolality is likely to have been driven by increased water consumption and the fall in plasma sodium and glucose concentrations, although the increased urea concentration would have compensated slightly. Blood oxygenation increased slightly and may reflect a decreased metabolic rate (Fig. 75).

Day 12 was the most extreme point of the metabolic response to high heat load. Mean blood pH was near neutral despite the reduction in both pCO_2 and HCO_3^- levels, and a large negative BE. Most of the cattle experienced a mixed acid-base disorder with a hyperchloremia; that is, a near neutral pH, markedly reduced pCO_2 levels, HCO_3^- concentrations and BE, with increased chloride concentrations and slightly increased AG. A transient increase in mean plasma potassium concentration probably reflected the kidneys' effort to generate HCO_3^- which involves retention of K^+ ions.

Recovery: While the mean blood pH was still within normal limits, it tended toward acidotic and lagged the recovery to near normal pCO_2 and HCO_3^- levels by two days. About 20% of the animals were acidotic (blood pH <7.35). The pCO_2 levels peaked on day 18 and the

mean was slightly increased although not significantly relative to the PreHOT mean. The elevated pCO_2 levels probably reflected the very low respiration rates observed during Recovery (< 70 bpm). The plasma bicarbonate concentration was normal. Together these results suggest that animals underwent a respiratory acidosis somewhat augmented in some animals by a metabolic acidosis as indicated by the elevated blood lactate concentrations and high AG values, and recovering BE.

PENs: Interestingly, the mean pH during this period stabilised at a relatively high pH, and the mean blood HCO_3^- concentration and BE maintained upward trajectories.

Table 9. A synopsis of the acid-base imbalances identified during the course of this heat load trial (based on the descriptors given by Reddi, 2014).

Day/period	Mixed acid-base disorder	pH	pCO_2	HCO_3^-	Cl^-	AG	BE
PreHOT Day 2	Mixed metabolic acidosis and respiratory acidosis	↓	↑/N	↓	N	↑	↓
HOT Day 12	Mixed respiratory alkalosis and metabolic acidosis	N	↓↓	↓↓	↑	↑	↓↓
Recovery Day 18	Mixed metabolic acidosis and respiratory acidosis	↓	↑/N	N	N	N	↓/N

N – no change; AG - anion gap; BE – base excess

4.1.3.3 Conclusions

The blood gas data from this trial has certainly extended our observations of the metabolic effects of high heat load and matured our understanding. Throughout the periods in the climate chambers and into PENs, the cattle are dealing with complex changes in acid-base balance.

A surprising finding is the very clear response to the change of conditions and management on entry into the climate chambers. There was a marked although transient metabolic and respiratory acidosis. Fortunately, the cattle achieved an acid-base stability of some level prior to the onset of the heat load challenge.

The increased respiration in response to high heat load directs the acid-base balance toward a respiratory alkalosis, however, a metabolic acidosis was also evident in many animals. Renal, and probably hepatic compensation mechanisms maintained blood pH near neutral. However, the kidney, lungs and liver are functioning under reduced blood flow and reduced feed intake.

In recovery, the acid-base balance swings back toward a chronic metabolic and respiratory acidosis. The acidic blood pH does not fully recover till some time back in PENs and on full feed.

4.1.4 Preliminary comparison of the two high heat load trials, CC7+8 and CC9-12

4.1.4.1 Introduction

The major difference in the climatic conditions between these two trials were parameters for the PreHOT and Recovery periods. The CC7+8 trial commenced with PreHOT set to thermoneutral conditions and finished with thermoneutral conditions in Recovery. The CC9-12 trial attempted to replicate a heatwave event during typical summer conditions. The maximum and minimum temperature and relative humidity parameters for PreHOT and Recovery periods were based on mid-summer data for Dalby, Qld. Additionally, during the HOT period, the CC9-12 regime maintained maximum THI at 90 for 3 days prior to the step down in temperatures conducted in both regimes.

In the following preliminary comparison between the two regimes some physiological and blood parameters are compared to see if the warmer conditions during the PreHOT and Recovery periods, and the extra days at maximally hot conditions in HOT, changed the responses in CC9-12. Moreover, the similarity of the responses to high heat load despite the slightly differing conditions should become apparent. For the purposes of this brief analysis, the data from all animals that proceeded to completion within the trial are pooled.

4.1.4.2 Physiological parameters

The DMI during PreHOT was ~12 kg/head/day for CC7+8, whereas it was ~10 kg/head/day for CC9-12 (Fig. 76). This reduced intake is likely to reflect the higher heat load in the CC9-12 animals. The minimum DMI was achieved on the third day into the HOT period for both trials; the DMI fell to ~3.6 kg/head/day in CC7+8, and ~1.7 kg/head/day for CC9-12. The actual quantum falls in both groups was ~ 8.3 kg/head/day. CC9-12 experienced a more linear response to recovery of feed intake (Fig. 76).

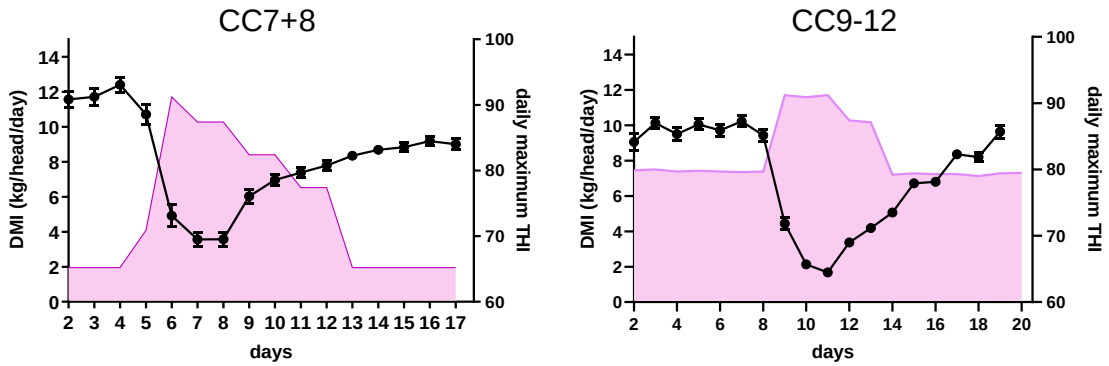

Fig. 76. Mean daily DMI (± SEM) for all animals in the CC7+8 and CC9-12 trials. The daily maximum THI is indicated by the filled area for each trial.

Respiration rate was affected by the differing conditions in PreHOT and Recovery also (Fig. 77). Mean respiration rates in CC7+8 were consistently lower than those of CC9-12 in all periods in the climate chambers, especially during PreHOT and Recovery.

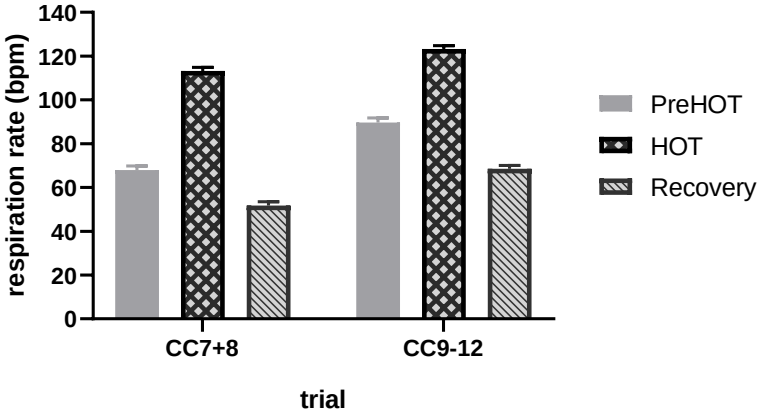

Fig. 77. Comparison of the mean respiration rates (± SEM) for the CC7+ 8 and CC9-12 trials during the three periods in the climate chambers (PreHOT, HOT and Recovery).

Rumen temperatures were impacted by the conditions in PreHOT and Recovery. For CC7+8, the diurnal range in PreHOT was 38.2 – 39.2°C, and in Recovery, the range was 37.8 – 38.8°C (Fig. 16). The PreHOT diurnal range for CC9-12 was a full degree higher at 39.2 - 40.4°C, despite the lower feed intake. In Recovery, the diurnal range of CC9-12 was 38.5 – 39.7°C (refer to Fig. 56). The mean maximum rumen temperature for CC7+8, 41.2°C, was recorded the first day of HOT, day 7, which had the highest environmental temperatures and THI. The mean maximum rumen temperatures for CC9-12 were 41.6 – 41.8°C, occurring during days 9-11.

4.1.4.3 Metabolic responses

Liver function

The amount of liver enzyme released into the blood increased during Recovery for both trials. This is particularly evident for AST and GLDH (Fig. 78A and B) where the AST and GLDH activities are very stable during PreHOT and HOT for both trials. As soon as conditions moderate, the plasma levels of both these enzymes rise, but more dramatically for CC9-12. The highest mean daily AST activity for CC9-12 was 2-fold higher than that of CC7+8. Likewise, the highest mean daily GLDH activity for CC9-12 was 3.3-fold higher than that of CC7+8.

In Recovery, elevated levels of daily mean plasma GGT activity were obvious for CC9-12, but not so clear in CC7+8 (Fig 78C). In both trials, the peak of GGT activity occurred on the last day of Recovery. This trajectory differed from the AST and GLDH trajectories in CC7+8 and CC9-12 which tended to peak and plateau on 2-3 days into Recovery (Fig. 78A and B).

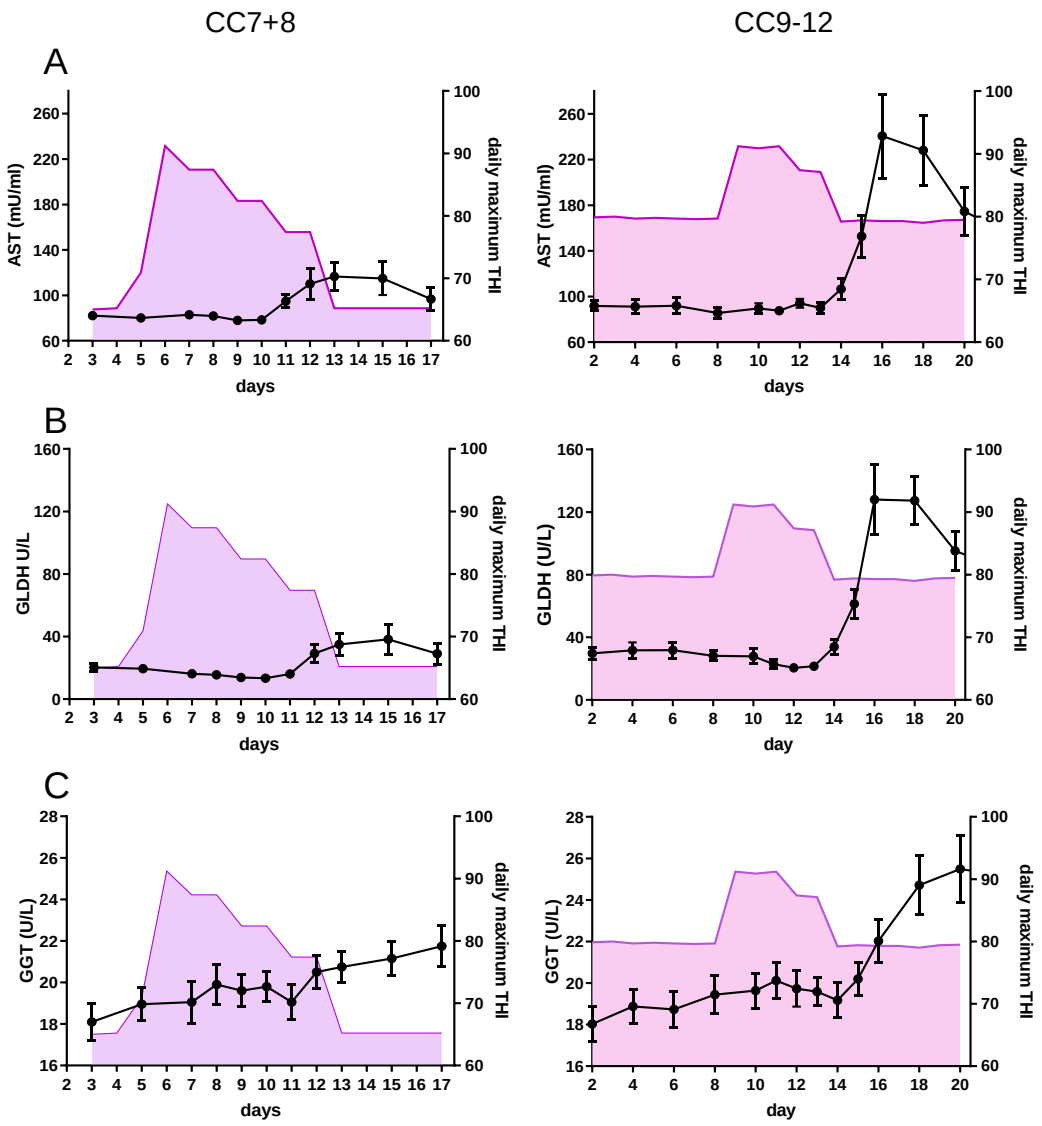

Fig. 78. Comparison of the time course trajectories of the mean activities (± SEM) of the liver function enzymes during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. Panel A. AST. Panel B. GLDH. Panel C. GGT. The daily maximum THI is indicated by the filled area for each trial.

The mean plasma ALP trajectories for CC7+8 and CC9-12 were remarkably comparable (Fig. 79). The minima were reached five days into the HOT period. The mean plasma ALP activity ascended gradually as conditions cooled in late HOT and in Recovery. The mean plasma ALP activities for both trials had not returned to PreHOT levels at the end of the Recovery period.

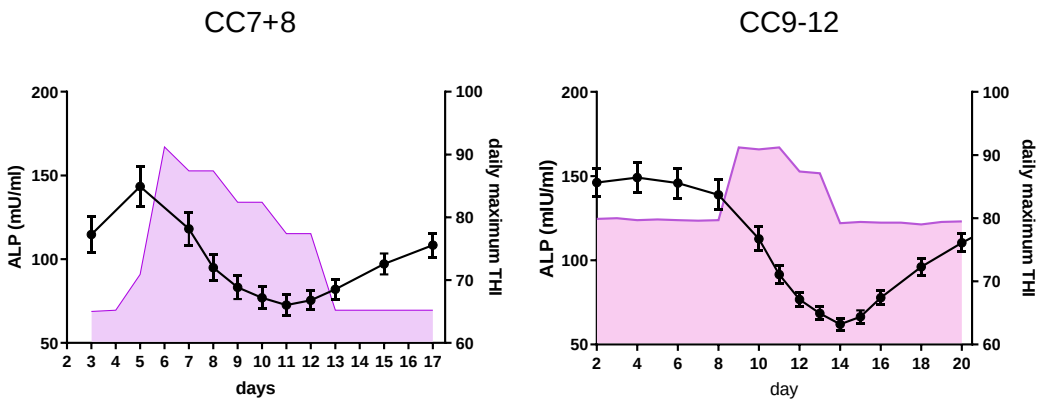

Fig.79. Comparison of the time course trajectories of the mean ALP activities (± SEM) during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. The daily maximum THI is indicated by the filled area for each trial.

As previously mentioned, the plasma bilirubin concentrations for CC9-12 during HOT was markedly elevated. The CC9-12 daily mean plasma bilirubin concentration peaked at day 12 at 7.0 mM, three days into the HOT period (Fig. 80A). The CC7+8 daily mean plasma bilirubin concentration appeared to have two maxima on days 6 and 12 (three and six days into HOT) at 5.2 and 4.9 mM respectively. Relatively high levels persisted throughout Recovery. By contrast, and despite the warmer conditions in Recovery, the excess plasma bilirubin was cleared rapidly in the CC9-12 trial during Recovery.

The daily mean plasma cholesterol concentrations fell in both trials and did not return to normal levels in Recovery (Fig. 80B). The PreHOT concentrations differed between the trials. The PreHOT mean for CC7+8 was 3.45 mM, and the PreHOT mean for CC9-12 was 2.66 mM. The differing concentrations may well reflect the different levels of feed intake between the two trials. Cholesterol is synthesised from acetate, one of the major products of rumen fermentation. The lowest cholesterol concentrations were seen at the latter days of the HOT period and were 22.0 and 17.8% reduced in CC7+8 and C9-12 respectively relative to their PreHOT means.

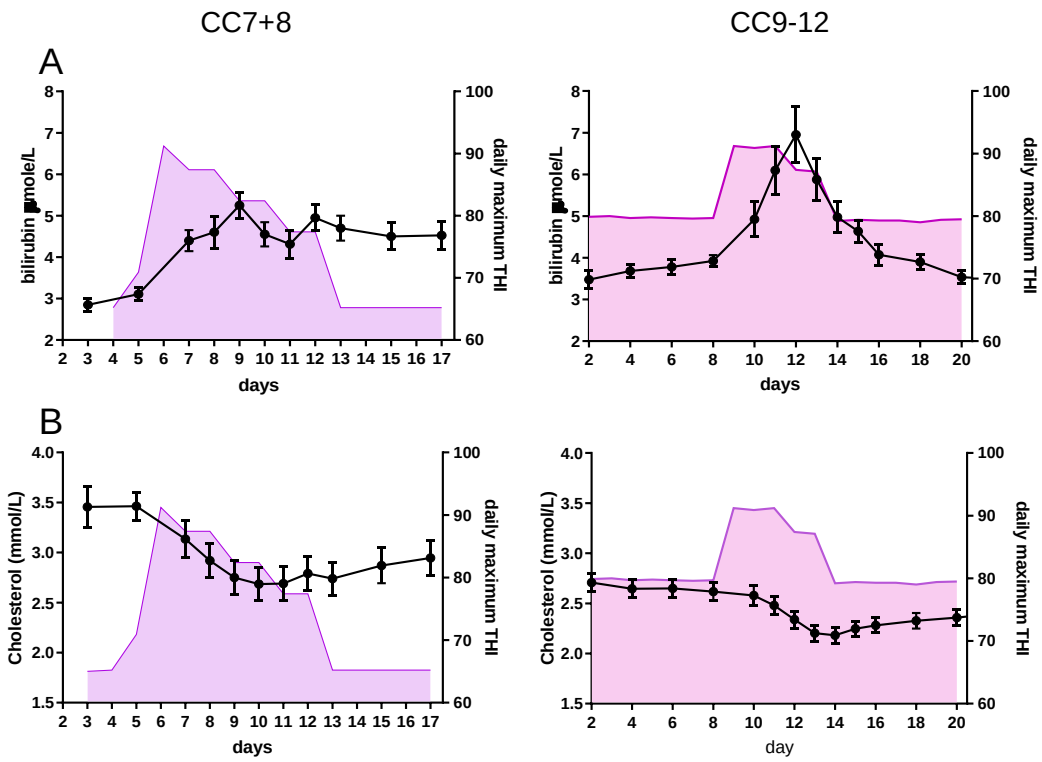

Fig. 80. Comparison of the time course trajectories of the mean plasma bilirubin and cholesterol concentrations (± SEM) during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. Panel A. bilirubin. Panel B. cholesterol. The daily maximum THI is indicated by the filled area for each trial.

Energy metabolism

The trajectories for the energy metabolites, glucose and β-hydroxybutyrate, were more dramatic in the CC9-2 trial (Fig. 81A and B). The PreHOT glucose concentrations of the two trials were similar. The two-step reduction in mean daily plasma glucose concentration seen in CC7+8 did not occur in CC9-12 where the glucose concentration plunged on the second day of HOT (Fig. 81A). The minimum glucose concentrations happened late into the HOT period for both trials. The minimum glucose concentration for CC7+8 was 3.6 mM on one single day, but the minimum for CC9-12 was 3.0 mM over two days. The return to normal glucose concentrations was rapid in both trials.

Interestingly, the mean PreHOT concentration of plasma β-hydroxybutyrate was higher in CC7+8 at 0.23 mM compared to the CC9-12 PreHOT mean of 0.18 mM (Fig. 81B). At the end of the Recovery period, the same difference in concentrations can be seen for the two trials. This difference in oxidation of fatty acids may indicate higher use of fatty acids in the cooler PreHOT and Recovery periods of CC7+8. The mean plasma β-hydroxybutyrate concentration for CC7+8 rose as soon as HOT was imposed and came to a plateau at 0.325 mM over the last 3 days of HOT. The CC9-12 trial saw a single rapid rise to a peak of mean plasma β-hydroxybutyrate concentration at 0.325 mM, and then just a rapid fall.

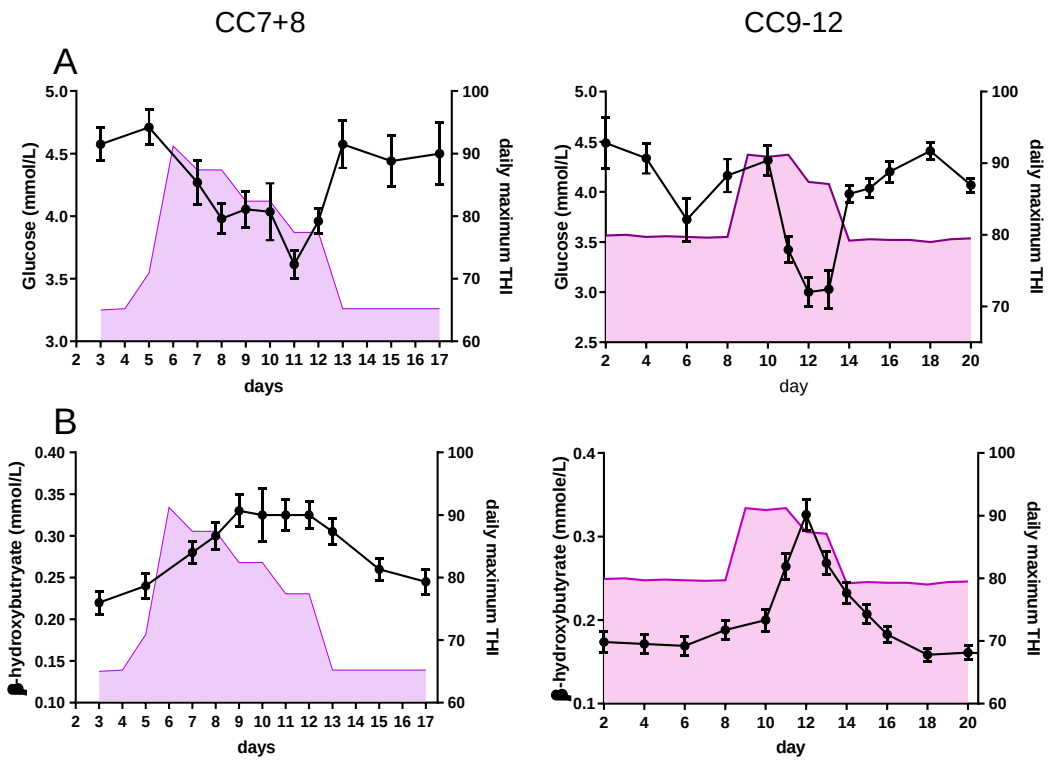

Fig. 81. Comparison of the time course trajectories of the mean plasma glucose and β-hydroxybutyrate concentrations (± SEM) during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. Panel A. glucose. Panel B. β-hydroxybutyrate. The daily maximum THI is indicated by the filled area for each trial.

Renal functions

The mean daily plasma chloride concentrations rose immediately with onset of HOT in both trials, but the response was more rapid in CC7+8 (Fig. 82A). The CC7+8 maximum plasma chloride concentration (104.35 mM) was achieved on the fourth day of HOT; the maximum plasma chloride concentration (104.00 mM) for CC9-12 was achieved on the fifth day of HOT. Both trials maintained these higher plasma chloride concentrations for three days, which was well into Recovery for CC9-12.

Plasma bicarbonate concentration is well known to reduce in hot conditions due to the increased respiration rate. The trajectories for daily mean plasma bicarbonate concentration in both trials was very similar with a rapid fall at onset of HOT and a rapid recovery as conditions moderated late in the HOT period (Fig. 82B). The minimum mean concentrations obtained were 22.2 mM and 19.0 mM for CC7+8 and CC9-12 respectively.

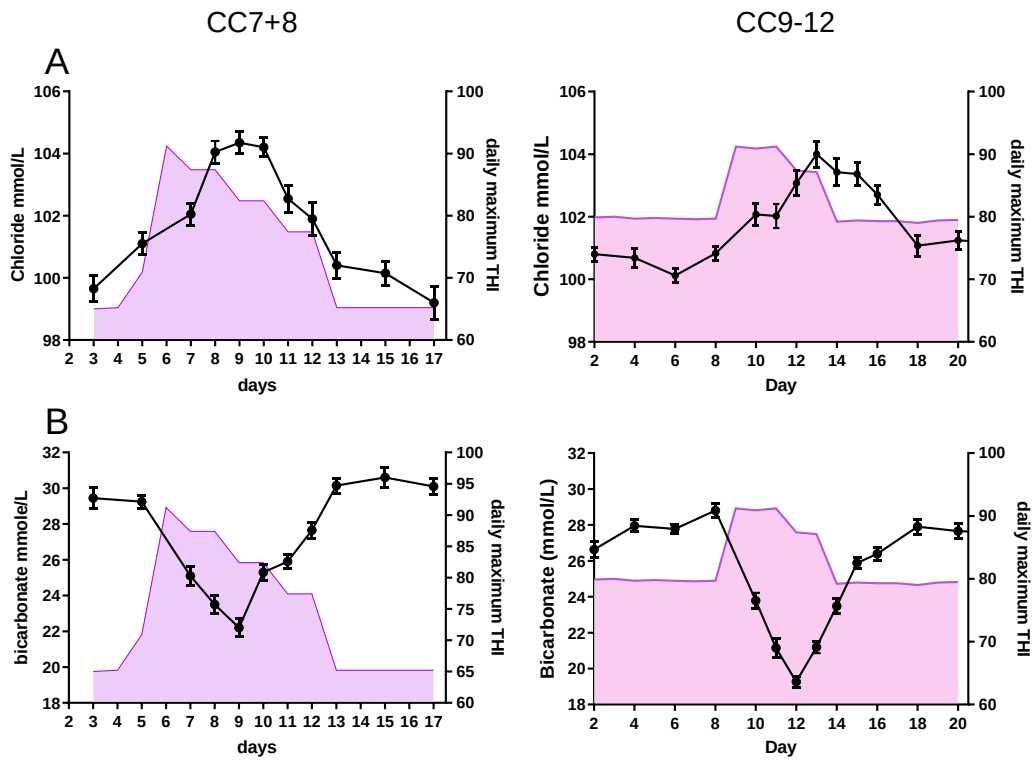

Fig. 82. Comparison of the time course trajectories of the mean plasma chloride and bicarbonate concentrations (± SEM) during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. Panel A. chloride. Panel B. bicarbonate. The daily maximum THI is indicated by the filled area for each trial.

We have found plasma urea and creatinine concentrations to be very consistent indicators of increased heat load. Daily mean plasma creatinine and urea concentrations rose rapidly with the onset of HOT in both trials (Fig. 83A and B). In CC7+8, probably due to the slightly reduced maximum ambient temperature and THI after the first days of HOT, the concentrations of the two metabolites came to a plateau during HOT on days 7-9. The mean creatinine concentration stabilised at 0.164 mM, and the mean urea concentration at 6.9 - 7.0 mM. In comparison, in CC9-12, the mean concentrations of creatinine and urea continued to rise to 0.176 and 9.3 mM respectively until the first step down in maximum ambient temperature and THI after 3 days of very hot conditions (Fig. 83A and B). A phenomenon detected in both trials was the persistent low urea concentrations during Recovery. The minimum mean urea concentration for CC7+8 was 3.9 mM, ~15% reduced from the PreHOT mean. The minimum mean urea concentration for CC9-12 was 3.8 mM, ~30% reduced from the PreHOT mean.

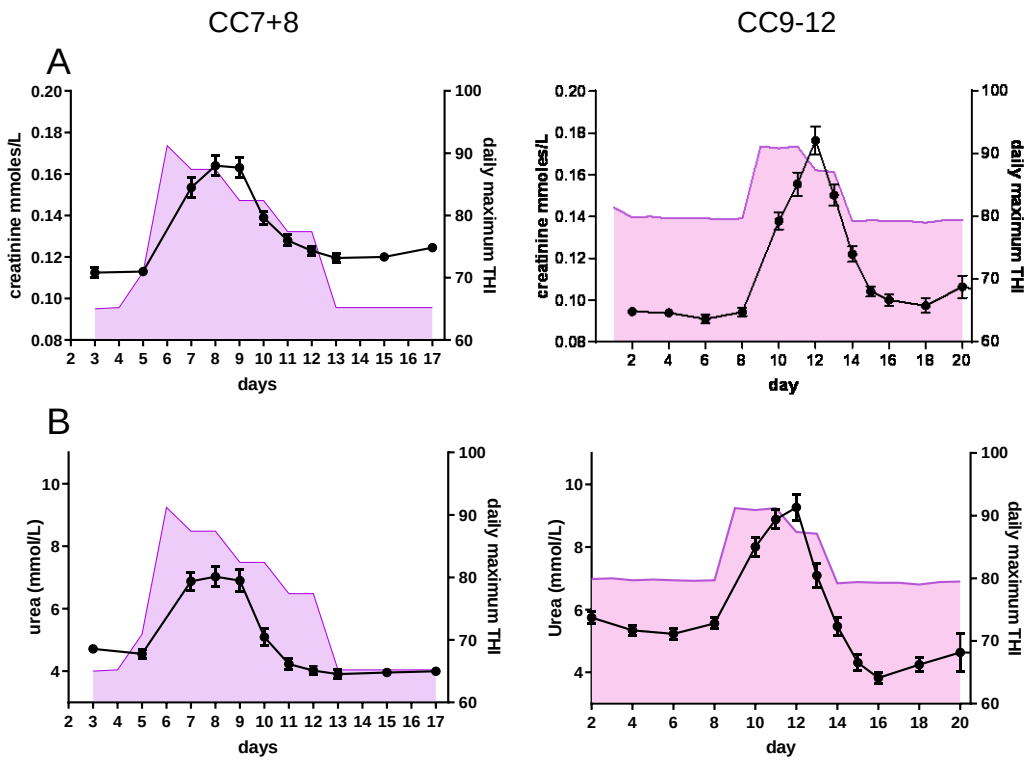

Fig. 83. Comparison of the time course trajectories of the mean plasma creatinine and urea concentrations (± SEM) during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. Panel A. creatinine. Panel B. urea. The daily maximum THI is indicated by the filled area for each trial.

4.1.4.4 Endocrine responses

Adiponectin and leptin, two protein hormones involved in energy metabolism and appetite regulation, registered falls in daily mean concentration during HOT (Fig. 84A and B). The drop in adiponectin concentration during HOT appears to have been delayed in CC9-12 as compared to the adiponectin trajectory for CC7+8. The adiponectin concentrations recovered to near the PreHOT concentrations for both trials for part of the Recovery period. Leptin concentrations remained reduced compared to the PreHOT means for both trials after HOT.

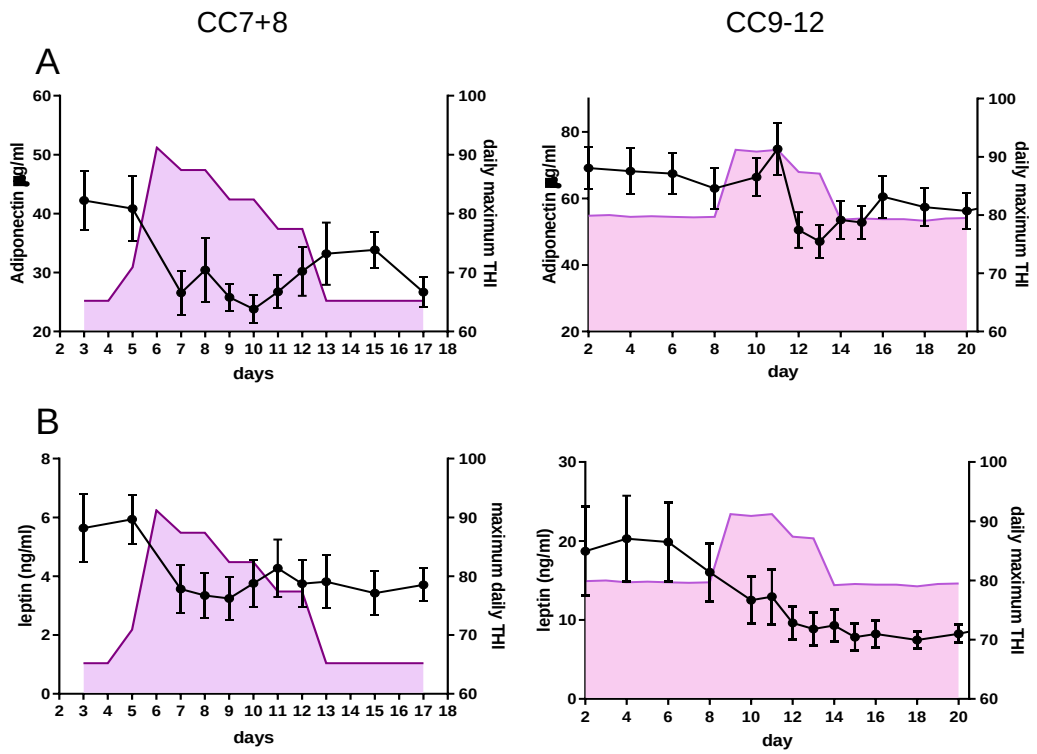

Fig. 84. Comparison of the time course trajectories of the mean plasma concentrations (± SEM) of the protein hormones, adiponectin and leptin, during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. Panel A. adiponectin. Panel B. leptin. The daily maximum THI is indicated by the filled area for each trial.

4.1.4.5 White blood cell changes and inflammatory responses

Altered white blood cell (WBC) numbers and populations have been another reliable response to high heat load. As seen in Fig. 85A and B, both trials experienced a mild leucophilia and neutrophilia as the daily mean WBC and neutrophil counts rose during the HOT period. The mean counts for CC7+8 came to a plateau during HOT, whereas they continued to rise to a peak in CC9-12. The counts fell markedly to below the PreHOT means as soon as the conditions improved. The drop in mean WBC and neutrophil counts was very evident in CC9-12; in Recovery, the minimum WBC and neutrophil counts were 25 and 40% less than the PreHOT mean respectively. For CC7+8, the minimum WBC and neutrophil counts were 15 and 14% less than the PreHOT mean respectively.

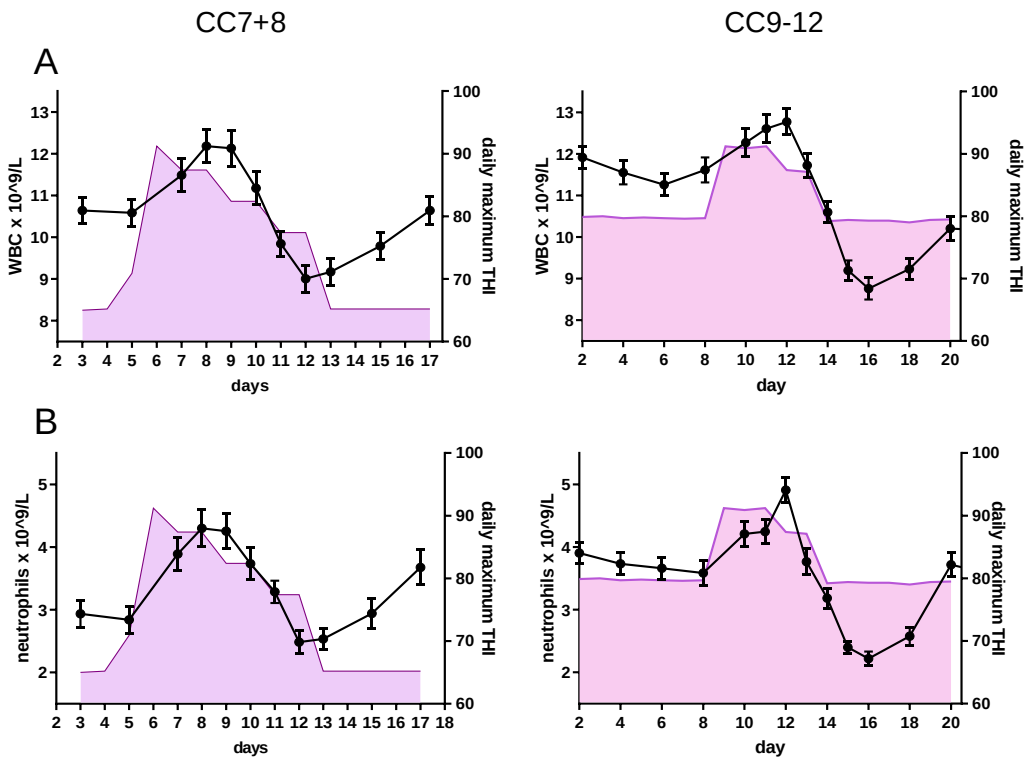

Fig. 85. Comparison of the time course trajectories of the mean white blood cell (WBC) and neutrophil counts (± SEM) during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. Panel A. WBC. Panel B. neutrophils. The daily maximum THI is indicated by the filled area for each trial.

Throughout this study, we have seen no indication of induction of inflammatory responses. Due to the very large variation between animals it can be difficult to detect subtle changes in cytokine responses at the group level. In Fig. 86A and B, the daily mean plasma IL-6 and TNFα concentrations of the CC7+8 trial revealed stable concentrations or even a small decrease (not significant) during the course of experiment. Intriguingly, for CC9-12, a small increase in concentrations of both cytokines was detected 4-5 days into the HOT period which quickly fell away as soon as conditions moderated. This small peak was not statistically significant, but it may indicate that some animals did release these cytokines into the plasma under the CC9-12 regime.

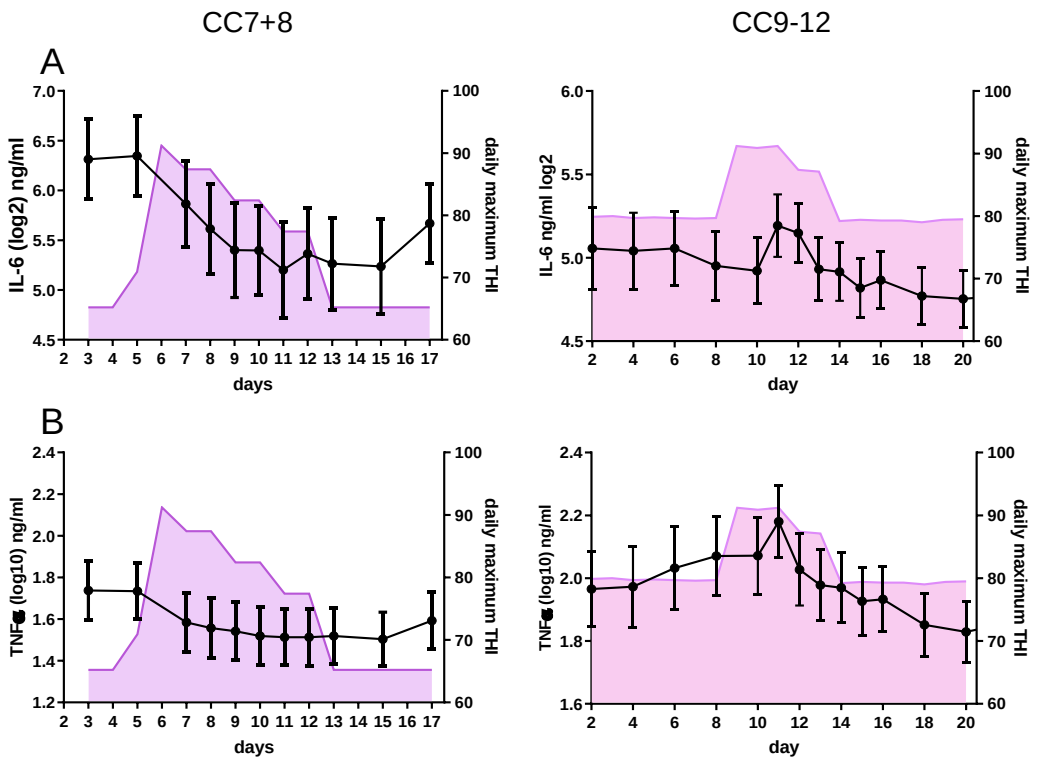

Fig. 86. Comparison of the time course trajectories of the mean plasma concentrations (± SEM) of two pro-inflammatory cytokines, TNFα and IL-6, during the days in the climate chambers for each of the trials, CC7+8 and CC9-12. Panel A. IL6. Panel B. TNFα. The daily maximum THI is indicated by the filled area for each trial.

4.1.4.6 Discussion

The CC9-12 regime succeeded in placing extra heat load on the steers throughout the course of the experiment in the climate chambers. Consequently, DMI was reduced, and respiration rate and rumen temperatures were increased in all periods relative to that associated with the CC7+8 regime. There were metabolic, haematological and endocrine consequences also. However, the primary ‘take home” message from this preliminary comparison of these two trials is the similarity in responses across so many dimensions. Most of the differences in responses between the trials pertain to the magnitude of the responses.

In the Recovery period, the amounts of the liver enzymes, AST, GLDH and GGT, shed into the plasma was markedly increased under the CC9-12 regime. This is a direct indicator of a higher level of cellular damage or death in the liver because of the higher heat load. The CC9-12 trial was able to provoke more release of GGT than any of the previous climate chamber regimes. However, the other “liver” enzyme, ALP, behaves very differently in that it is consistently lower in plasma during the HOT period and into Recovery in all of our climate chamber regimes. Its levels were at their lowest during the CC9-12 protocol although the magnitude of this decrease was not so different to that of CC7+8. Cholesterol is another liver product produced from acetate. Like ALP activity, plasma cholesterol consistently falls under increased heat load, and like ALP, there was not a great difference in the reduction in concentration between CC9-12 and CC7+8.

The build up of bilirubin in plasma was observed in both trials, but it was particularly acute under the CC9-12 conditions. This result directs attention to liver function and hepatic blood flow, since bilirubin should be expelled from the liver into the bile stream (and into the gall bladder). The upward trajectory of the plasma bilirubin concentration in CC9-12 only ceased on the first of step-down cooler days of HOT.

Plasma glucose and β-hydroxybutyrate report to some extent on energy metabolism. The plasma glucose concentration is closely regulated and defended by the action of insulin and other hormones. During the HOT period in both trials, plasma glucose falls considerably, but the trajectories in the two trials differ. In CC7+8, there appears to be a two staged decrease in plasma glucose that is invoked at the onset of HOT. In CC9-12, plasma glucose concentration appeared to be defended on the first day of HOT and then collapsed. Interestingly, the lowest blood glucose in both trials occurred as feed intake had resumed to some extent and conditions were cooling. This might be an indication of the time taken to re-establish rumen production and transport of VFAs, and gluconeogenesis in the liver of these substrates.

β-hydroxybutyrate is one of the end products of fatty acid oxidation and can be used by the brain for energy. Given the reduced glucose concentrations, fatty acid oxidation would have been invoked to supply energy in the liver and kidney, and to provide β-hydroxybutyrate and acetoacetate for uptake by the brain. In both regimes, the plasma β-hydroxybutyrate trajectory was the inverse of the plasma glucose trajectories, rising during high heat load. In CC7+8, the rise was gradual and came to a plateau late into the HOT period. In CC9-12, the rise in plasma β-hydroxybutyrate did not occur till glucose concentration fell after the first day of HOT. Interestingly, both trials arrived at the same maximum plasma β-hydroxybutyrate concentration, 0.325 mM.

In both trials, plasma concentrations of leptin and adiponectin fell with the onset of HOT and reduced feed intake. Normally reduced feed intake is associated with an increase in adiponectin to encourage re-feeding and slow energy use. Leptin concentrations also fell during HOT and remained at lowered levels in Recovery. Leptin acts as a longer term appetite suppressant, so its reduced concentration in Recovery should be beneficial.

The increased heat load associated with the C9-12 regime impacted glomerular filtration. As noted, the plasma creatinine and urea concentrations rose in both trials during HOT. The upward trajectories of these metabolites were contingent on the number of maximally hot days. In CC7+8, the plasma creatinine and urea concentrations rose for one day only, and then came to a plateau. In CC9-12, the concentration continued to rise during the three maximally hot days, only descending when conditions moderated. In Recovery, plasma urea concentrations were lower than the PreHOT levels in both trials, but particularly notable in CC9-12. Whether this is due to retention of urea as a N source to repair tissue and resume growth or “overcompensation” by the kidney to rapidly remove excess urea is unknown at this stage.

The slightly different behaviour in renal function between the two trials was confirmed by the differing plasma chloride trajectories during HOT and Recovery. As with plasma creatinine and urea concentrations, the plasma chloride concentration rose quickly in CC7+8 and then stabilised before falling as temperatures and THI dropped. In CC9-12, the plasma chloride concentration maintained its ascent until the last day of HOT. Even as conditions cooled well

into Recovery, the chloride concentration remained high. The kidney, along with the lungs, are involved in acid-base balance. The lungs can remove excess bicarbonate as carbon dioxide, and the kidney can reabsorb or secrete bicarbonate to maintain acid-base balance. As described in the previous section, the low plasma bicarbonate encourages renal retention of chloride ions. The altered trajectory for plasma chloride in CC9-12 was probably governed by the much reduced plasma bicarbonate in this trial.

Along with more pronounced physiological and metabolic response in CC9-12 compared to CC7+8, were the consecutive increase in HOT and then decrease during Recovery in the white blood cell counts especially the neutrophil population. This would indicate that the more severe the heat load, the higher the vulnerability to infection in the days immediately following the heatwave event.

4.1.5 Acute moderate heat load climate chamber study - CC4-6

4.1.5.1 Introduction

In brief, three cohorts of six steers (HOT) were exposed to five days with a daily maximum temperature of 35°C and cycled to a night-time minimum temperature of 28°C in climate controlled rooms. The temperature and THI regime for the HOT treatment are given in Fig 87. For four days prior (PreHOT) and six days after (Recovery) this thermal challenge the animals were maintained at 22°C for 24 hours to achieve thermoneutrality (Fig. 87). On exiting the climate rooms on day 20, the cattle were housed in feedlot pens, and monitored for a further 10 days. The first cohort, CC4, was terminated at day 13 to allow post-mortem tissue collection. The analyses presented in the following sections pertain to the data collected from the CC5 and 6 cohorts only. These two cohorts proceeded through the entire regime shown in Fig. 87.

Each group of six steers that were exposed to the thermal challenge were accompanied by weight matched six steers that remained at 22°C for 24 hours for the 19 days in the climate controlled rooms. These thermoneutral Controls were pairfed: that is, each control steer was offered the amount of feed that its weight matched pair consumed the previous day. The rationale for the Control group was to provide data as to the physiological and metabolic impact of limit feeding alone, and to compare with the combined effects of moderate heat load and the voluntary reduction in feed intake.

All animals were on a finisher ration throughout (offered twice daily). Intensive recording of behavioural and physiological data was augmented with haematological and biochemical data obtained from blood and plasma collected by frequent bleeds. The bleed schedule is given in Fig 87.

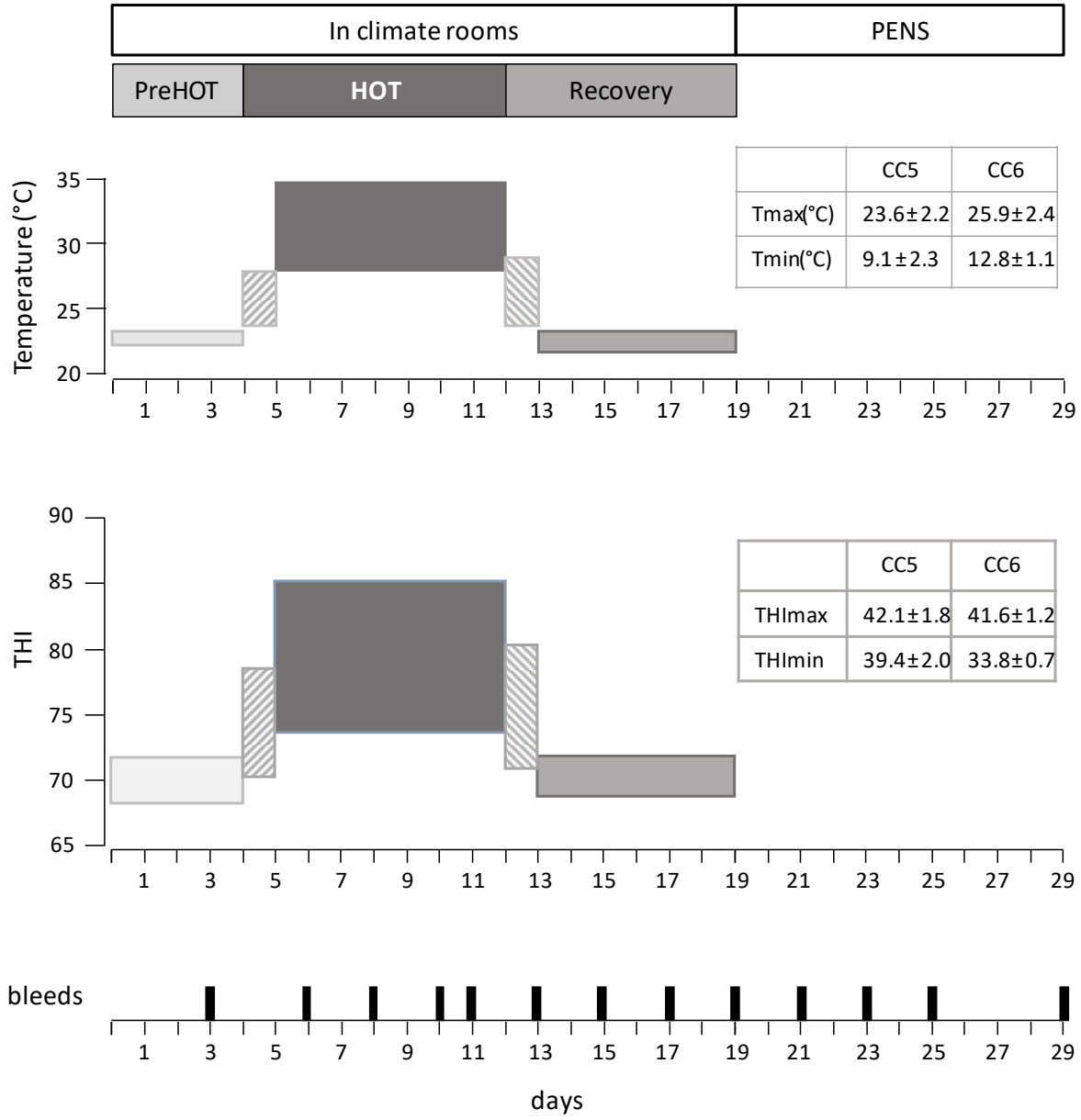

Fig. 87. The climatic regime of the acute moderate heat load challenge for the HOT treatment group. The range of the mean daily ambient temperature and Temperature-Humidity Index (THI), and duration of each period is depicted for the 29 days of the experiment. The PreHOT, HOT and Recovery periods were conducted in climate controlled rooms. The PENS interval occurred in outdoor feedlot pens; the climatic conditions (mean ± SD) for each replicate is presented in the inserted tables. Blood sampling days are indicated also.

4.1.5.2 Animal Performance

Both treatment groups experienced significantly reduced DMI during the HOT and Recovery periods relative to the PreHOT interval (Fig. 88A); obviously this was not a voluntary reduction by the Control group. The mean DMI of the HOT group was 4 kg less than the pre-HOT mean, and 2 kg less than that of the thermoneutral feed restricted Controls. The HOT

group's mean DMI rose by 22% during Recovery ($p < 0.0001$, Fig. 88B). The mean DMI in PENS was significantly higher than the HOT and Recovery periods but remained 0.8 kg lower than the PreHOT mean.

Applying the restricted feeding regime to the Control group during the HOT period ensured that the two treatment groups had a similar weight loss trajectory during the HOT and Recovery periods (Fig. 88C and D). The Controls tended to a slightly higher live weight during HOT ($p = 0.093$). Once back out in PENS, both groups markedly and similarly increased their mean live weights.

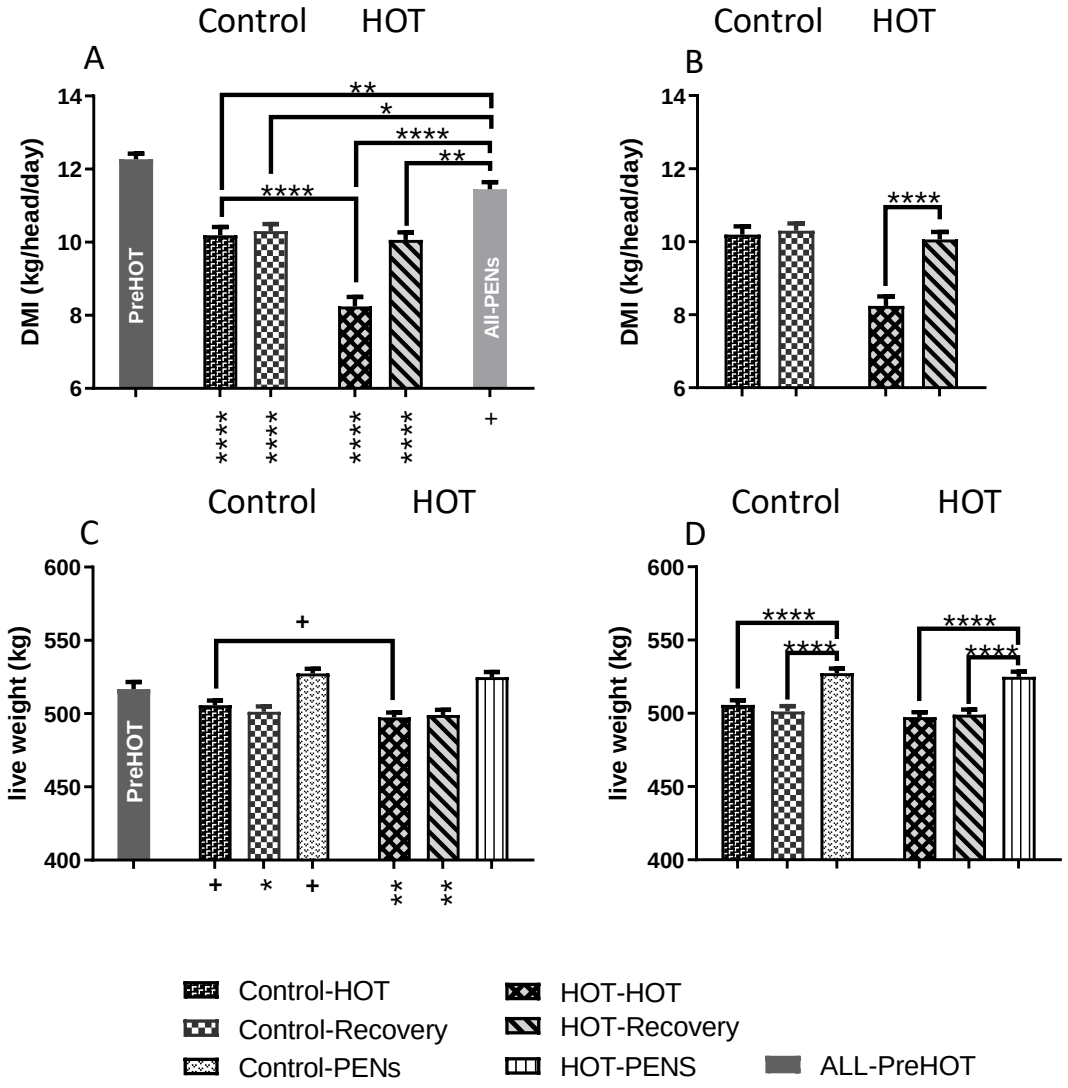

Fig. 88. Animal performance before (PreHOT), during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The PreHOT value in each histogram is the mean ± SEM of all animals during that interval. A. Between periods and treatment comparisons of the mean DMI (± SEM) of the Control and HOT groups. B. Within treatment comparisons of the mean DMI (± SEM) of the Control and HOT groups during the HOT and Recovery periods. Only pen DMI (All-PENS) (i.e. not individual intake) was available during PENS. C.

Between periods and treatment comparisons of the mean live weight (± SEM) during all periods. D. Within treatment comparisons of the mean live weight (± SEM) of the Control and HOT groups during the HOT, Recovery and PENS periods. The asterisks under the x-axis indicate statistically significant difference with PreHOT. +, p <0.1; *, p <0.05; **, p <0.01; ****, p <0.0001.

4.1.5.3 Physiological responses

With reduced feed intake during the HOT period, the Control group correspondingly reduced water usage (Fig. 89), whereas the HOT group, with even lower feed intake, maintained water usage at PreHOT levels. The water usage of the HOT animals significantly increased ($p \leq 0.003$) during HOT period compared with Control animals as shown in Fig. 89. The HOT animals remained higher in their water usage in Recovery although this difference compared with Control animals was not significant. On average, during HOT, the HOT animals' water usage was an extra 6.2 L/head/day than that of the Control. During Recovery, the HOT group substantially reduced water usage, converging with the levels of the Control group along with feed intake.

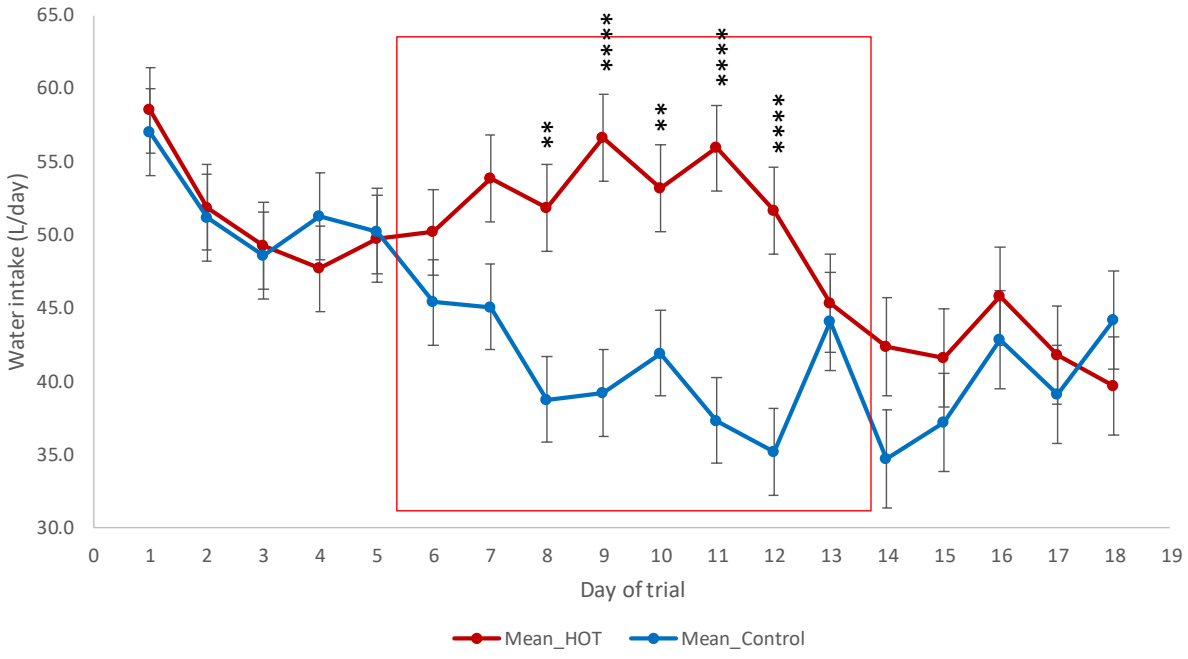

Fig. 89. Mean (± SEM) water usage of all cattle across the entire period in climate controlled rooms. The red boxed area indicates the HOT period in climate controlled rooms. The asterisks indicate levels of significant difference: **, p <0.01; ****, p <0.0001.

Respiration rate (RR) was significantly higher ($p \leq 0.0001$) for HOT animals compared with Controls for all HOT days during the day time hours of 0600 to 1800 h. Fig. 90 presents the mean RR between 1000 to 1400 h, when it was at its highest for the HOT animals during the

HOT period. The RR of all animals is lower in Recovery compared with PreHOT (days 1-4); this may be due to an adaptation to the housing for all animals or an alteration in the breathing pattern in HOT animals. The HOT animals may alter their respiratory pattern to slower, deeper breathing after the fast panting that was utilised in the HOT period. The mean RR of the HOT animals remained elevated in the first 2 days of Recovery due to the previous day's heat load and/or as a result of the increase in feed intake (metabolic heat) in the Recovery period.

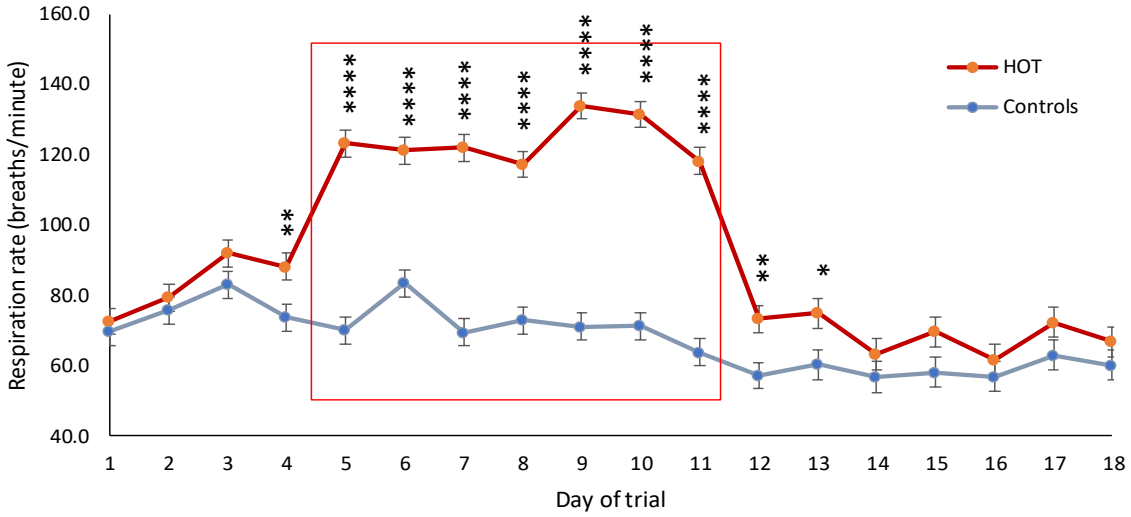

Fig. 90. Mean respiration rate (± SEM) of all cattle across all days for 1000-1400 h time period in the climate controlled rooms. The red boxed area indicates the HOT period in climate controlled rooms. The asterisks indicate levels of significant difference: *, p <0.05; **, p <0.01; ****, p <0.0001.

Fig. 91 summarises the mean daily rumen temperature for HOT and Control treatments. The rumen temperature of HOT animals was elevated in the HOT period (p ≤0.0001) compared with Control animals and remained elevated for 2 days after HOT period. This indicates that the HOT animals still had high heat load during the first days of the Recovery period. The HOT animals had high mean RR (Fig. 90) and elevated surface temperatures (data not shown) in the first 2-3 days of the Recovery period also. The higher rumen temperature of HOT animals in first days of the HOT period compared with second half of the HOT period (days 9-11) suggest that it takes up to 3 days for the cattle to fully invoke effective heat dissipation methods. The decrease in rumen temperature in days 8-11 coincides with an increase in respiration rate and surface temperature, indicating the animals have fully enabled heat dissipation mechanisms and have offloaded some heat.

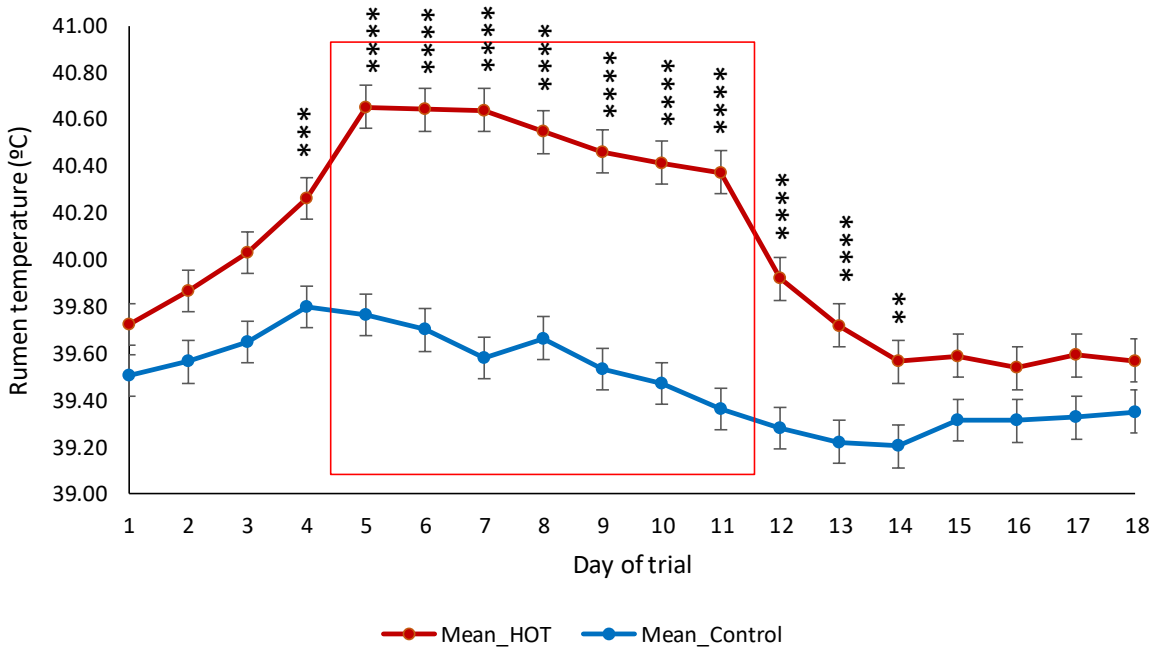

Fig. 91. Mean rumen temperature (± SEM) of all animals across entire trial period in climate controlled rooms. The red boxed area indicates the HOT period in climate controlled rooms. The asterisks indicate levels of significant difference: **, p <0.01; ***, p <0.001; ****, p <0.0001.

4.1.5.4 Metabolic responses

Plasma buffering and electrolytes

The mean plasma bicarbonate level for Control group during HOT was not different to that of the PreHOT interval; in contrast, the mean of the HOT group was significantly depressed (~15%) (Fig. 92A). The mean plasma bicarbonate concentrations rose rapidly and similarly in both groups during Recovery and in PENS relative to the means of the HOT period and were significantly higher than the PreHOT mean (Fig. 92A and B).

There were subtle but significant changes in plasma electrolyte concentrations in response to high heat load and restricted feed intake in thermoneutral conditions. The mean plasma chloride concentrations during HOT was higher than PreHOT for both treatment groups (Fig. 92C) and the HOT group means for each period were significantly higher (1 - 1.5%) than the PreHOT mean (Fig. 92C). During the HOT and Recovery periods, the HOT means were higher than the Control. Interestingly, when the animals recovered from their treatments, the plasma chloride concentration was at its lowest of the three periods for both groups (Fig. 92D).

The mean plasma sodium concentrations were similar in both cohorts over the three periods; highest during the Recovery period, and lowest in PENS period (Fig. 92E and F). The HOT and Recovery mean plasma sodium levels were slightly higher (~1%) than the PreHOT mean for both treatment groups (Fig. 92E), but the HOT group's means were lower relative to their Control counterparts. The plasma potassium concentrations showed little effect of

either feed restriction or high heat load (data not shown). The plasma phosphate concentrations increased 8-12% in the Control group during HOT and Recovery relative to the PreHOT mean, but this was not replicated in the HOT group (data not shown).

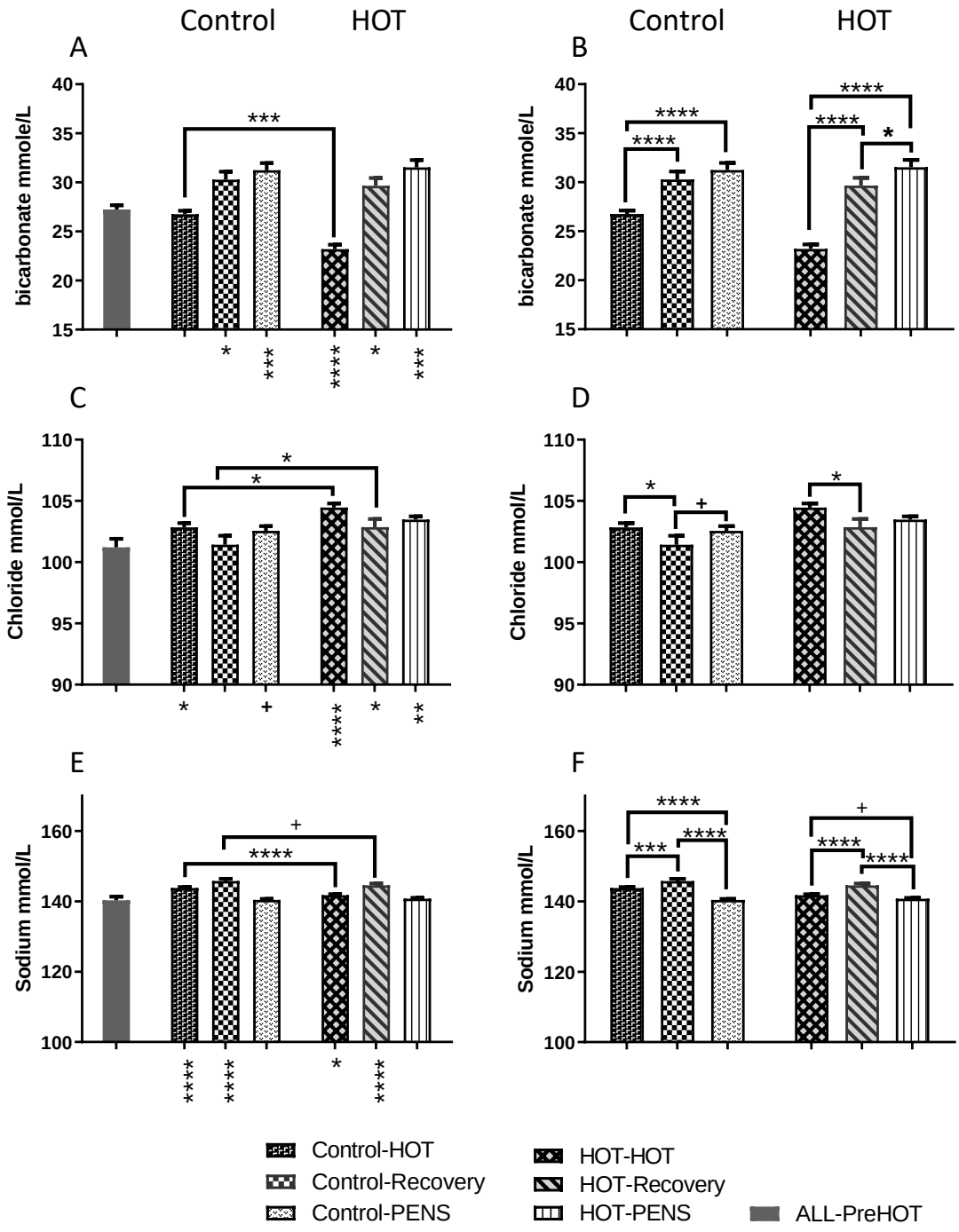

Fig. 92. Changes in plasma bicarbonate, chloride and sodium concentrations in response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A, C and E. Between treatment group and ALL-PreHOT comparisons of the mean plasma

bicarbonate, chloride and sodium concentrations (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B, D and F. Within treatment group comparisons of the mean plasma bicarbonate, chloride and sodium concentrations (± SEM) respectively of the Control and HOT groups during the HOT, Recovery and PENS periods. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Urea and creatinine

Plasma urea and creatinine concentrations are informative of renal function. The mean plasma creatinine concentrations of the Control group during HOT was similar to that of the PreHOT interval, but then rose during the Recovery period (7%) and remained higher than PreHOT while in PENS (Fig. 93A and B). The mean plasma creatinine of the HOT group was higher (approximately 20%) during the HOT period relative to the PreHOT interval, and subsequently fell significantly over the consecutive periods. The mean plasma creatinine concentrations of the Control group during HOT was 13% lower than that of their thermally challenged counterparts.

Both groups experienced similar responses in plasma urea concentrations which rose 5-6% during the HOT period (relative to the PreHOT interval), and then fell significantly during the two later periods of the study (Fig. 93C and D). While the mean plasma urea concentrations were not significantly different between the two treatments during HOT, the means of the HOT group were about 10% lower than those of the Control group over Recovery and PENS periods (Fig. 93C). That is, the fall in mean plasma urea levels was more extreme after high heat load in the HOT animals relative to the Control thermoneutral animals, and this persisted for some time while in PENS.

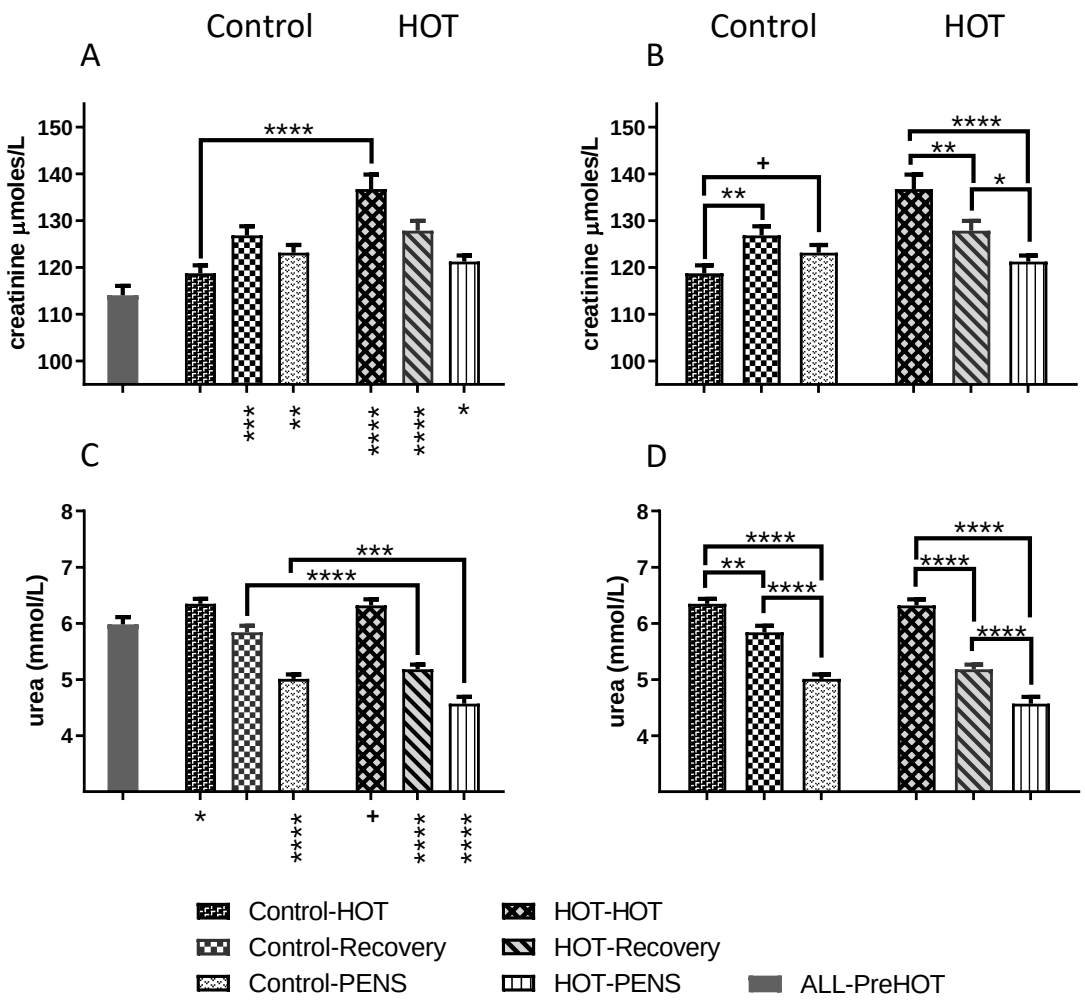

Fig. 93. Changes in plasma creatinine and urea concentrations during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A and C. Between group and ALL-PreHOT comparisons of the mean plasma creatinine and urea concentrations (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B and D. Within treatment group comparisons of the mean plasma creatinine and urea concentrations (± SEM) respectively of the Control and HOT groups during the HOT, Recovery and periods. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Liver function enzymes, cholesterol and triglycerides

The mean plasma activities for ALP, AST and GGT were reasonably stable across the sequential periods for the Control group (Fig. 94). Plasma GLDH activities behaved similarly in the Control group (data not shown). In contrast, there were clear responses to the heat load challenge by the HOT group for all enzymes.

The PreHOT interval produced the highest mean plasma ALP activity for trial. The Control group experienced a 14% reduction in mean plasma ALP activity with feed restriction during

HOT, which persisted (~17%) during Recovery and in PENS ($p < 0.05$) (Fig. 94A and B). For the HOT group, mean plasma ALP activities during HOT, Recovery and PENS were approximately 33, 44 and 18% reduced compared to the PreHOT mean. When comparing the means between the groups, the HOT cohort's mean plasma ALP activities were 22 and 33% lower than that of the Controls for the HOT and Recovery periods (Fig. 94A and B). Thus, mean plasma ALP activity was depressed in the HOT animals during the heat load challenge and in Recovery, but similar to the Control group once back in PENS.

The mean plasma AST activity during the PreHOT period was significantly higher (9 – 14%) than those of the Control group during all periods (Fig. 94C). The mean plasma AST activities for the HOT group were not different to that of the PreHOT interval, although they increased with each sequential period. The mean activity during HOT was 7 – 9% below that of the means for the Recovery and PENS periods (Fig. 94D). All the HOT group's mean plasma AST activities tended to or were significantly higher (7-15%) than those of the Control group for the corresponding period. Fig. 94E and F shows that mean plasma GGT activity was increased in both groups and all periods relative to the PreHOT mean, with the highest mean activities achieved during Recovery. However, the rise of the Control group during restricted feeding was almost twice that of the rise in the HOT group while subjected to thermal stress.

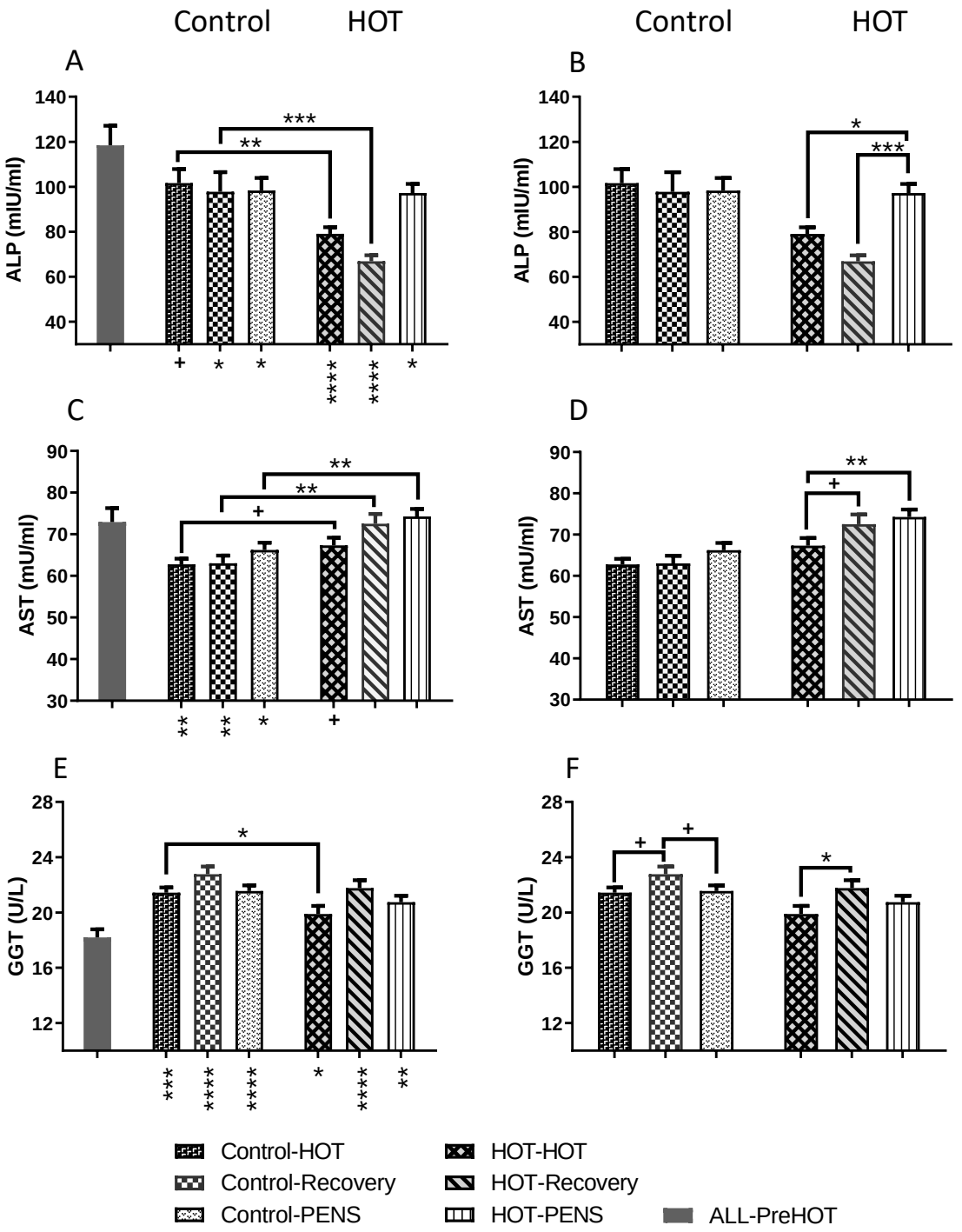

Fig. 94. Changes in plasma liver enzyme activity in response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A, C and E. Between group and ALL-PreHOT comparisons of the mean plasma ALP, AST and GGT activities (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B, D and F. Within group comparisons of the mean plasma ALP, AST and GGT activities (± SEM) respectively of the

Control and HOT groups during the HOT, Recovery and PENS periods. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

The Control animals significantly increased their mean plasma cholesterol concentrations by 10 - 15% during the HOT and Recovery periods relative to the mean during the PreHOT interval in PENS and (Fig. 95A and B). The HOT animals experienced stable mean plasma cholesterol concentrations over the course of the trials, but these levels were 15 – 30% lower ($p < 0.003$) than the means for the Control group for the same periods.

The mean plasma triglyceride concentrations were higher in both groups relative to the PreHOT mean during HOT and Recovery (Fig. 95C). Both groups experienced quite different trajectories in the two groups over the course of the study although they both experienced the lowest means during PENS (Fig. 95C and D). The Control mean plasma triglyceride level during HOT was 15 - 20% higher than that of the subsequent periods and 40% higher than the PreHOT mean. That is, restricted feeding induced an elevation of circulating triglycerides. For the HOT group, the mean plasma triglyceride concentration in PENS was 20-25% lower than the two earlier periods, and the only period that was equivalent to the PreHOT interval. On comparing the groups, the HOT cohort's mean plasma triglyceride levels during HOT and PENS were significantly lower than those of the Control group during the same periods (Fig. 95D).

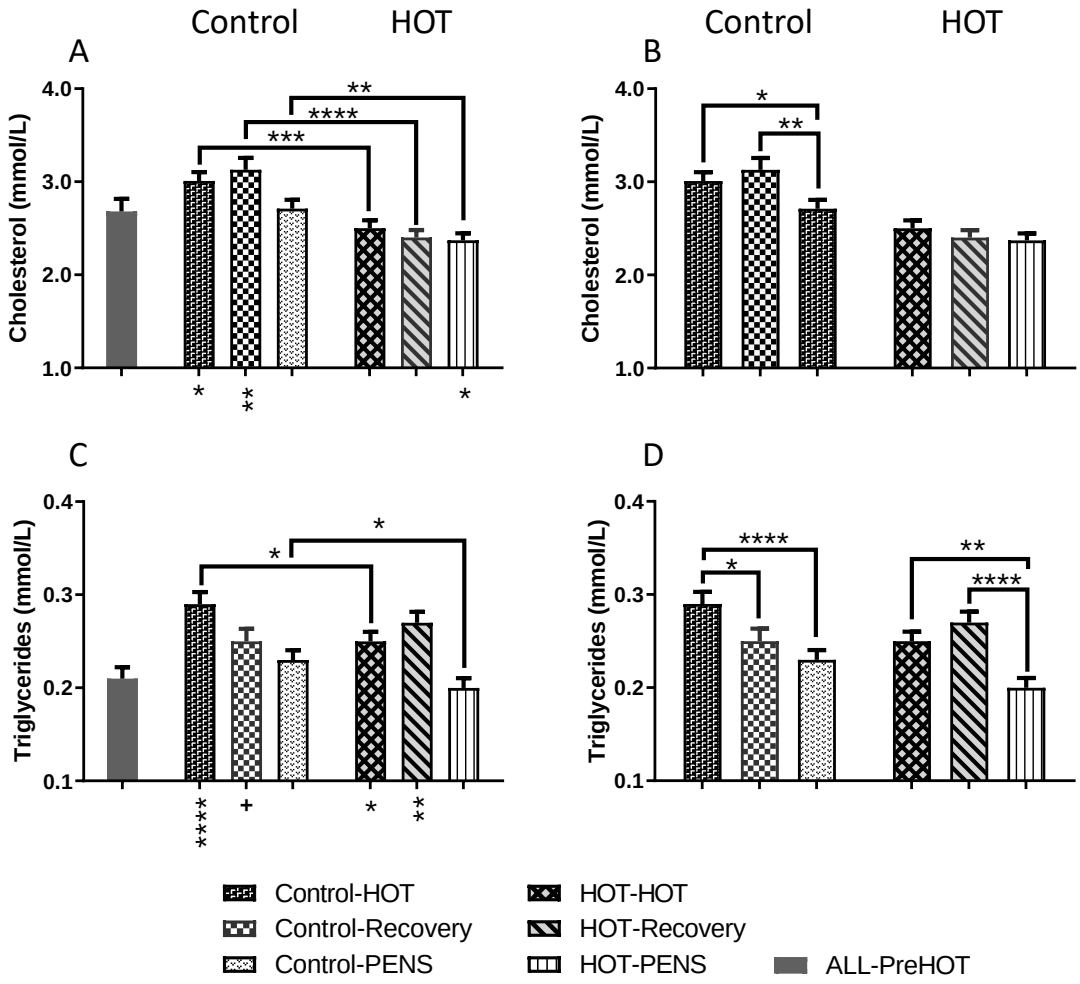

Fig. 95. Changes in plasma cholesterol and triglyceride concentrations during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A and C. Between group and ALL-PreHOT comparisons of the mean plasma cholesterol and triglyceride concentrations (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B and D. Within treatment group comparisons of the mean plasma cholesterol and triglyceride concentrations (± SEM) respectively of the Control and HOT groups during the HOT, Recovery and periods. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

Energy Metabolism

There were significant changes in mean plasma glucose and NEFA concentrations between periods for the HOT group only (Fig. 96A and B). Mean plasma glucose concentrations of either group was not significantly different from the PreHOT mean at any time, although the 4% decrease in the mean of the HOT group during Recovery tended toward significance (p = 0.075). The PENS mean of the Control group trended higher relative to the HOT and Recovery periods also, but this inclination was stronger in the HOT group where the mean plasma glucose concentration was 5-6% higher in PENS relative to the HOT and Recovery

periods (p <0.003). On average, the mean plasma NEFA levels of both groups for all periods were 60 – 120% higher than the PreHOT mean (Fig. 96B). For the HOT group, the mean plasma NEFA concentration during HOT was 25% lower than during PENS (p <0.05) but not different with the Recovery period. There were no significant differences between the treatment groups for any of the periods.

Plasma insulin is the main regulator of blood glucose, and negatively influences mobilisation of fatty acids (NEFAs) from fat stores. The mean plasma insulin levels during this trial mirrored plasma glucose concentrations in that when glucose rose, so did insulin and vice versa. It is interesting to note, that when plasma glucose and insulin levels were low in the HOT animals during HOT, the NEFA response was muted (Fig. 96C).

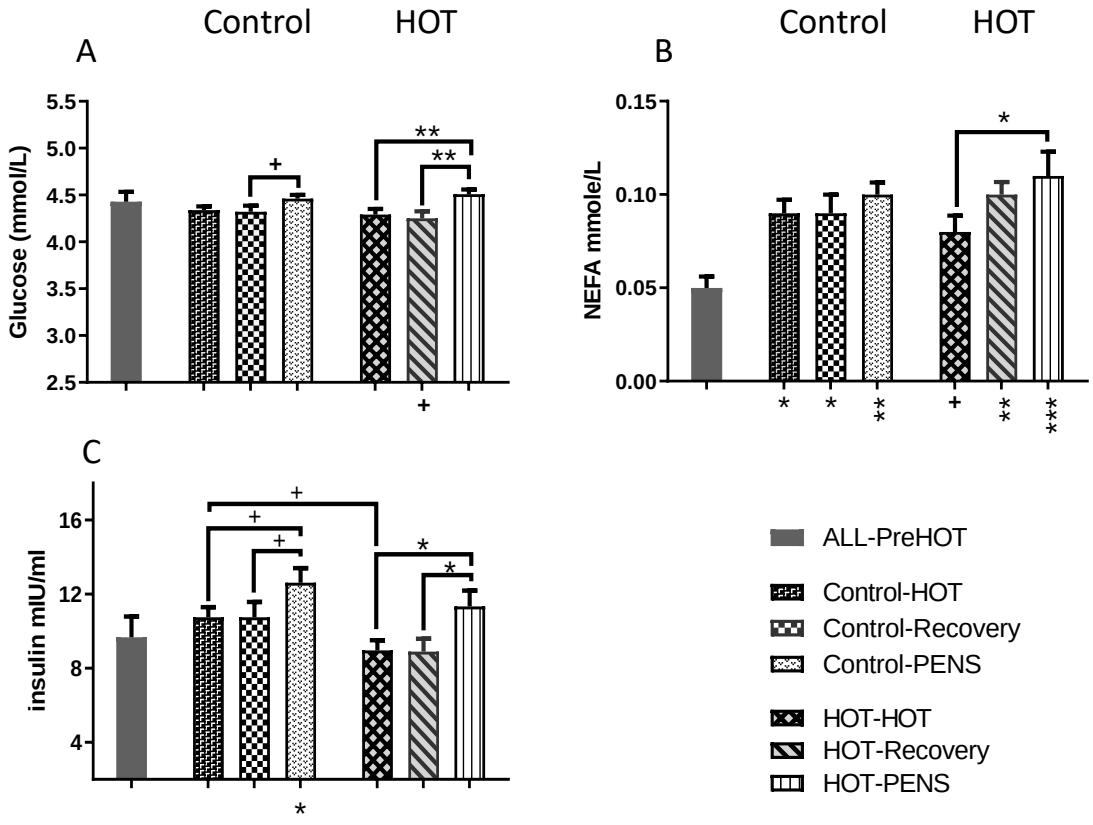

Fig. 96. Changes in plasma glucose, NEFA and insulin in response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A, B and C. Within group, and between group and ALL-PreHOT comparisons of the mean plasma glucose, NEFA and insulin concentrations (± SEM) respectively of the Control and HOT groups during all periods. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001.

Glutamine, the most abundant of the amino acids in blood and most tissues can be used as an energy metabolite in many cells and through several biochemical pathways. The Control group while feed restricted experienced a significant 18% rise in mean plasma glutamine concentration relative to the PreHOT mean, and then a further 8% rise during Recovery (Fig. 97A and B). The HOT group saw a 9% decrease in mean plasma glutamine concentration during HOT relative to the PreHOT ($p = 0.085$) but increased quickly during Recovery to be 16% higher than the PreHOT mean. When HOT, the HOT treatment group had 146 µM less plasma glutamine than their feed restricted thermoneutral Controls.

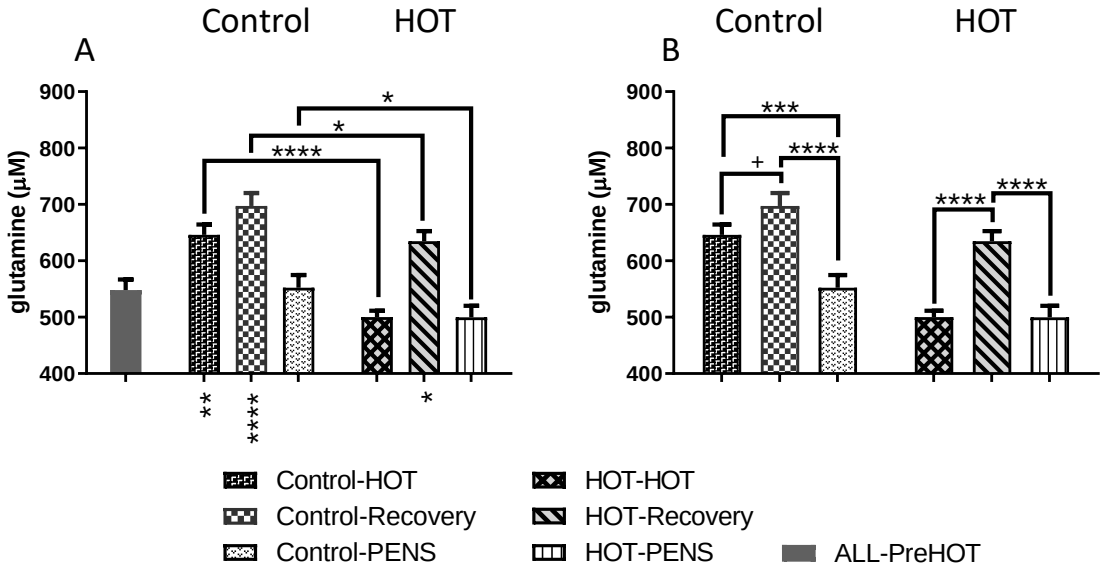

Fig. 97. Changes in plasma glutamine response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panel A. Between group and ALL-PreHOT comparisons of the mean plasma glutamine concentration (± SEM) of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panel B. Within treatment group comparisons of the mean plasma glutamine concentration (± SEM) respectively of the Control and HOT groups during the HOT, Recovery and periods. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

4.1.5.5 Haematological changes

Overall, the white blood cell (WBC) counts were lowest in PENs for both treatment groups, probably reflecting a decreased count with age and weight (Fig. 98A and B). The mean WBC counts of the HOT animals were higher than the Control means for the HOT period and in PENs. The neutrophil numbers were the major influence on the WBC counts. The mean neutrophil counts behaved differently between the two treatment groups (Fig. 98C and D). The mean neutrophil counts of the HOT group did not significantly change relative to the PreHOT mean at any time. In contrast, the feed restricted Controls experience a fall during

HOT, and again in PENs, and were significantly lower than the means of the HOT group for those periods.

The mean PBL and monocyte counts behaved similarly in both treatment groups (data not shown). The mean of eosinophil count in the HOT group tended to be lower during the Recovery period and higher in PENs but not significantly different to the Control group (data not shown).

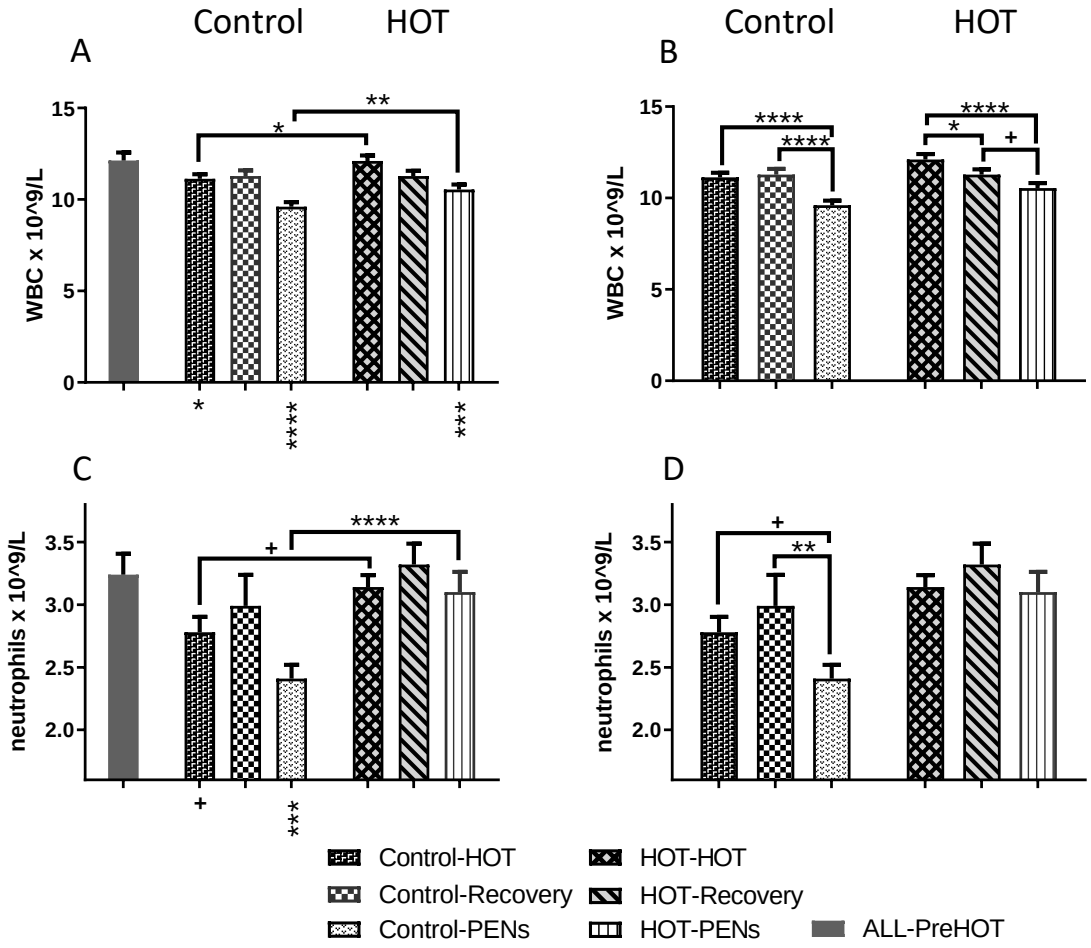

Fig. 98. Changes in WBC and neutrophil counts during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A and C. Between group and ALL-PreHOT comparisons of the mean WBC and neutrophil counts (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B and D. Within treatment group comparisons of the mean WBC and neutrophil counts (± SEM) respectively of the Control and HOT groups during the HOT, Recovery and periods. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

There was a tendency for a slight increase (3.7%) in mean RBC count in HOT animals during HOT relative to the Controls; otherwise there were no other differences. In PENs, the mean RBC counts were lowest for both treatment groups (data not shown). The total haemoglobin (Hb) concentration confirmed the increased RBC content with the mean total Hb concentration 5-7% higher in the HOT animals during thermal challenge as compared to the PreHOT period and the Recovery counterparts. As with the RBC counts, the lowest total Hb concentration was seen in PENs (Fig. 99A and B). The haematocrit (HCT) reflected this result also (Fig. 99C and D). The mean platelet counts were not different during periods and between groups (data not shown).

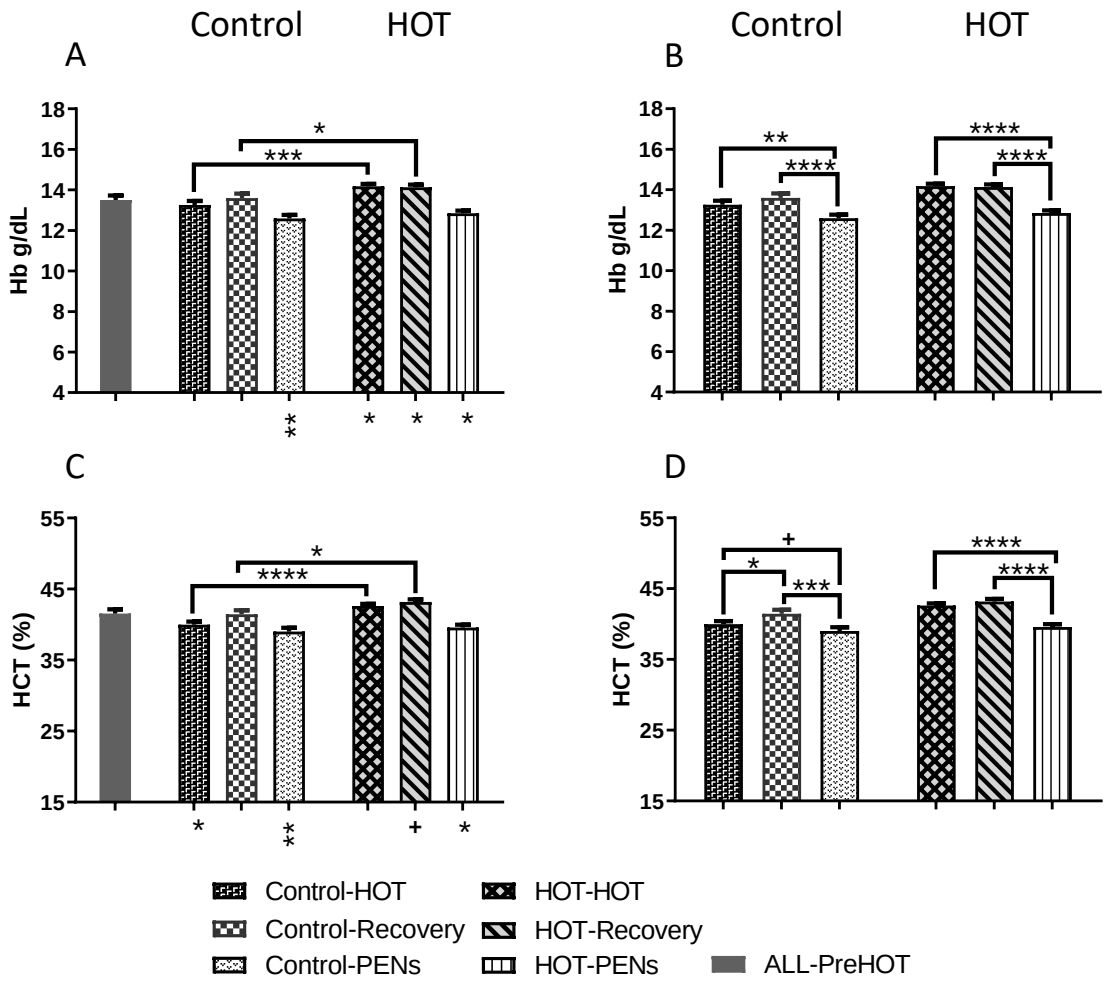

Fig. 99. Changes in total haemoglobin (Hb) concentration and haematocrit (HCT) during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A and C. Between group and ALL-PreHOT comparisons of the mean Hb concentration and HCT (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B and D. Within treatment group comparisons of the mean Hb concentration and HCT (± SEM) respectively of the Control

and HOT groups during the HOT, Recovery and periods. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

4.1.5.6 Endocrine changes

Leptin is a negative regulator of appetite. The mean leptin concentrations fell significantly in both treatment groups in all periods relative to the PreHOT mean (Fig. 100A and B). The HOT animals maintained significantly higher mean leptin concentrations relative to the Control group, even in PENs. The leptin response appeared opposite that of the insulin response (Fig. 96C).

Adiponectin concentrations responded differently in the two treatment groups also (Fig. 100C and D). In the Control group, the mean adiponectin concentration was not affected by the reduced feed intake, rose slightly in Recovery, and fell again in PENs. In the HOT group, the mean adiponectin concentration fell during HOT, rose in Recovery and fell in PENs. The means of the HOT group were consistently lower than those of the Controls at all times.

Prolactin, another hormone implicated in energy balance and water regulation showed no change in plasma concentration between periods or treatments in this experiment (data not shown).

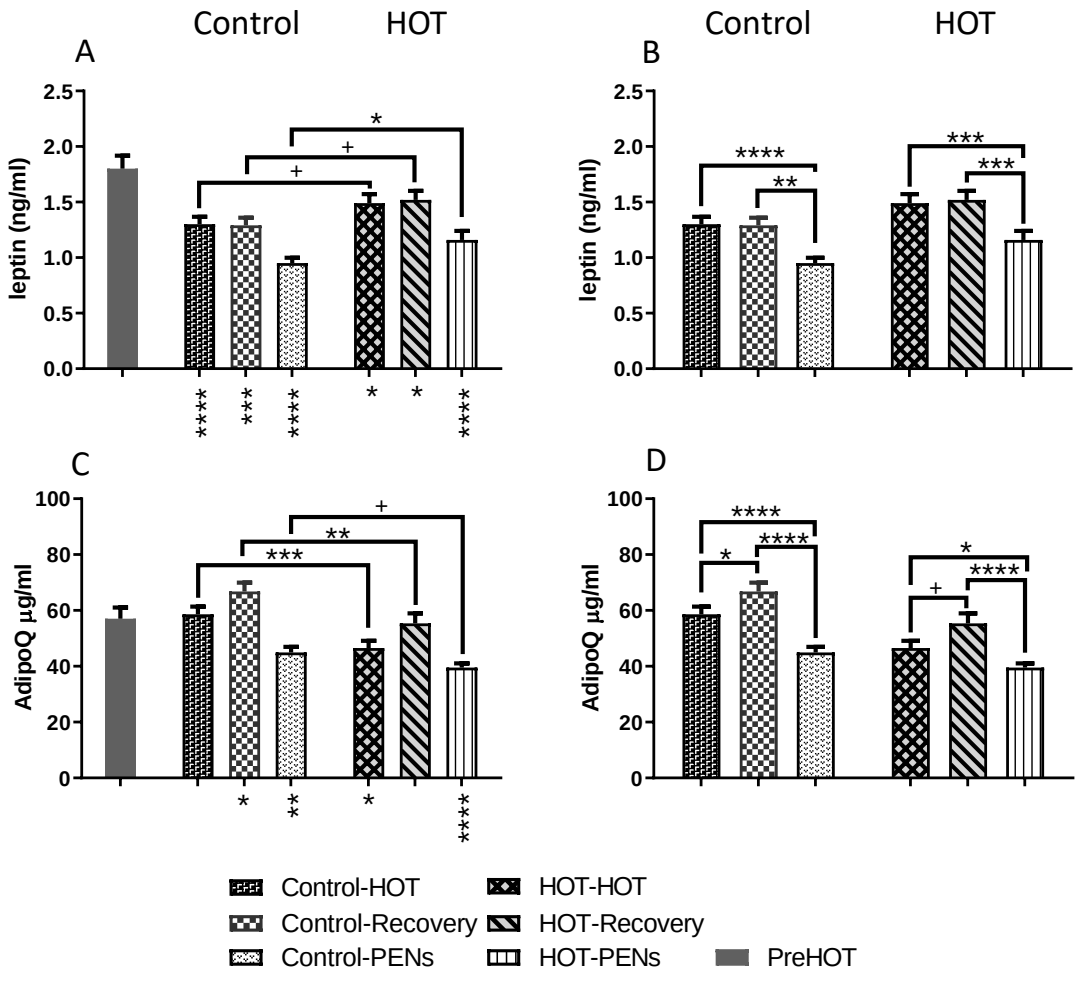

Fig. 100. Changes in plasma leptin and adiponectin (AdipoQ) concentrations during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A and C. Between group and ALL-PreHOT comparisons of the mean plasma leptin and AdipoQ concentrations (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B and D. Within treatment group comparisons of the mean plasma leptin and AdipoQ concentrations (± SEM) respectively of the Control and HOT groups during the HOT, Recovery and periods. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

The two thyroid hormones, T3 and T4, which influence energy metabolism and thermoregulation, were also examined for changes in plasma concentrations. The mean T3 concentrations of the Control group rose during HOT and Recovery relative to the PreHOT mean and the HOT group during those periods (Fig. 101A and B). There was no T3 response in the HOT group at any period. The mean T4 concentrations of the Control group mirrored their T3 concentrations (Fig. 101C and D). T3, the more active of the two hormones, is produced by the loss of an iodine atom, a function performed by a deiodinase in the liver. For the HOT group, there is no change in mean T4 concentration during HOT relative to the PreHOT mean but it is much lower than the mean T4 concentration for the Control group. In Recovery and PENs, the HOT group's mean T4 concentrations are high relative to PreHOT mean and the Control mean in PENs.

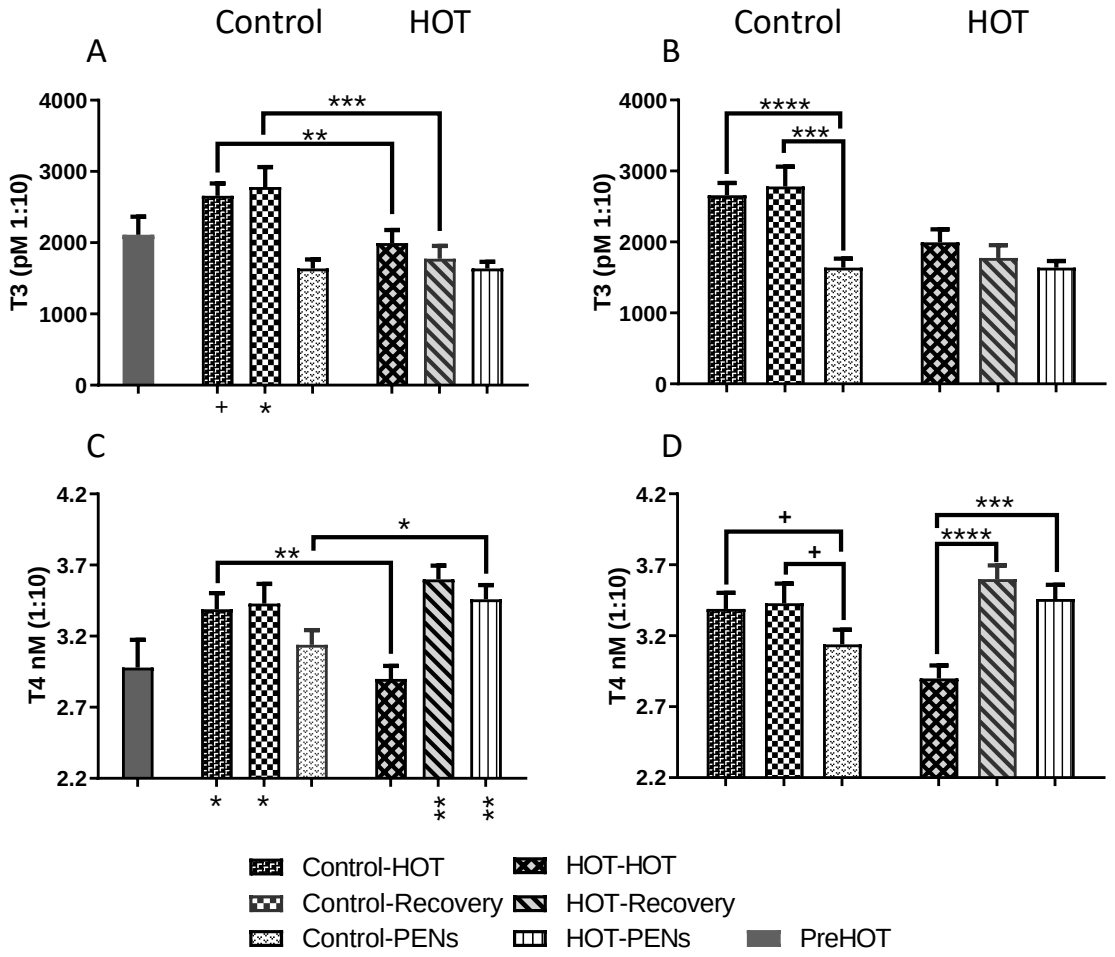

Fig. 101. Changes in plasma T3 and T4 concentrations during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A and C. Between group and ALL-PreHOT comparisons of the mean plasma T3 and T4 concentrations (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B and D. Within treatment group comparisons of the mean plasma T3 and T4 concentrations (± SEM) respectively of the Control and HOT groups during the HOT, Recovery and periods. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

4.1.5.7 Inflammatory response and endotoxin levels

Two of the pro-inflammatory major cytokines, TNFα and IL-1β, responded to high heat load with reduced plasma concentrations during HOT, Recovery and PENs, relative to the PreHOT means and to the Control means (Fig. 102A and B). IL-6, a cytokine implicated in the regulation of TNFα and IL-1β, behaved similarly (data not shown). In the Control animals, there was no change in mean TNFα and IL-1β concentrations at any time relative to the PreHOT mean. Interferon-γ (IFNγ), another major cytokine, behaved differently (Fig. 102C). During Recovery, the mean IFNγ concentration of the Control group rose. There was

no such rise in the HOT group. The mean of the HOT group fell during HOT (relative to the PreHOT mean). Increases in plasma endotoxin levels are known to set off strong inflammatory responses. As seen in Fig. 102D, the mean endotoxin levels were always low and in both treatment groups. There was a small non-significant rise in the HOT group during Recovery, but this level is too low to stimulate an inflammatory response.

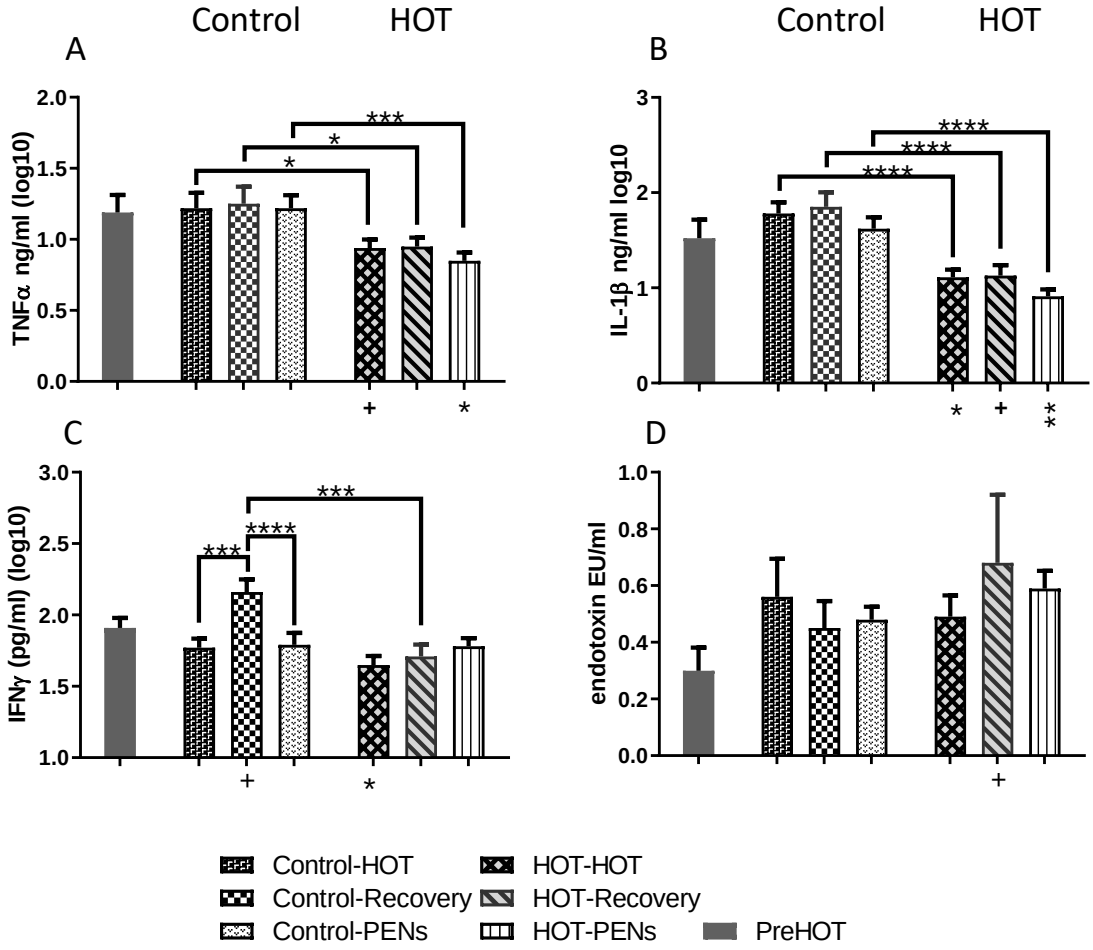

Fig. 102. Changes in plasma cytokine and endotoxin concentrations during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A - D. Within treatment group, and between treatment group and ALL-PreHOT comparisons of the mean plasma TNFα, IL-1β, IFNϒ and endotoxin concentrations (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

4.1.5.8 Discussion

The trial investigated the metabolic responses of an acute moderate heat load challenge to Black Angus feedlot steers (490 – 540 kg live weight). Comparisons were made to a Control group of animals maintained in thermoneutral conditions (TN) and pair fed but feed restricted during the period when the HOT cohort underwent thermal challenge. For the HOT group, the conditions in the climate chambers induced typical physiological responses: increased respiration rate, body and surface temperatures, and water consumption and reduced feed intake and live weight.

Under the conditions of this experiment, the thermally challenged animals maintained similar live weight during HOT and Controls, despite the much reduced feed intake. This weight parity by the HOT cohort may reflect the increased water uptake (an extra 6.2 L/head/day) and water retention in the tissues during the heat stress challenge. The increased water intake counteracts the escalation of water loss to cooling mechanisms, mainly evaporative sweating and increased respiration or panting which bring evaporative cooling deep into the core. Likewise, the HOT cohort in this study maintained their mean plasma protein concentrations relative to the Controls thus some of the subtler changes in plasma analytes discussed below are not due to haemoconcentration or haemodilution.

Restricted feeding (Controls) and voluntary feed restriction (HOT) induced a similar increase in plasma urea concentrations. The HOT animals experienced reduced plasma urea during recovery at TN and in PENS relative to the Controls.

The 8 – 11% increase in mean creatinine plasma concentrations of the Control group during TN and in PENS may reflect increased muscle turnover due to compensatory gain and movement after the period of restricted feed intake and movement in chambers. The marked 20% rise in mean creatinine plasma concentrations of the HOT group in response to high heat load also may have some contribution from increased muscle protein turnover or mobilisation during heat challenge but renal control has a strong influence on both circulating creatinine and urea concentrations. In the kidney, creatinine is freely excreted and while much urea is secreted, at least 50% of the urea is reabsorbed along with water. A scenario with reduced blood flow and glomerular filtration in the kidney resulting in less creatinine secretion and urea resorption explains the significantly reduced urea:creatinine ratio experienced by the HOT animals while under thermal stress.

The marked reduction in plasma bicarbonate concentrations in the HOT group while under heat load reflects the elevated respiration rate in an attempt to cool the body with evaporation from the mucosal surfaces of the respiratory system. Respiration rates of the HOT animals were 73% higher during the hottest 4 h of the HOT days in chamber compared to their TN counterparts. The loss of CO2 resulted in a 15% depression in plasma bicarbonate levels and possibly provoked the small 1 – 2% increase in plasma chloride concentration as a means to conserve circulating anion levels.

Under the conditions of this trial, the plasma concentrations of cation electrolytes were relatively stable with only subtle changes induced by high heat load and voluntary reduced intake or limited feed intake in thermoneutral conditions. The reduced intake was associated with increases in plasma sodium concentrations during the HOT and TN periods, but the increased levels in the HOT cohort were lower than that of the Controls. This suggests increased excretion of sodium by the kidney and in sweat as a response to increased body

temperatures. The steers in this study experienced no overt alteration in plasma potassium levels at any time despite the anticipated loss of this ion to perspiration, implicating cellular and renal mechanisms in preserving plasma potassium levels.

The plasma 'liver 'enzyme' profiles was used as indicators of changes in systemic and tissue metabolism in responding to feed restriction and thermal challenge. The activities of the four enzymes showed different behaviours in the two treatment groups. In the feed restricted thermoneutral control animals, plasma ALP activity decreased by approximately 15% and did not return to pretreatment levels. The reduction in plasma ALP activity in the thermally challenged cohort was much greater and remained suppressed on return to thermoneutral conditions.

Plasma AST activity was reduced in the Control steers, probably as a response to reduced feed intake. The HOT animals showed higher plasma activity than the Controls during all three periods, that is, in the HOT animals there was change in plasma AST activity as feed intake reduced. Generally rising plasma AST activity is indicative of liver damage, but these levels of activity were not higher than those of the PreHOT period. Thus, the higher values in the HOT animals during recovery and in PENs may reflect elevated expression of hepatic AST and/or increased release of with increased cell turnover or sustained ER stress.

Altered rates of cholesterol metabolism has been reported in association with thermal stress. In this trial, the low plasma cholesterol in the HOT animals persisted well into Recovery and despite resumption of normal feed intake. This persistence in low plasma cholesterol has been observed when monitoring metabolism in dairy cows during summer heat waves.

Reports of reductions of plasma glucose and NEFA concentrations or lack of an overt rise in plasma NEFA in the face of reduced feed intake during heat stress in cattle are not entirely consistent however, the general view is that these two energy metabolites are metabolised but not replenished at the systemic level, however, there was no clear glucose or NEFA response to heat challenge by the HOT animals when compared to the Control cohort. Given the markedly reduced feed intake during that period, it is surprising to see that the HOT group maintained their mean plasma glucose concentration to that of the Controls. Since ruminal propionate was limited, the defence of the plasma glucose must have depended on other gluconeogenic substrates such as lactate and amino acids and/or utility of hepatic glycogen stores.

The lower concentration of circulating triglycerides in the HOT group during HOT implies a limited response by the adipose depots. Defence of adipose stores have been noted in a number of heat stress models and in livestock produced in warm environments. During Recovery, the HOT group's mean plasma triglyceride level rose increasing the availability of fatty acids to all tissues; the rise in circulating triglycerides coincided with a rise in circulating NEFA in this group. The plasma triglyceride level then fell below that of the Control group in PENS while the plasma NEFA continued to rise suggesting increased circulatory and tissue level LPL activity. Clearly there are sustained effects even from moderate heat load on lipid metabolism.

Free glutamine has a multitude of functions in cells and tissues. Amongst these is to act as a vital energy substrate in rapidly proliferative and high metabolically active cells. Glutamine, through glutaminolysis interacts with the TCA cycle. In response to reduced feed intake, the

Control animals are likely to have released hepatic and skeletal muscle glutamine to act as a glucose precursor for the gut and kidneys. The significantly lower plasma glutamine concentrations achieved by the HOT groups during HOT and Recovery attest to increased consumption of free plasma glutamine or reduced release and possibly synthesis in the liver and skeletal muscle.

The protein hormones, leptin and adiponectin, were impacted by both feed restriction and increased heat load. Leptin which acts as a brake on appetite, was reduced in the Control group while under feed restriction (HOT) and in Recovery; an appropriate response. In the HOT group, leptin concentration was only slightly reduced in HOT and Recovery, thus imparting a signal to reduce feed intake. Adiponectin levels of the HOT group were reduced in HOT and Recovery relative to the Control group, limiting its positive influence on glucose metabolism and lipolysis. The thyroid hormones acted in concert in the feed restricted Control during feed restriction by the upregulation of both T3 and T4 during HOT and Recovery. In contrast, T4 was markedly suppressed in the HOT group during HOT, implicating decreased production and release by the thyroid. However, the hepatic conversion to T3 was apparently unaffected in these conditions. The overall endocrine scenario in the HOT animals was to reduce feed intake, fatty acid and glucose metabolism and metabolic rate.

In assessing the inflammatory status in these moderate heat load steers, all cytokines were found be of reduced concentration during HOT and Recovery. Except for a rise in IFNγ in Recovery, there was no change in inflammatory status in the Control group during HOT and Recovery.

4.1.6 Chronic moderate heat load climate chamber study - CC1-3

4.1.6.1 Introduction

In brief, three cohorts of six steers (HOT) were maintained at thermoneutral conditions in the climate controlled rooms, cycling between 16 and 22°C as minimum and maximum temperatures for three days. Over the subsequent three days they were gradually taken to a night-time minimum temperature of 22°C, and over seven days to a daytime maximum temperature of 32°C (Fig.103). This treatment group remained in these conditions for a further seven days, before being returned to the thermoneutral conditions. The first cohort, CC1, was terminated at day 17 to allow post-mortem tissue collection. The analyses presented in the following sections pertain to the data collected from the CC2 and 3 cohorts only. These two cohorts proceeded through the entire regime shown in Fig. 103. On exiting the climate rooms on day 21, the cattle were housed in feedlot pens, and monitored for a further 17 days.

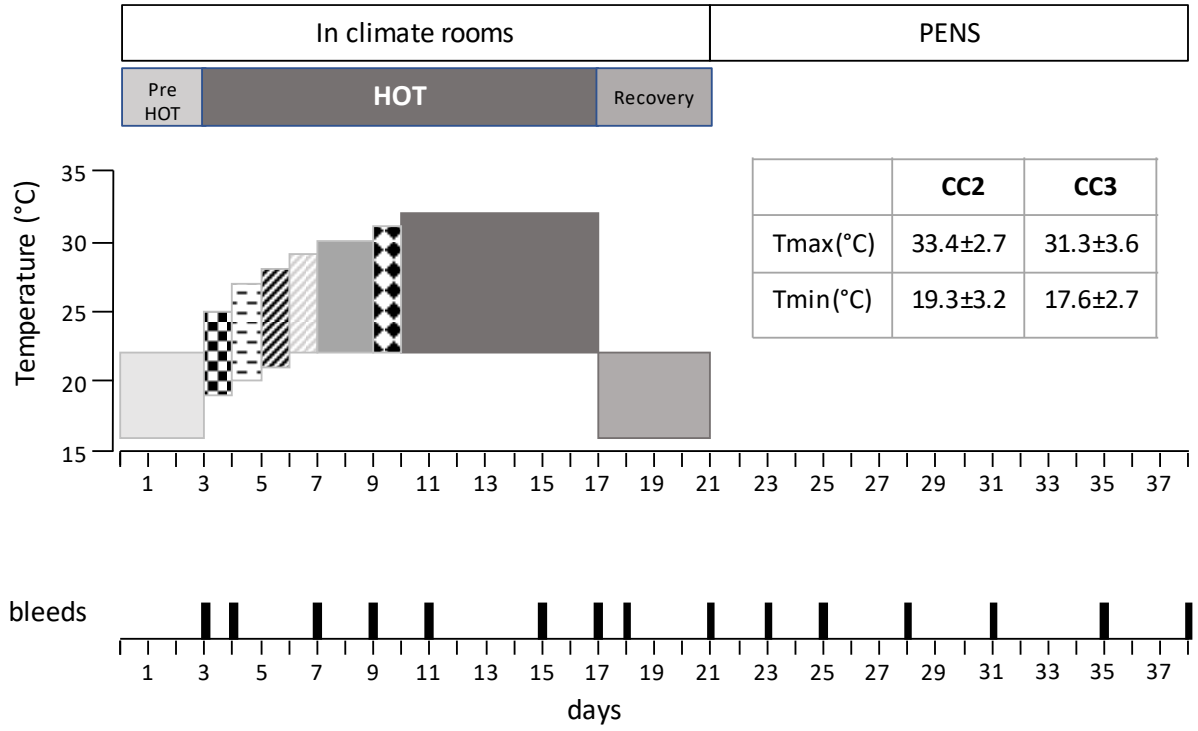

Fig. 103. The climatic regime of the chronic moderate heat load challenge. The range of the mean daily ambient temperature and duration of each period is depicted for the 38 days of the experiment. The PreHOT, HOT and Recovery periods were conducted in climate controlled rooms. Blood sampling days are indicated also. The PENS interval occurred in outdoor feedlot pens and the mean temperature conditions for those periods were calculated from Bureau of Meteorology Gatton weather station data.

Each group of six steers that were exposed to the thermal challenge were accompanied by six steers of matching weight that remained between 16- 22°C for 24 hours for the 21 days in the climate controlled rooms. The thermoneutral Controls were pairfed: that is, each control steer was offered the amount of feed that its weight pair consumed the previous day. All animals were on a finisher ration throughout (offered twice daily). Intensive recording of behavioural and physiological data was augmented with haematological and biochemical data obtained from blood and plasma collected at frequent bleeds. The bleed schedule is given in Fig. 103.

4.1.6.2 Animal Performance

During PreHOT, the mean DMI was 9.9 kg/head/day. There was a small but significant fall (0.3 kg/head/day) in the HOT group during HOT, which tended to be lower than intake during Recovery (Fig. 104A). There was no change in mean DMI for the Control during HOT or Recovery.

The mean live weight during PreHOT was 428.5 kg/head. Neither treatment group gained weight during HOT despite the minimal change in feed intake (Fig. 104B). While the Control group gained 13.3 kg/head on average during Recovery (relative to the HOT mean), the

HOT group had a non-significant increase of 8.5 kg/head. Once in PENs, both groups approached 470 kg/head live weight.

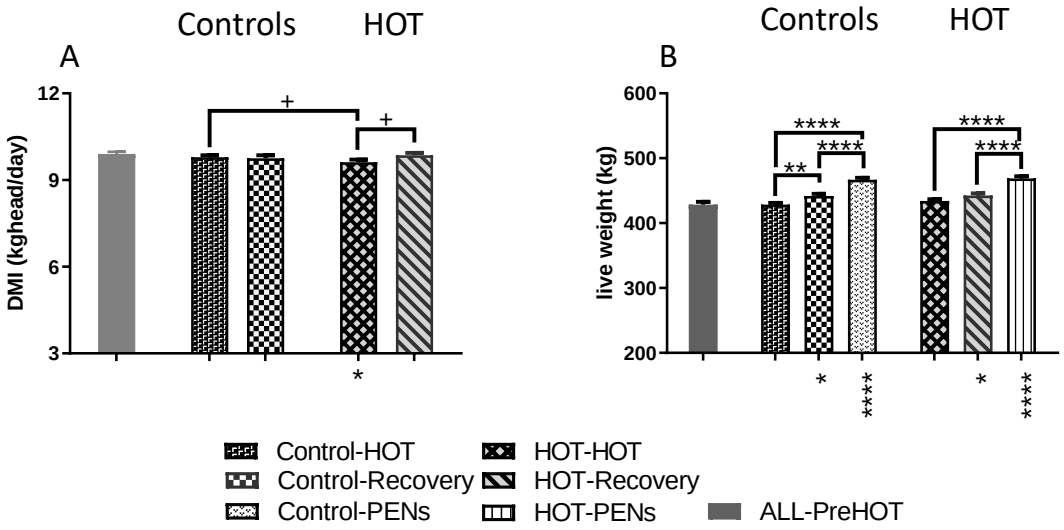

Fig. 104. Animal performance before (PreHOT), during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panel A. Between periods and within treatment comparisons of the mean DMI (± SEM) of the Control and HOT groups. Panel B. Between periods and within treatment comparisons of live weight (± SEM) of the Control and HOT groups during the HOT and Recovery periods. The asterisks under the x-axis indicate statistically significant difference with PreHOT. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$.

4.1.6.3 Physiological responses

In the PreHOT period, water usage averaged at 41 L/head/day (Fig. 105A). For the Control group who remained in thermoneutral conditions, mean water usage actually fell to 31-33 L/head/day during HOT and Recovery. The HOT group had a ~10% rise in mean water usage during HOT to about 45 L/head/day ($p = 0.106$), and water usage fell to ~37 L/head/day in Recovery for this treatment group (Fig. 105A and B). Therefore, the HOT group not only used more water during the HOT period but also in Recovery relative to the Controls.

After three days at thermoneutral conditions in the PreHOT interval, the mean rectal temperature was 38.89°C (Fig. 105C and D). For the Control group there was no significant change in mean rectal temperature during HOT, but if fell slightly to mean of 38.63°C in Recovery, and rose again in PENs to mean of 39.15°C. The HOT group experienced a small rise in mean rectal temperature to 39.06°C in HOT, which fell during Recovery to 38.58°C,

and rebounded to 39.20°C in PENs. There was no significant difference in the mean rectal temperatures between Control and HOT groups in Recovery and PENs (Fig. 105C).

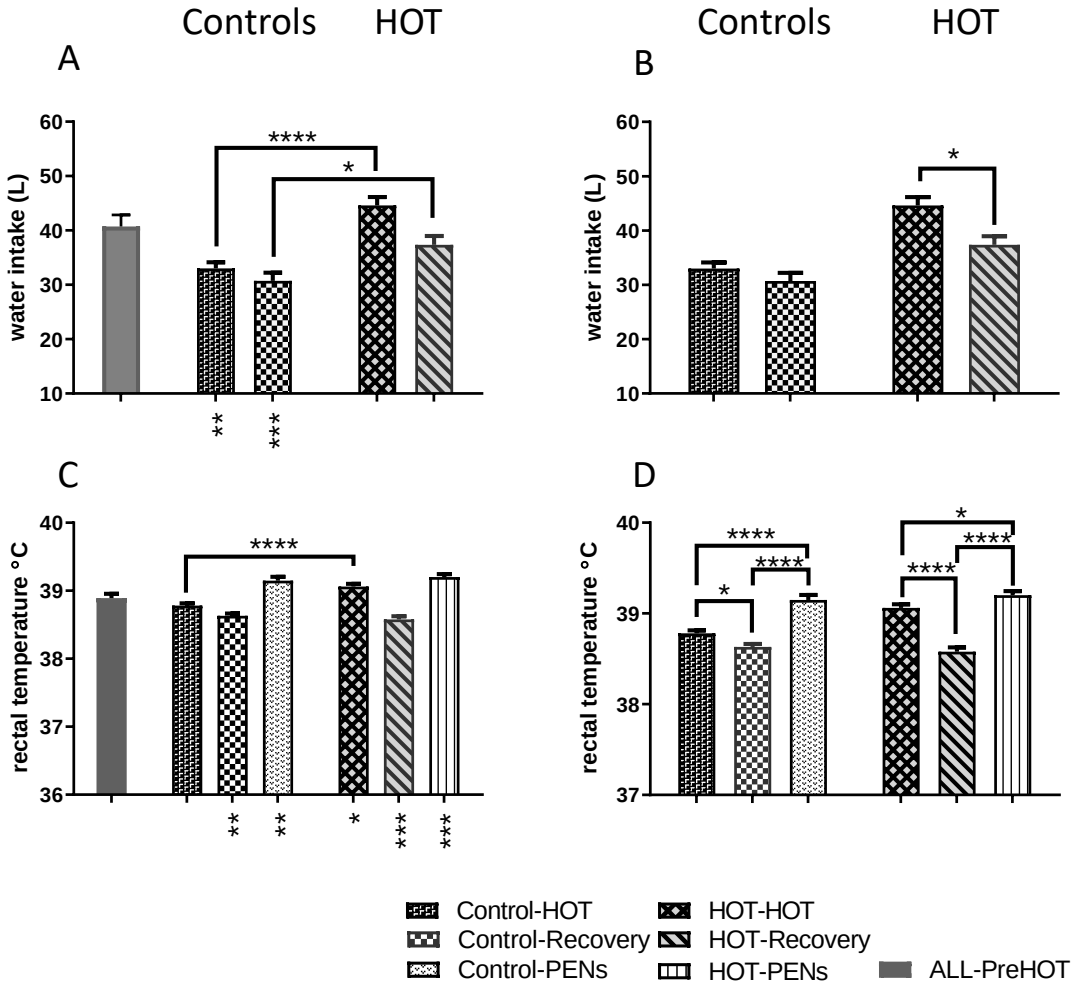

Fig. 105. Changes in water usage and rectal temperatures in response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Water usage was not recorded when in PENs. Panel A. Between group and ALL-PreHOT comparisons of water intake. Panel B. Within group comparison of water intake during HOT and Recovery. Panel C. Between group and ALL-PreHOT comparisons of the mean rectal temperatures (± SEM) of the HOT and Control groups during the HOT, Recovery and PENS periods. Panel D. Within group comparison of rectal temperatures during the HOT, Recovery and PENS periods. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

The mean respiration rate (RR) was ~69 bpm during PreHOT (Fig. 106A). For the Control group, it fell to 48-53 bpm during HOT and Recovery, whereas the HOT group increased their mean RR to 116 bpm during HOT and recovered to ~60 bpm in Recovery (Fig. 106A and B).

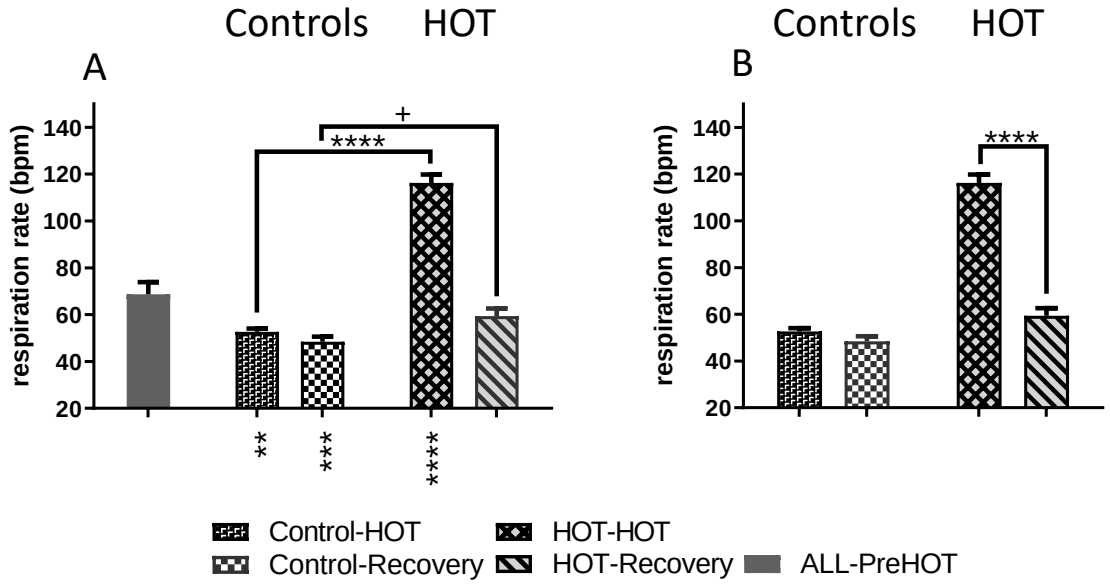

Fig. 106. Changes in respiration rate in response to thermal challenge (HOT) and during Recovery. The ALL-PreHOT value in the first histogram is the mean ± SEM of all animals during that interval. Panel A. Between group and ALL-PreHOT comparisons of the mean respiration rate (± SEM) of the HOT and Control groups during the HOT and Recovery periods. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panel B. Within group comparisons of the mean respiration rate (± SEM) of the HOT and Control groups. +, p <0.1; **, p <0.01; ***, p <0.001; ****, p <0.0001.

4.1.6.4 Metabolic responses

Plasma buffering and electrolytes

There were subtle and similar changes in mean plasma bicarbonate concentration in both groups across the three periods (data not shown). The Control group experienced a small (~5%) but significant increase in mean plasma bicarbonate concentration in the HOT period relative to the PreHOT mean; there was no change in the HOT group at this time. The mean plasma bicarbonate concentration in both groups fell marginally and progressively during Recovery and PENs.

There were small changes in mean plasma potassium concentration in both treatment groups also. In the Control group, relative to the PreHOT mean, the mean plasma potassium concentration was reduced by ~4% in Recovery and PENs (Fig. 107). For the HOT group, the mean plasma potassium concentration was 5.1-7.3% lower than the PreHOT mean over the three periods. In the HOT period, the HOT animals had lower mean plasma potassium concentration than the Control animals (Fig. 107). Both groups displayed a small decrease

in mean sodium concentration in Recovery, however there was no alterations in chloride concentrations in the treatment groups at any period (data not shown). There was a small but significant rise of 5% in the mean plasma phosphate concentration by the HOT animals during HOT relative to the Control mean for that period. Otherwise, mean plasma phosphate concentrations were stable (data not shown).

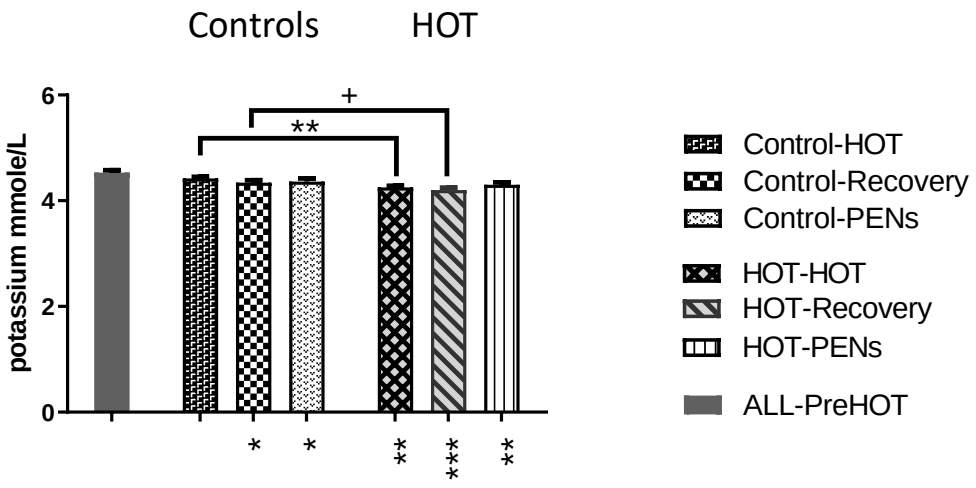

Fig. 107. Changes in plasma potassium concentration during (HOT) and after thermal challenge (Recovery and PENS). The ALL-PreHOT value in the histogram is the mean ± SEM of all animals during that interval. Between group and ALL-PreHOT comparisons of the mean plasma potassium concentrations (± SEM). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Urea and creatinine

The mean plasma creatinine concentration of both groups was highest in PENs and not different to each other (Fig. 108A), probably reflecting the higher muscle mass as weight and age increased. The Control group mean plasma creatinine concentration did not change in response to feed restriction during HOT and Recovery. The HOT group saw significant rises in plasma creatinine concentrations in HOT and Recovery. The mean of the HOT group during HOT was 8-10% higher than the PreHOT mean and the Control mean (Fig. 108A). The mean of the HOT group during Recovery was 5.7 and 7.7% higher than the PreHOT mean and the Control mean at Recovery respectively.

Plasma urea concentrations behaved differently to those of creatinine (Fig. 108B and C). For the Control group, the mean plasma urea concentrations were very stable across the periods but there was some movement in concentration in the HOT group. The mean plasma urea concentrations of the HOT group during HOT was not different from the PreHOT mean but was ~9% higher than the Control mean. During Recovery, the mean of the HOT group was 11.2 and 10.4% greater than the PreHOT mean and the Control mean (at Recovery)

respectively. Fig. 108C shows that the HOT group mean at Recovery was higher than the means at HOT and in PENs. There was no difference in the treatment group once in PENs.

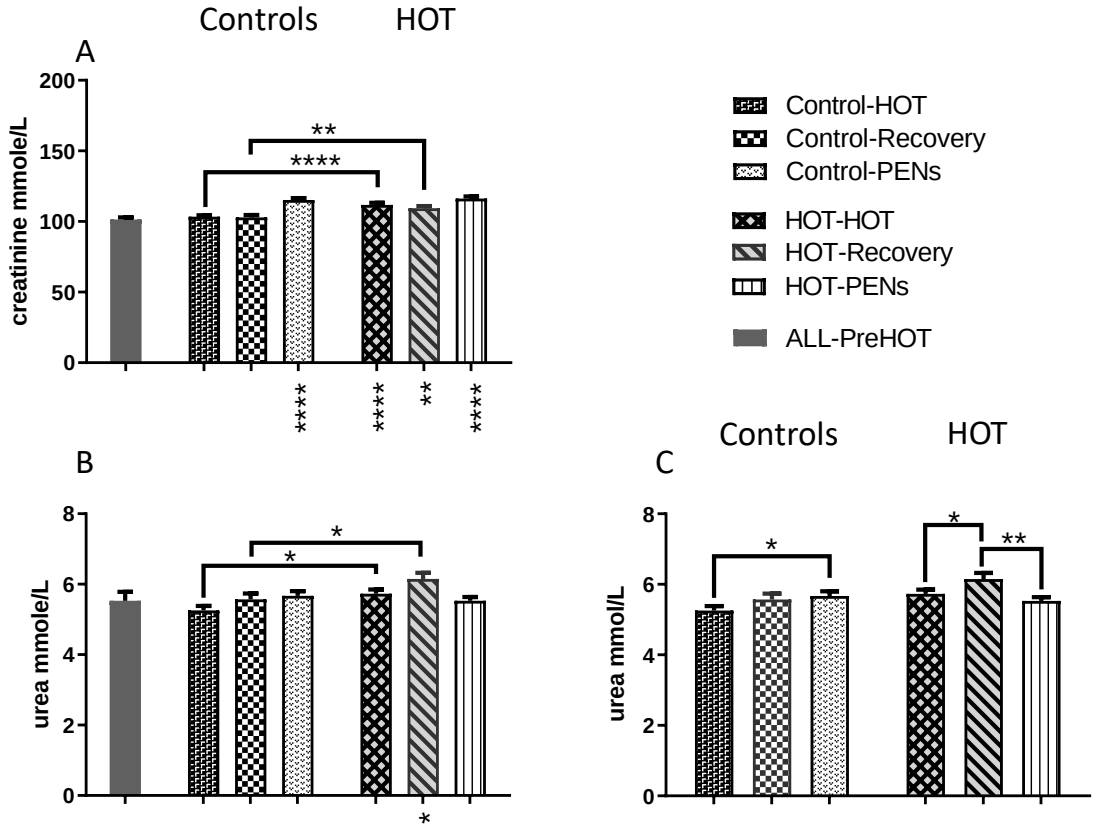

Fig. 108. Changes in plasma creatinine and urea concentrations during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panel A. Between group and ALL-PreHOT comparisons of the mean plasma creatinine concentrations (± SEM). Panel B. Between group and ALL-PreHOT comparisons of the mean plasma urea concentrations (± SEM). The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panel C. Within group comparisons of the mean plasma urea concentrations (± SEM) of the HOT and Control groups. *, p <0.05; **, p <0.01; ****, p <0.0001.

Liver function enzymes, cholesterol and triglycerides

The ALP activities of the two groups behaved very differently across HOT, Recovery and PENs. Generally, the mean plasma activities for ALP increased in the Control group and fell in the HOT group during the three different periods (Fig. 109A and B) and the means of the Control group were all significantly higher than those of the HOT group for three periods and the PreHOT mean. The Control group means during HOT and Recovery were 32-36% higher than the PreHOT mean, and in PENs, it was ~15% higher. In contrast, the HOT group means during HOT and Recovery were 15.5 and 26.5% lower than the PreHOT mean

respectively. In PENs, the mean plasma ALP activity of the HOT group returned to the PreHOT level but did not match that of the Control.

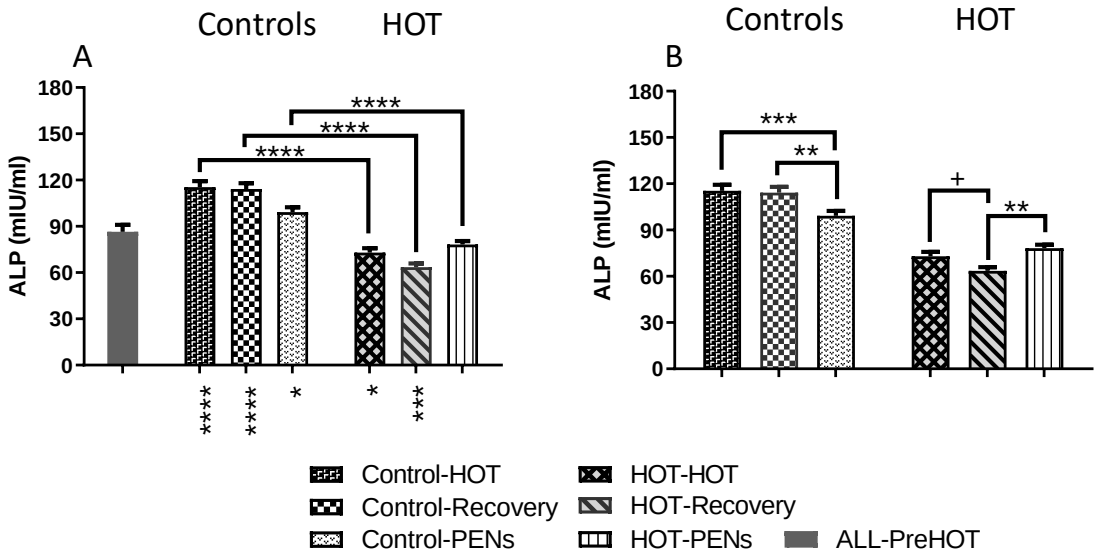

Fig. 109. Changes in ALP activity in response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panel A. Between group and ALL-PreHOT comparisons of the mean plasma ALP activity (± SEM) of the HOT and Control groups during the HOT, Recovery and PENS periods. Panel B. Within group comparisons of the mean plasma ALP activity (± SEM) of the HOT and Control groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

The mean plasma AST activity dropped by about 12% in both groups during the Recovery period relative to the PreHOT mean and their own HOT period means (data not shown). There was no difference in plasma AST response in the two treatment groups.

The mean plasma GGT activity of the Control group during the three periods was stable and was not different to the PreHOT mean (Fig. 110A). In contrast, the mean plasma GGT activity of the HOT group was 12-18% higher than the PreHOT mean during the three periods. The mean of the HOT group during HOT was ~11% greater than the mean of its thermoneutral feed restricted Control (Fig. 110A). The mean GLDH activity behaved quite differently to GGT activity in both treatment groups (Fig. 110B). It rose by 46 and 56% as feed restriction was imposed on the Control group during HOT relative to the PreHOT mean and the HOT group's mean during HOT. Following this rise, the mean plasma GLDH activity of the Control group returned to levels close to the PreHOT mean (Fig. 110B).

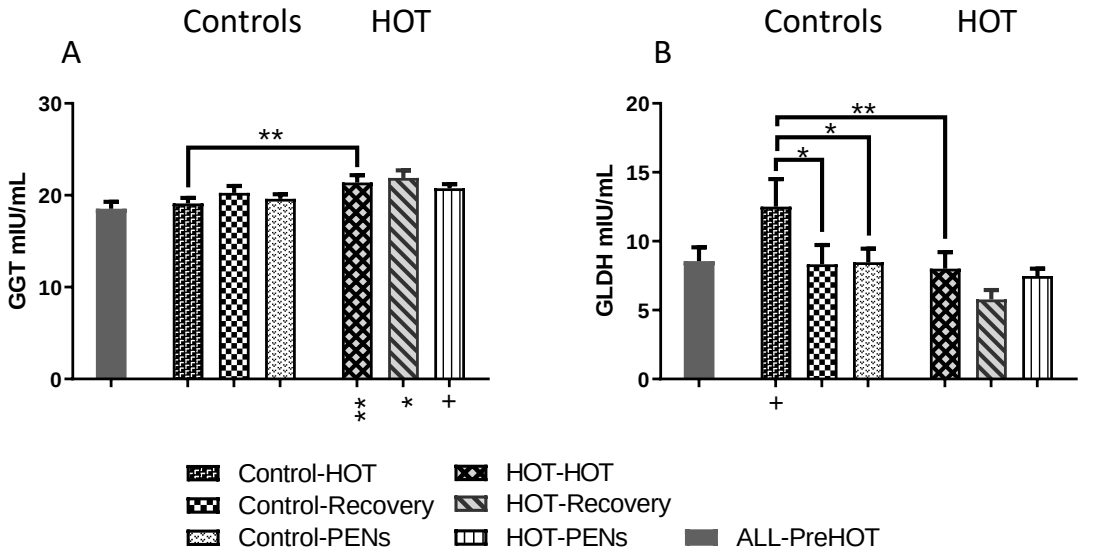

Fig. 110. Changes in plasma GGT and GLDH activities during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panel A and B. Between group and ALL-PreHOT comparisons of the mean plasma GGT and GLDH activities (± SEM) respectively. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. +, p <0.1; *, p <0.05; **, p <0.01.

The plasma cholesterol concentrations responded similarly in both treatment groups during the three periods, increasing during Recovery and PENs (data not shown). Relative to the PreHOT mean, the mean cholesterol concentrations were 18.5-21% and 26-29% higher during Recovery and PEN respectively. The mean triglyceride concentrations did not differ with the PreHOT mean at any period for either treatment group (Fig. 111). However, there was an apparent response detected in the Control group relative to the HOT group during HOT when the Control mean triglyceride concentration was ~14% higher than that of the HOT group.

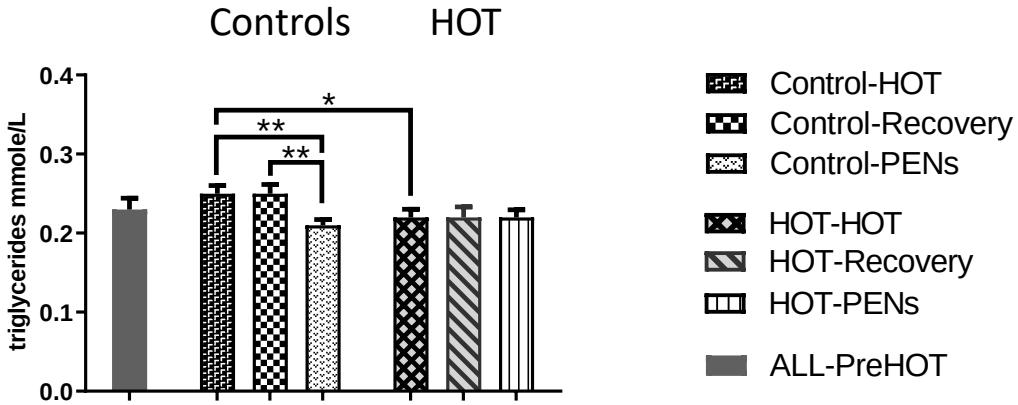

Fig. 111. Changes in plasma triglyceride concentrations in response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Between group and ALL-PreHOT, and within group comparisons of the mean plasma triglyceride concentrations (± SEM) respectively of the HOT and Control groups during all periods. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. *, $p < 0.05$; **, $p < 0.01$.

Energy Metabolism

The mean plasma glucose concentrations were stable across treatment groups and all periods (Fig. 112A). The mean plasma insulin concentrations responded differently between the treatment groups (Fig. 112B). In the Control group during HOT, the mean plasma insulin concentration fell ~27% relative to the PreHOT mean and was ~18% lower than the HOT group's mean. Thereafter, the mean plasma insulin concentrations decreased in both groups relative to the PreHOT mean (~50% in the Control group and 38-40% in the HOT group).

Unlike the glucose concentration, but like the insulin concentrations, plasma NEFA concentrations responded differently to the treatments (Fig. 112C and D). The mean plasma NEFA concentrations of the Controls were 31, 54 and 23% higher than the PreHOT mean during HOT, Recovery and PENS respectively. The HOT group's mean during HOT was not different to the PreHOT mean but was 30% lower than the Control mean during HOT. In the subsequent periods, the HOT group means increased and were similar to those of the Control group and 31-39% higher than the PreHOT mean (Fig. 112C and D).

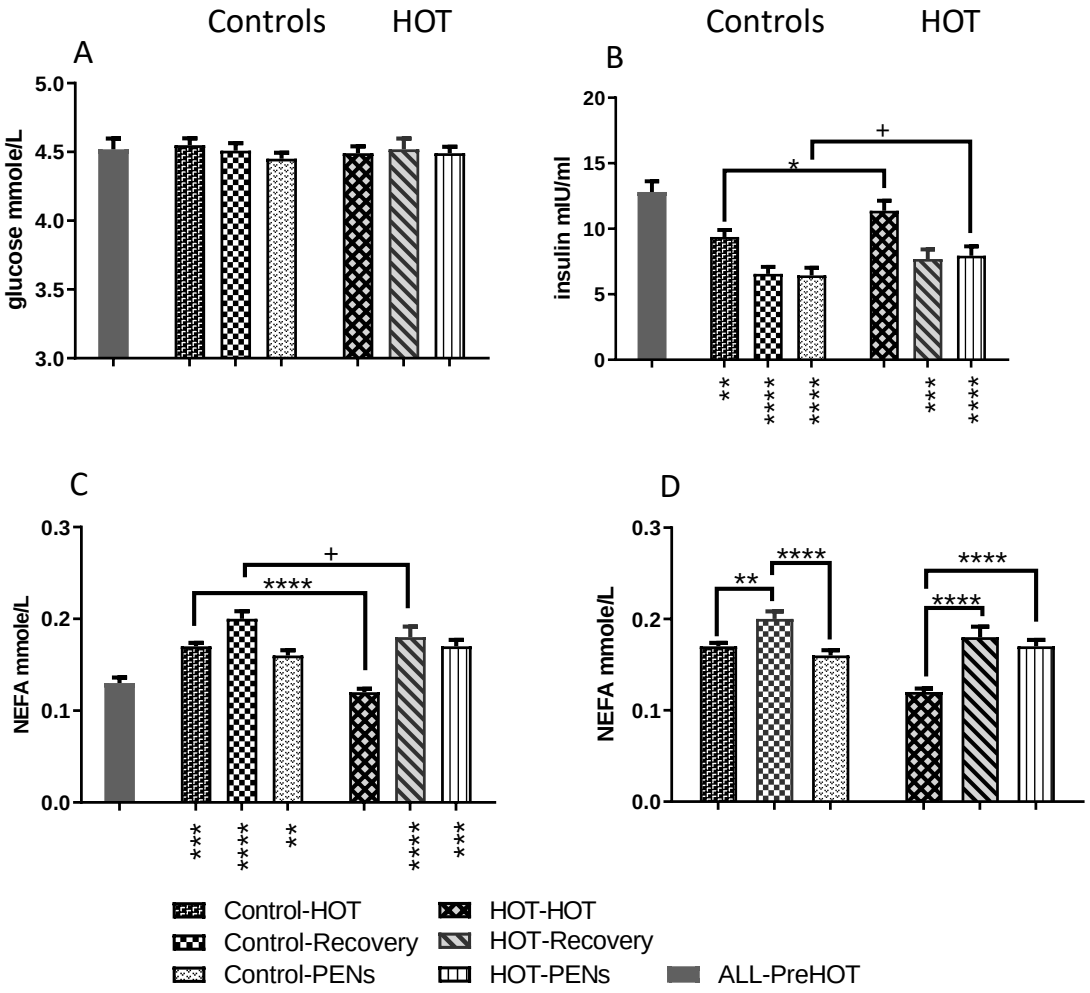

Fig. 112. Changes in plasma glucose, NEFA and insulin concentrations in response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A and B. Within group, and between group and ALL-PreHOT comparisons of the mean plasma glucose and insulin concentrations respectively. Panel C. Between group and ALL-PreHOT comparisons of the mean plasma NEFA concentration (± SEM) of the HOT and Control groups during the HOT, Recovery and PENS periods. Panel B. Within group comparisons of the mean plasma NEFA concentration (± SEM) of the HOT and Control groups.

4.1.6.5 Haematological changes

There was no change in the mean white blood cell (WBC) counts for the Controls over the three periods (Fig. 113A and B). After the HOT period, the HOT group experienced reduced mean WBC counts that were ~15% and 9-11% lower than the PreHOT and Control means respectively. The HOT group had reduced mean PBL counts while in Recovery and PENs relative to Control means (data not shown). The mean neutrophil numbers were stable for the Control group during all periods but were 23-24% lower than the PreHOT mean. In contrast, the HOT group saw a rise in neutrophils during the HOT relative to the Control

mean but not the PreHOT mean (Fig. 113C and D). The HOT group's mean eosinophil count was significantly lower (77%) than the Control mean (data not shown). Monocyte counts were lower in all groups in all periods relative to the PreHOT (data not shown).

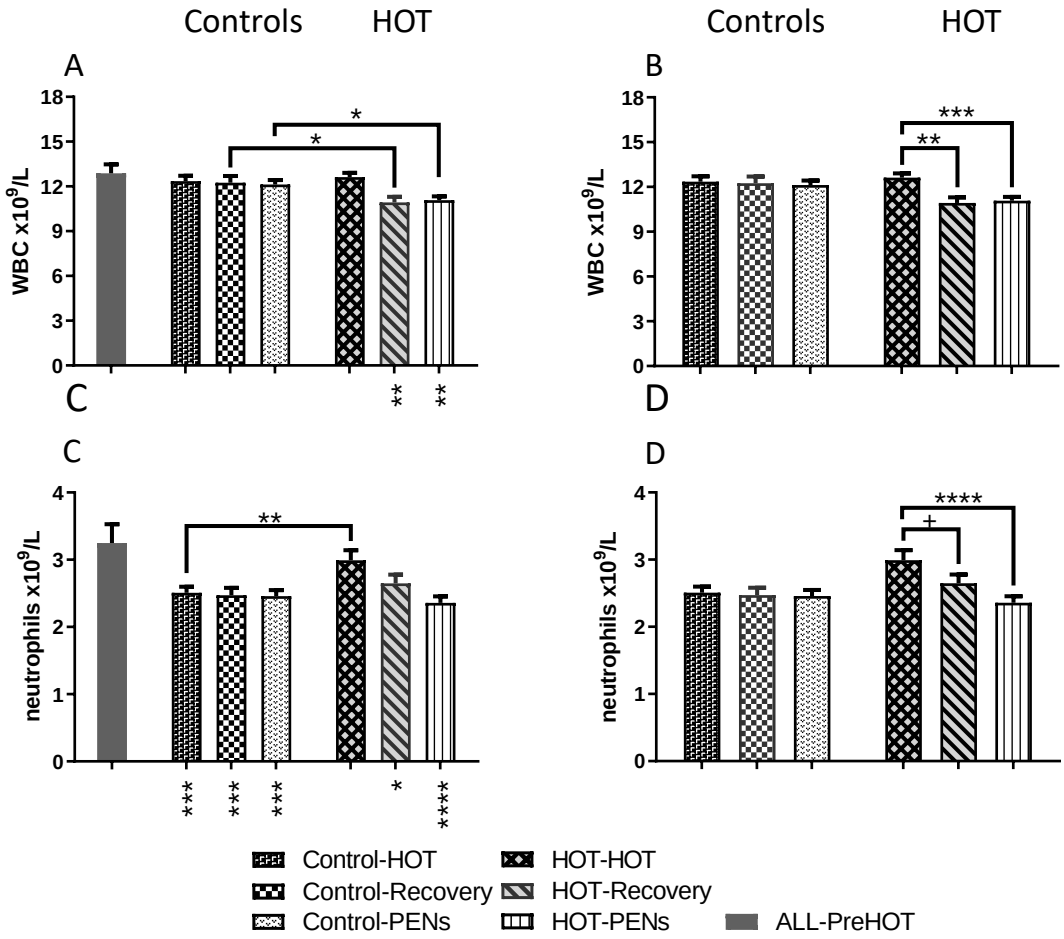

Fig. 113. Changes in WBC and neutrophil counts during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panels A and C. Between group and ALL-PreHOT comparisons of the mean WBC and neutrophil counts (± SEM) respectively of the Control and HOT groups. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. Panels B and D. Within treatment group comparisons of the mean WBC and neutrophil counts (± SEM) respectively of the Control and HOT groups during the HOT, Recovery and periods. +, p <0.1; *, p <0.05; **, p <0.01; ***, p <0.001; ****, p <0.0001.

The mean RBC counts were or tended to be higher than the PreHOT mean and the Control means for the respective periods (Fig. 114A and B). During HOT, the HOT group's mean was 5-7% increase over the PreHOT mean and the Control during HOT. In Recovery and PENs, the HOT group means were 17% higher than the PreHOT mean, and 10-12% higher

than the Control means. Mean haematocrit and total haemoglobin concentration followed a similar pattern (data not shown).

During HOT and Recovery, the mean platelet counts for the Control group did not change relative to the PreHOT mean but rose by 40% in PENs (Fig. 114C and D). The HOT group means in HOT and Recovery were 30 and 50% higher than the PreHOT mean respectively, and 32% and 35% higher than the respective Control means.

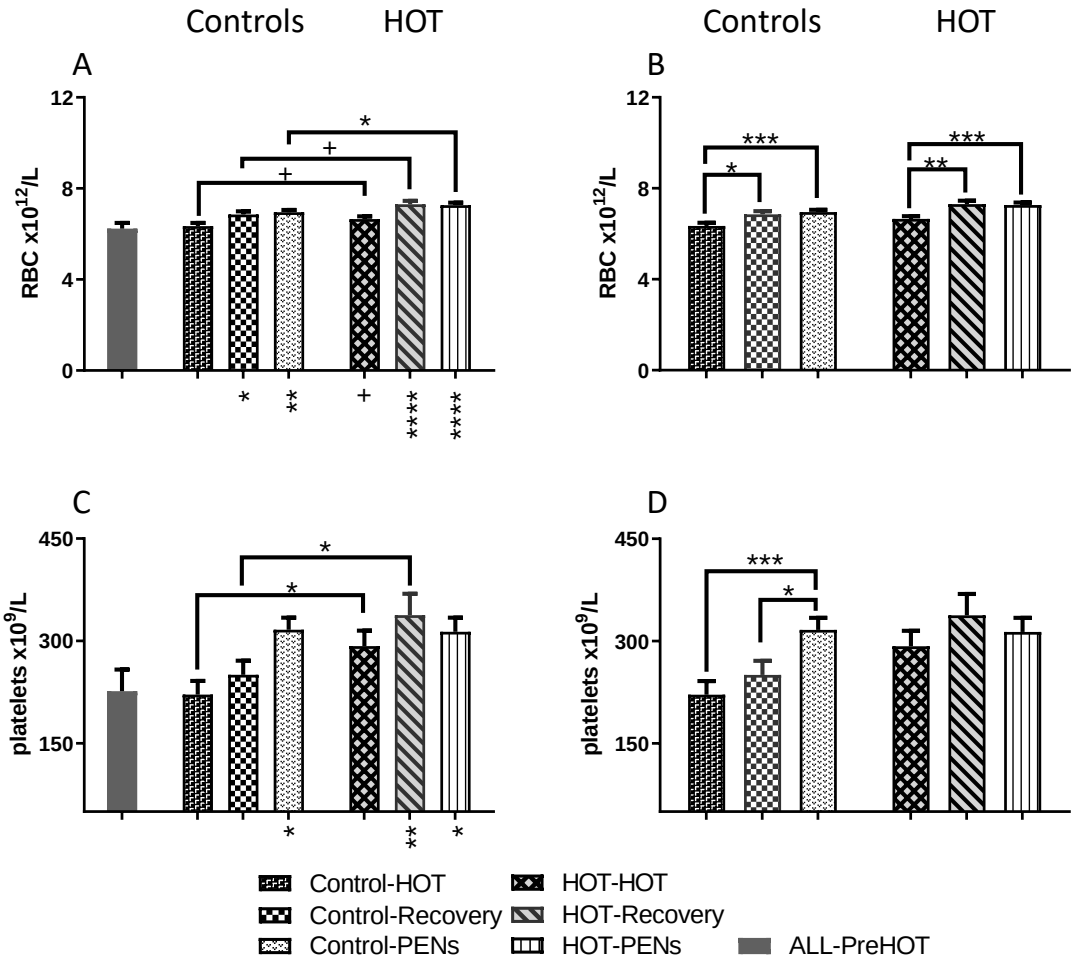

Fig. 114. Changes in circulating red blood cell (RBC) and platelet numbers in response to thermal challenge (HOT) and during recovery (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. Panel A and C. Between group and ALL-PreHOT comparisons of the mean RBC and platelet numbers (± SEM) respectively of the HOT and Control groups. Panel B and D. Within group comparisons of the mean RBC and platelet numbers (± SEM) respectively of the HOT and Control groups during the HOT, Recovery and PENS periods. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. +, p<0.1; *, p <0.05; **, p <0.01; ***, p <0.001.

4.1.6.6 Endocrine changes

Only leptin, adiponectin and insulin were measured for this climate chamber experiment. The insulin response has been described above. The mean leptin concentrations were unchanged in both treatment groups during HOT and Recovery but rose ~20% in PENs relative to the PreHOT mean in both groups (data not shown). Adiponectin levels showed no response to treatments also, but in both groups dropped 20-28% in PENs (relative to the PreHOT mean, data not shown).

4.1.6.7 Inflammatory response and endotoxin levels

There was no change to mean TNFα concentration with treatments, although within the groups, the concentration was highest in PENs (data not shown). Similarly, the mean IL10 concentration was not altered by treatments, but the HOT group's mean was lower in Recovery than that of the Control while at Recovery (data not shown). The mean IL-1β concentrations of the HOT group were slightly higher than the PreHOT in Recovery and PENs (Fig. 115A). The HOT mean in PENs was higher than its Control mean also. As seen in Fig. 115B, the mean endotoxin levels were always low in both treatment groups, and lower at all times and both groups relative to the PreHOT mean.

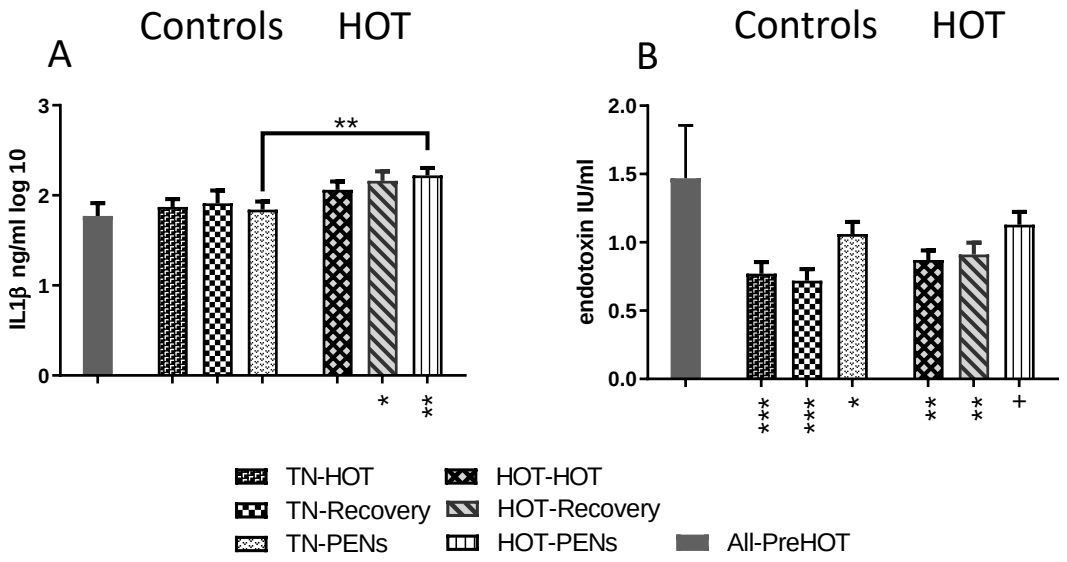

Fig. 115. Changes in plasma IL1β and endotoxin concentrations during (HOT) and after thermal challenge (Recovery and PENS). Panel A. Between group and ALL-PreHOT comparisons of the mean plasma IL1β concentrations (± SEM) during (HOT) and after thermal challenge (Recovery and PENS). Panel B. Between group and ALL-PreHOT comparisons of the mean plasma endotoxin concentrations (± SEM) during (HOT) and after thermal challenge (Recovery and PENS). Control animals were feed restricted and maintained at thermoneutral conditions until PENS. The ALL-PreHOT value in each histogram is the mean ± SEM of all animals during that interval. The asterisks under the x-axis indicate statistically significant difference with ALL-PreHOT. +, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

4.1.6.8 Discussion

CC1-3 was the first climate chamber experiment of the research program and very much a learning experience. As with CC4-6, comparisons were made to a Control group of steers maintained in thermoneutral conditions and pair fed during the period when the HOT group underwent thermal challenge. The HOT group was gradually stepped up to the warm conditions (HOT) over six days which would have permitted some adaptation. The 24 h temperature range was 22 – 32°C; these are very mild conditions compared to the later heat load regimes.

Furthermore, the responses of both treatment groups are likely to have been (experimentally) compromised by their previous experience of hot weather. In the four weeks prior to entry to climate chambers the CC2 cohort had already experienced mild and moderate-strong heatwaves. In PENs, CC2 were subjected to another strong heatwave. The CC3 cohort only saw a mild heat wave three weeks prior to entering the climate chambers.

Nonetheless, there was a small but significant fall in DMI in the HOT group during HOT, however there was no change in mean DMI for the Control during HOT or Recovery. Despite this, neither treatment group gained weight during HOT despite the minimal change in feed intake. The HOT group showed increased levels of water usage, rectal temperature and respiration rate during HOT when compared to Controls.

In spite of the increased respiration rate, plasma bicarbonate levels were not affected in the HOT group during HOT, suggesting that any increased exhalation of CO_2 was being completely compensated for. In terms of other metabolic responses, some of the usual suspects were affected by these relatively mild conditions. Plasma creatinine was increased in the HOT group during HOT, implicating a renal response, but with no change in plasma bicarbonate there was no requirement to retain chloride. Confirming instigation of the renal involvement, was the slight increase in plasma urea in the HOT group relative to the feed restricted Controls during the HOT period.

Consistent with our other heat load experiments in the climate chambers was the fall in ALP and its persistently low levels during Recovery and into PENs. This plasma enzyme derived from liver and bone is very sensitive to the heat load.

The Control group displayed a textbook response to feed restriction during HOT and Recovery. With the reduction of plasma insulin, plasma glucose levels were maintained and NEFA mobilised from fat stores as a secondary energy source. The HOT group also maintained glucose levels throughout HOT and Recovery, but insulin was not reduced and NEFA was not mobilised.

While the acute moderate and high heat load trials saw increases in circulating white blood cells during HOT, this did not occur in the HOT in this experiment. However, the fall in both total white blood cell count and the neutrophil count in Recovery demonstrates the constancy of this response to heat load.

4.2 Feedlot Trials

4.2.1 Gatton summer feedlot trials

4.2.1.1 Weather conditions

The three Gatton summer feedlot trials commenced in late November or December and were completed in March of the following year. The mean maxima and minima for air temperature, THI and HLI are given in Table 10 below. Overall, summer 2 delivered the warmest conditions.

Mean maximum air temperatures and THI were not very different between the summers, however summer 2 produced significantly higher mean maximum HLI (Tables 10 and 11). Summer 1 produced significantly lower mean minimum air temperature, THI and HLI values relative to summers 2 and 3, which were not different based on these minima. Nor were Summers 2 and 3 significantly different for maximum and minimum air temperature and THI but were discriminated on the mean maximum HLI (Tables 10 and 11).

Table 10 Average daily weather conditions of the Gatton summer 1-3 feedlot trials (includes the week prior to first bleed).

	Gatton summer 1	Gatton summer 2	Gatton summer 3
Start date	27 November 2013	2 December 2014	15 December 2015
Finish date	11 March 2014	3 March 2015	29 March 2016
Maximum temperature			
Mean ± SD	31.8 ± 3.6	30.8 ± 3.1	31.1 ± 3.0
Highest value	43.7	39.6	38.7
Lowest value	23.4	22.2	23.0
Minimum temperature			
Mean ± SD	19.1 ± 2.3	20.3 ± 1.7	19.8 ± 1.9
Highest value	24.7	23.6	23.6
Lowest value	12.9	16.2	15.1
Maximum THI			
Mean ± SD	78.3 ± 3.5	79.4 ± 2.8	79.1 ± 2.7
Highest value	86.7	86.1	84.7
Lowest value	70.5	71.7	71.7
Minimum THI			
Mean ± SD	66.0 ± 3.8	67.8 ± 2.9	67.0 ± 3.2
Highest value	75.7	73.8	73.6
Lowest value	55.6	61.0	59.1
Maximum HLI			
Mean ± SD	89.7 ± 7.2	103.9 ± 8.4	95.9 ± 8.2
Highest value	103.5	117.4	119.1
Lowest value	74.3	67.3	72.4
Minimum HLI			
Mean ± SD	55.8 ± 4.1	58.3 ± 2.9	57.2 ± 3.2
Highest value	66.3	63.6	64.9
Lowest value	39.0	51.0	50.4

Table 11 Levels of significant difference amongst the overall weather conditions for the Gatton summer 1-3 feedlot trials (determined by paired t-test). Values for the minima are given above the diagonal, and values for the maxima are below the diagonal.

p-values		minima		
	Air temperature	Summer 1	Summer 2	Summer 3
maxima	Summer 1		0.0001	0.037
	Summer 2	n.s.		n.s.
	Summer 3	n.s.	n.s.	
	THI			
	Summer 1		<0.0001	0.084
	Summer 2	0.036		n.s.
	Summer 3	n.s.	n.s.	
	HLI			
	Summer 1		<0.0001	0.020
	Summer 2	<0.0001		0.048
	Summer 3	<0.0001	<0.0001	

4.2.1.2 Gatton heatwave events and animal performance and physiological responses

Mean values for weather conditions are of limited use in describing responses to acute heat wave events. Heatwaves and their severity were identified using the framework developed by Hahn et al. (1999). As seen in Table 12 below, under this framework, heatwave events are categorised on the basis of daytime and night-time THI and duration in days.

Table 12 Heatwave categories as developed by Hahn et al., 1999.

Category	Duration	Total hours, THI>79	hours/day, THI>84	Night-time recovery hours/night, THI<72
Slight	3-4 days	10-25	0	5-10
Mild	3-4 days	18-40	< 5	3-8
Moderate	More persistent	25-50	< 6	1-6
Strong	Increased persistence	33 -65	< 6	0-4
Severe	Very persistent	40-80	3-15 hours/day for 3 or more days	0-2

Animal performance and physiological responses during these events were examined for each feedlot trial and are described below. The performance measures include ADG and live

weights, and daily DMI where available. The physiological measures inspected were daily maximum and minimum rumen temperatures, rectal temperatures, and panting scores taken at 2 pm (PS at 2 pm). This timepoint for the panting score generally gave the highest values. Not all the feedlot trials results given below will have the full set of parameters. In these cases, the data was not fully processed in time for submission of this report.

Gatton summer 1

Despite being the coolest summer relative to summers 2 and 3, summer 1 delivered four heatwave events, two of which were classified as mild and two as moderate (to strong). The most persistent and intense event, which was preceded by a slight heatwave, occurred in mid-February toward the end of the trial. See Table 13.

Table 13 Gatton summer 1 heatwave events (categorised according to the scale of Hahn et al., 1999).

Event number	dates	Heatwave category
1	26 – 29 December 2013	mild
2	2 – 6 January 2014	moderate to strong
3	20 – 22 January 2014	mild
4	15 – 21 February 2014	strong

As evident in Fig. 116, the mean maximum rumen temperatures were raised during every heatwave event, and peaked toward the end of each event. Event 2 induced the largest increase in mean maximum rumen temperature (1.29°C), and event 4 caused the largest increase in mean minimum rumen temperature (0.52°C) (relative to the prior day's maximum). Note the consistently lower rumen temperatures following event 4.

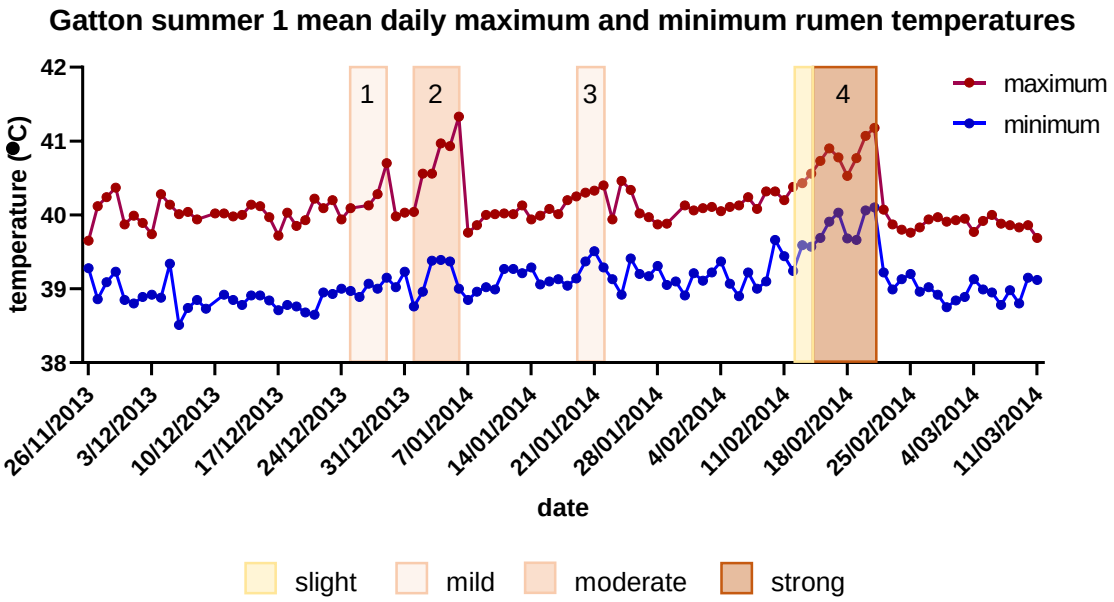

Fig. 116. Mean daily maximum and minimum rumen temperatures during the Gatton summer 1 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks.

Unexpectedly, there was little correspondence between the weekly rectal temperatures and rumen temperatures between 7th January and about 18th February. The rectal temperature data indicated a very strong rise in body temperature during event 3, a mild heatwave (Fig. 117). This response was not detected in the rumen (Fig. 116). Pearson correlations of mean weekly rectal temperature with mean daily maximum and minimum rumen temperatures were 0.54 and 0.55 respectively (p = 0.047 and 0.032).

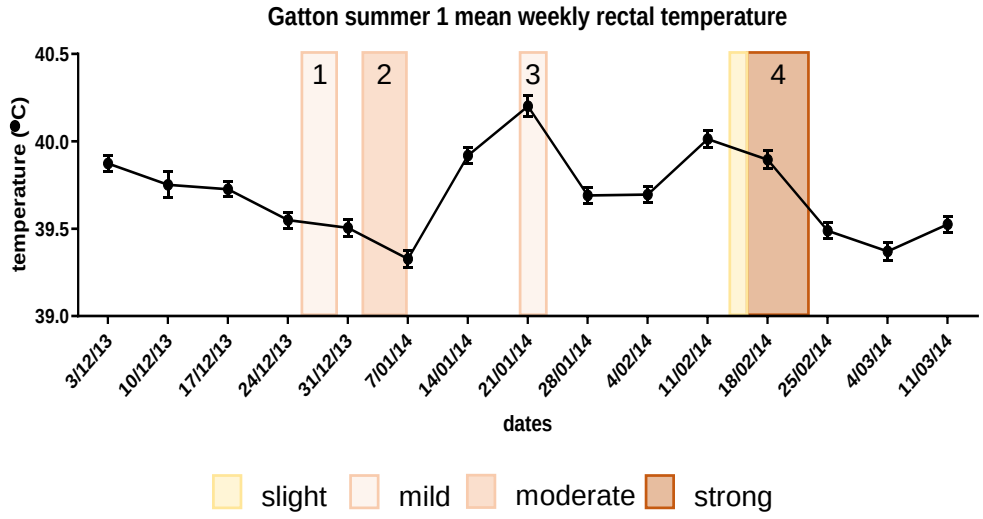

Fig. 117. Mean weekly rectal temperatures during the Gatton summer 1 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

The mean daily PS at 2 pm rose at some point during each heatwave event, most obviously in event 2 when it peaked with a mean PS of 3.0 on January 6 (Fig. 118). During event 4, the mean PS did not respond as strongly despite the higher intensity heatwave (and increased rumen temperatures, Fig. 116). After event 4, the mean PS were lower than at the start of the trial.

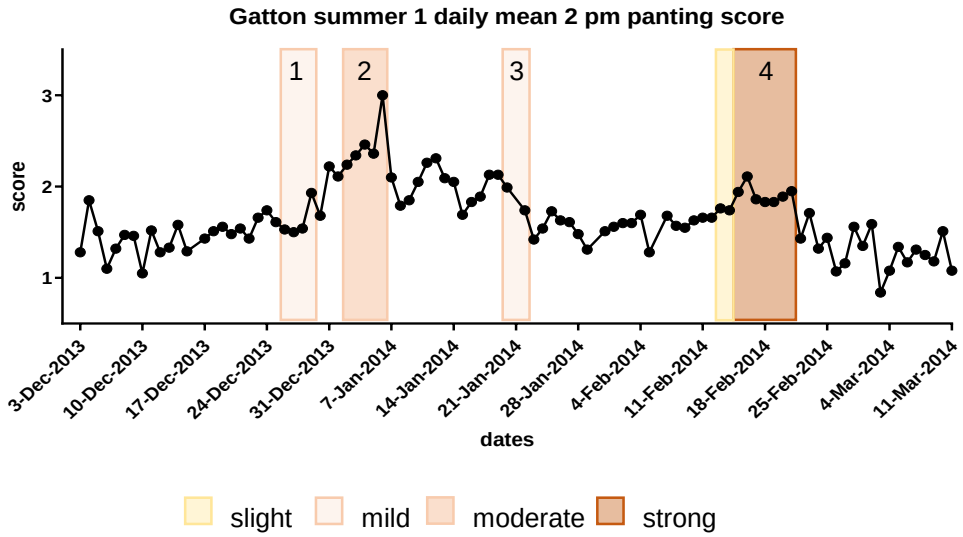

Fig. 118. Mean daily panting score at 2 pm during the Gatton summer 1 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks.

The daily mean PS at 2 pm correlated best with the daily mean maximum rumen temperature (Pearson r = 0.612, p <<0.0001). The correlations between the mean PS with the daily mean minimum rumen temperatures were highly significant but weaker (Table 14). There was no significant correlation with the weekly mean rectal temperature.

Table 14 Correlation of daytime mean panting scores with mean daily maximum and minimum rumen temperatures during the Gatton summer 1 feedlot trial.

Panting scores at	Daily minimum rumen temperature			Daily maximum rumen temperature		
	r	p-value	N	r	p-value	n
8 am	0.424	<<0.0001	83	0.570	<<0.0001	80
12 noon	0.450	<<0.0001	94	0.547	<<0.0001	92
2 pm	0.398	<<0.0001	94	0.612	<<0.0001	93
4 pm	0.369	<0.0001	96	0.495	<<0.0001	94

r: Pearson correlation regression; n: number of observations

The average weekly DMI increased gradually and through the first heatwave event but stalled with event 2 at around the 13 kg/head/day level (Fig. 119). Intake resumed and peaked between 15-16 kg/head/day before levelling out and then decreasing after the onset of a mild heatwave event (event 3) in mid to late January. This decrease in DMI continued into the only strong heatwave of the trial (event 4) after which it increased gradually until the end of trial.

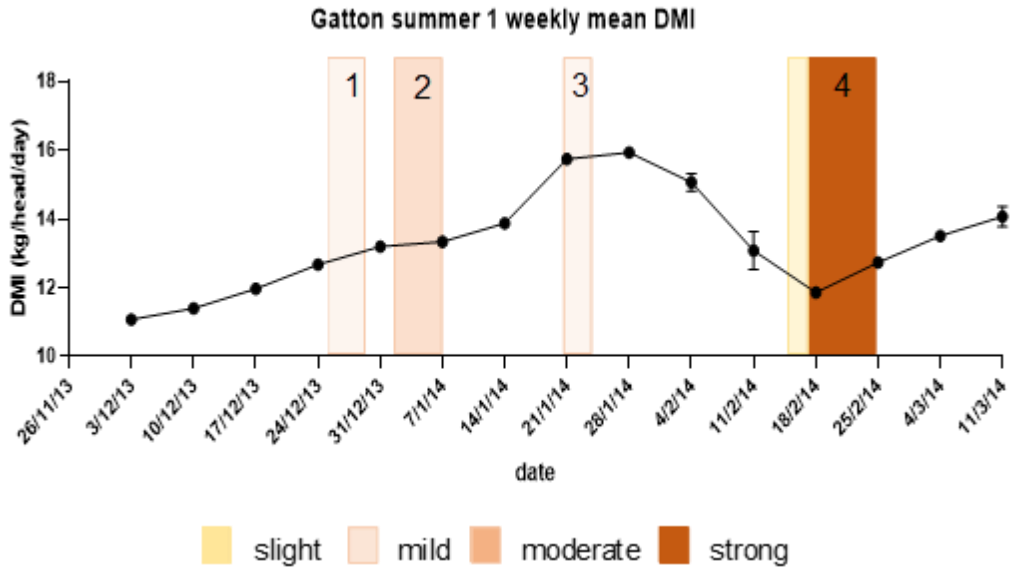

Fig. 119. Mean weekly DMI during the Gatton summer 1 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Live weight gain clearly stalled as a consequence of the late February strong heatwave (event 4, Fig. 120A). Weekly mean ADG fell during each of the two moderate-strong heatwaves (events 2 and 4), and was lower still in the subsequent week, so that the fall in ADG over the two week period was about 1 kg/head/day (Fig. 120B). Mean ADG was close to zero following event 4 (a strong heatwave).

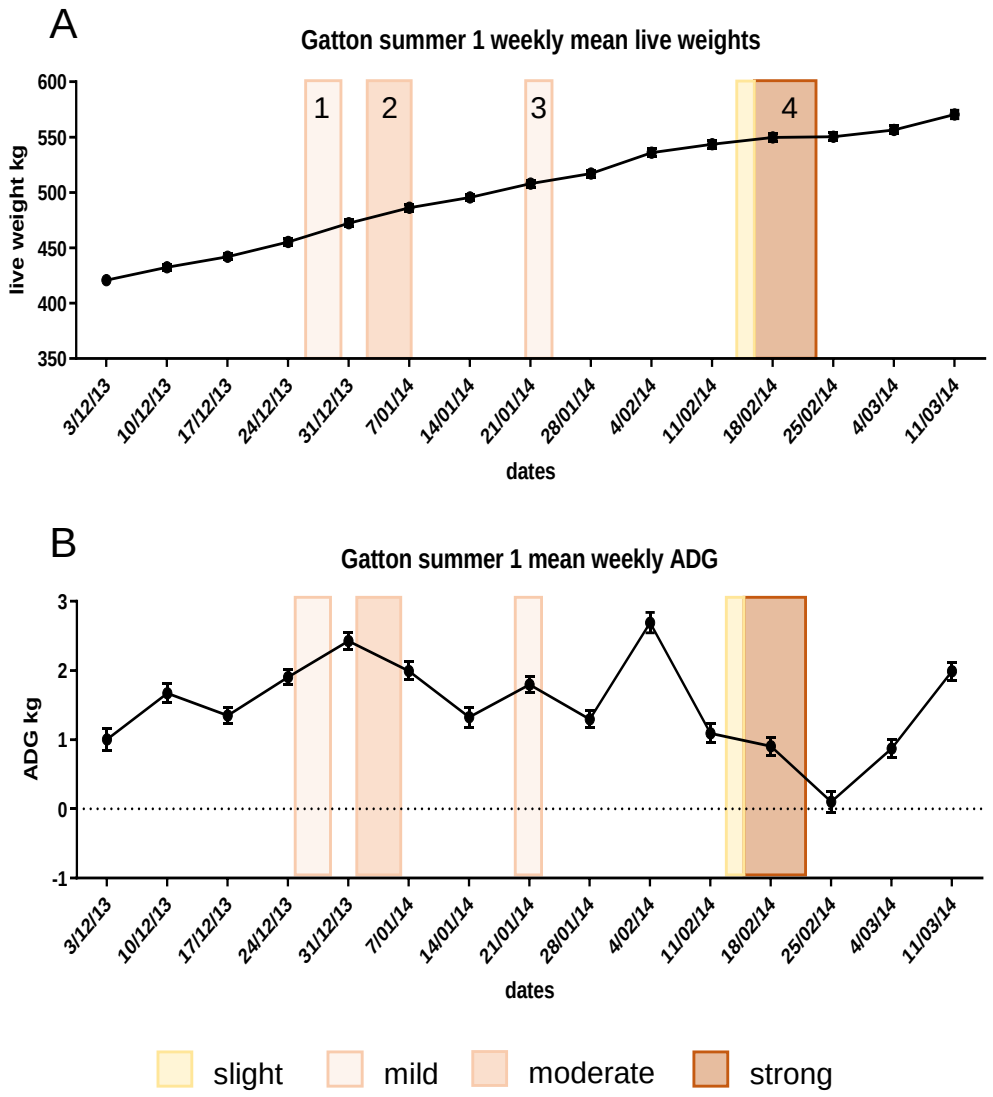

Fig. 120. Mean weekly live weights (A) and weekly ADG (B) during the Gatton summer 1 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Gatton summer 2

The summer of 2014/15 was the warmest summer experienced in this study. Eight heatwave events were detected using the Hahn framework (Hahn et al., 1999), but none fell into the strong or severe categories (Table 15). There were 2-3 moderate events.

Table 15 Gatton summer 2 heatwave events (categorised according to the scale of Hahn et al., 1999).

Event number	dates	Heatwave category
1	9 – 11 December 2014	slight
2	16 – 18 December 2014	mild
3	30 December 2014 – 2 January 2015	mild to moderate
4	13 – 16 January 2015	mild
5	17 – 19 January 2015	moderate
6	25 – 27 January 2015	moderate
7	22 – 24 February 2015	slight to mild
8	28 February – 2 March 2015	slight to mild

Summer 2 was characterised by robust responses in mean minimum rumen temperature regardless of heatwave category (Fig. 121). Events 2 and 7, classified as mild and slight, were associated with rises of 1.2 and 1.6°C, respectively. Maximum rumen temperatures also rose with each heatwave. Events 2 and 3 induced rises of >1°C in the mean maximum rumen temperatures.

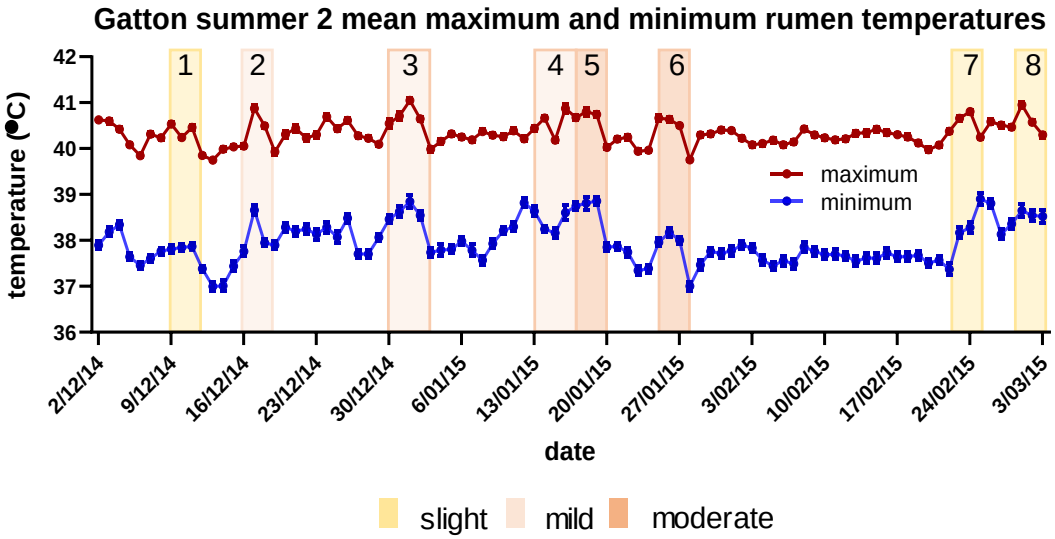

Fig. 121. Mean daily maximum and minimum rumen temperatures during the Gatton summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Elevation of mean rectal temperature was observed for events 6 and 7 only, with increases of 0.57 and 0.69°C respectively. The 7-day interval between rectal temperature measurements resulted in not recording the likely increases provoked by the other heat events. Overall, there was good correspondence between the mean maximum rumen temperatures and rectal temperature in this trial (Pearson r = 0.80, p=0.001; Fig. 121 and Fig. 122).

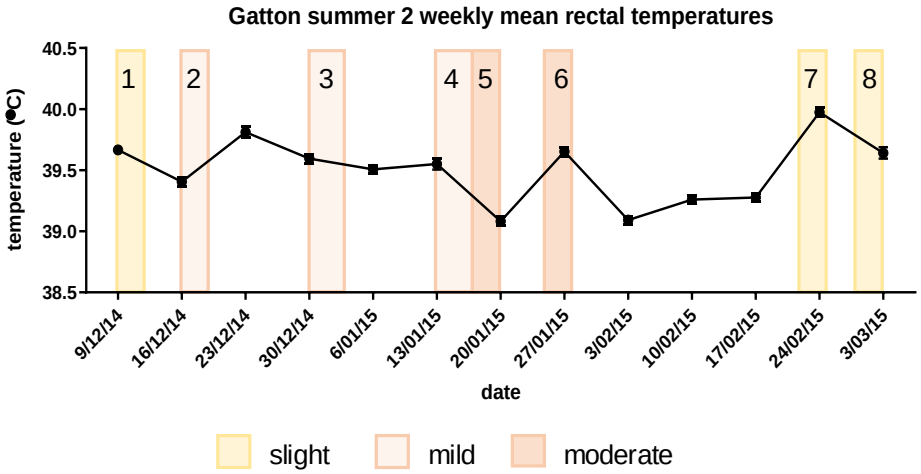

Fig. 122. Mean weekly rectal temperatures during the Gatton summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

The mean PS at 2 pm increased with most heatwave events although there were intermittent spikes apparently not associated with hot weather (Fig. 123). The highest mean PS at 2 pm (1.69-1.79) was recorded during events 2, 3, 6 and 7. The mean PS at 2 pm were persistently high during the slight heatwave events at the end of the trial.

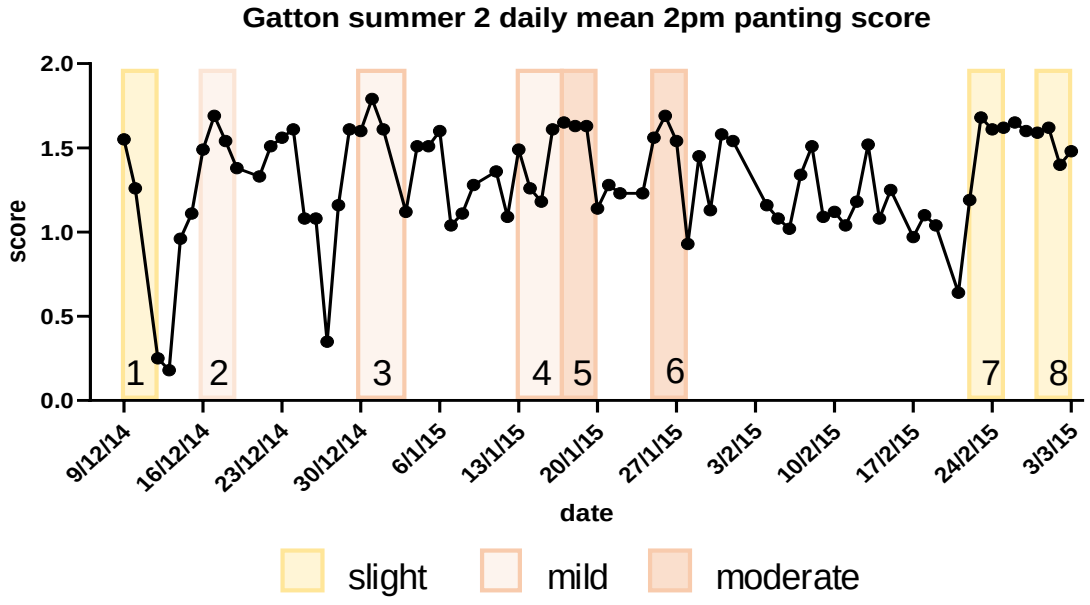

Fig. 123. Mean daily panting score at 2 pm during the Gatton summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks.

In this feedlot trial, correlations with PS and body temperatures were strong (Table 16). Mean PS at noon correlated well with mean maximum rumen temperature (r = 0.70, p <<0.0001). The mean minimum rumen temperature had moderate correlations (~0.6) with PS at noon, 2 and 4 pm. The highest correlation (r = 0.88) was achieved by the noon PS and weekly rectal temperature.

Table 16 Correlation of daytime mean panting scores with mean daily maximum and minimum rumen temperatures and mean weekly rectal temperatures during the Gatton summer 2 feedlot trial.

Panting scores at	Daily minimum rumen temperature			Daily maximum rumen temperature			Weekly rectal temperature		
	r	p-value	n	r	p-value	n	r	p-value	n
12 noon	0.624	<<0.0001	78	0.700	<<0.0001	78	0.883	0.001	10
2 pm	0.628	<<0.0001	78	0.649	<<0.0001	76	0.800	0.002	12
4 pm	0.599	<<0.0001	79	0.540	<<0.0001	79	0.688	0.013	12

r: Pearson correlation regression; n: number of observations

The mean daily DMI for the second summer feedlot trial (Fig. 124) shows an overall increase in intake until late February. A sharp drop in DMI is evident at the end of December with heatwave event 3, and a smaller decrease can be seen in mid-January as the animals enter a series of heatwave (events 4, 5 and 6). DMI increased throughout February, peaked at around 14 kg/head/day until the onset of two late mild heatwaves (events 7 and 8). This causes a sudden large drop in DMI, possibly as the animals are full of feed and heavy at this stage and may be unable to cope with this late heatwave event.

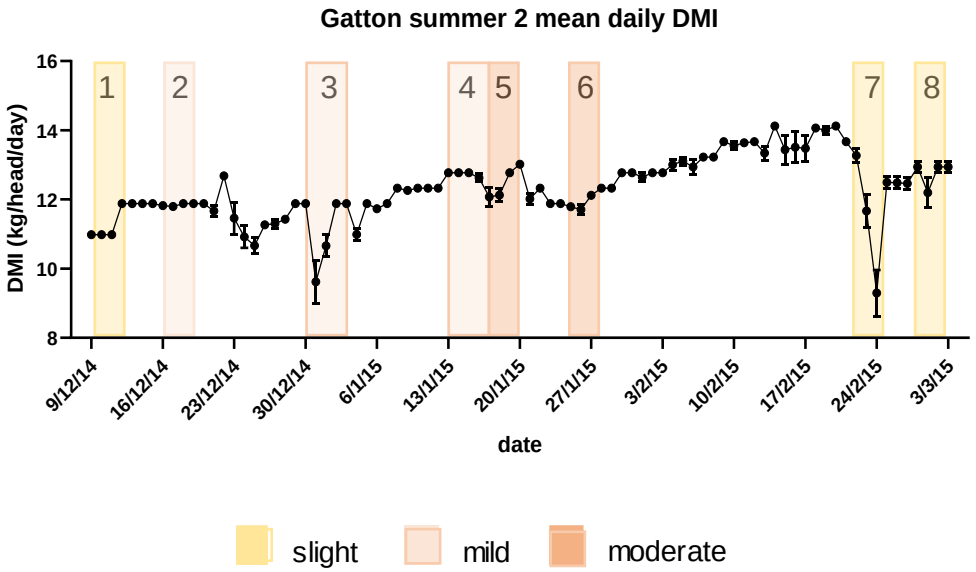

Fig. 124. Mean daily DMI during the Gatton summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

The growth trajectory of the mean live weight stalled during events 5 and 6 (both moderate) but regained momentum thereafter (Fig. 125A). Mean ADG fell following the heatwave events irrespective of intensity, with the exception of the two slight heatwave events (events 7 and 8) toward the end of the trial (Fig. 125B). Mean ADG was below zero after event 5 (moderate) and at the beginning of event 6 but recovered well.

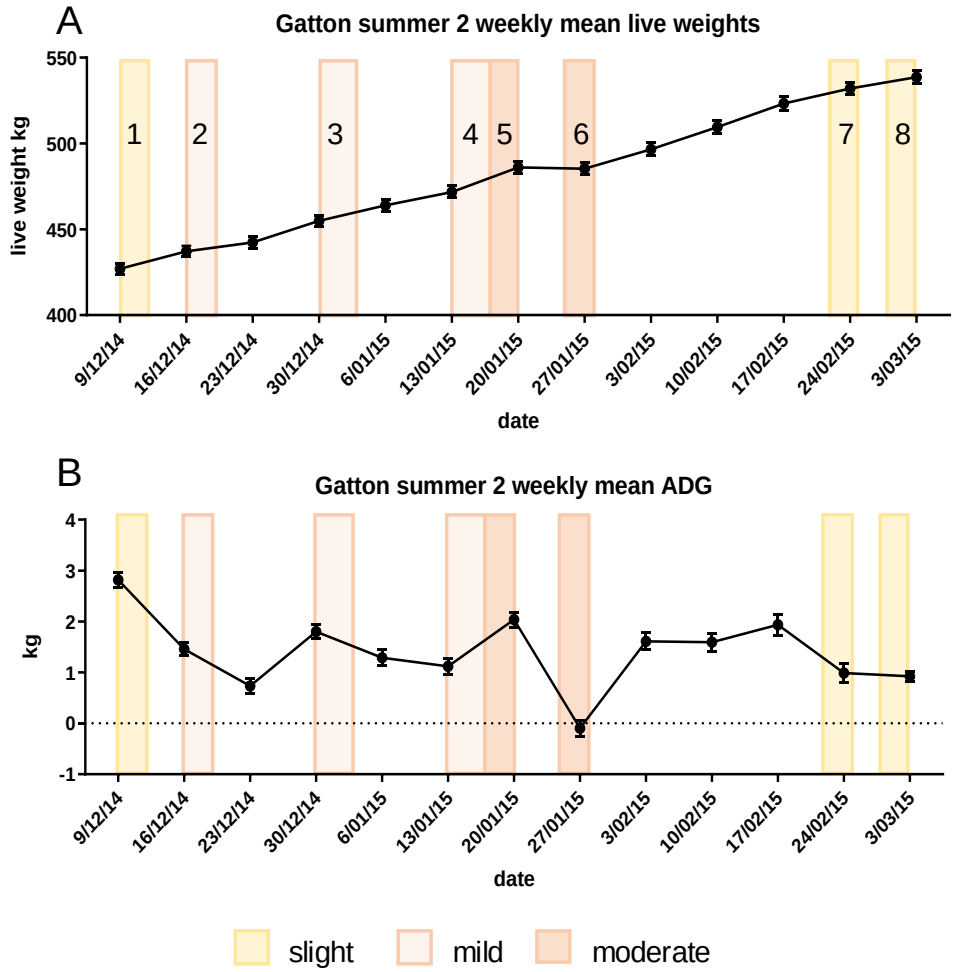

Fig. 125. Mean weekly live weights (A) and weekly ADG (B) during the Gatton summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Gatton summer 3

While on average summer 3 was similar to summer 2, it delivered a moderate-strong heatwave event (event 3) with a mild event before and after (Table 17).

Table 17 Gatton summer 3 heatwave events (categorised according to the scale of Hahn et al., 1999).

Event number	dates	Heatwave category
1	11 – 14 January 2016	slight
2	22 – 25 January 2016	mild
3	29 January – 2 February 2016	moderate to strong
4	16 - 19 February 2016	mild
5	26 - 29 February 2016	slight

The weekly rectal temperature measurements captured temperature rises in response to events 1, 2 and 4 (Fig. 126). The anticipated rise in response to event 3, a strong heatwave, may have been missed. Event 4 induced an increase of 1.0°C, and events 1 and 2 were each associated with an increase of 0.66° C.

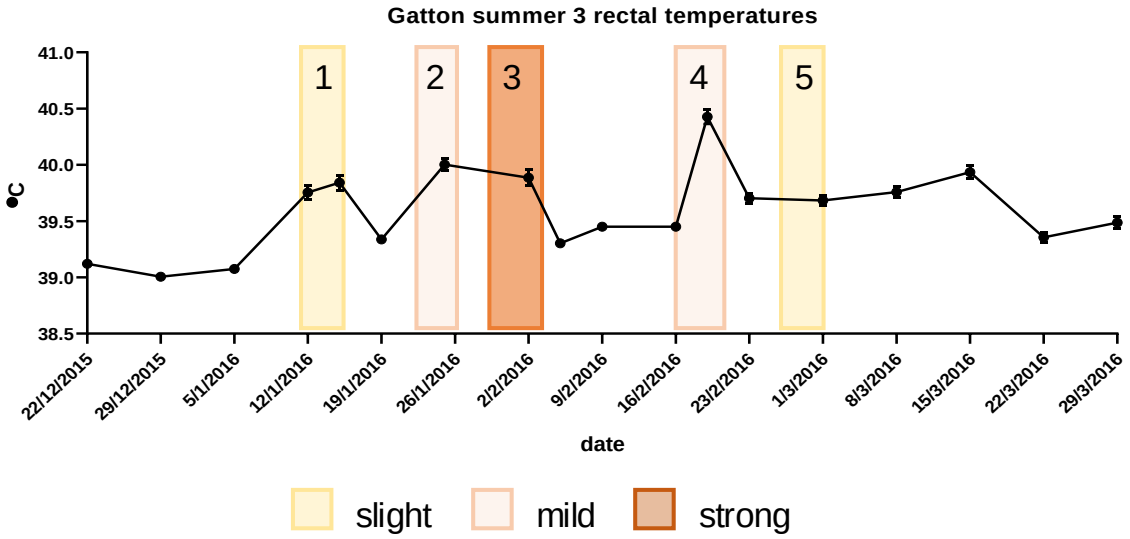

Fig. 126. Mean weekly rectal temperatures during the Gatton summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

The mean PS at 2 pm increased with each heatwave event although the timing of the rise was variable (Fig. 127). The highest mean PS, 1.95, occurred during event 4. The correlations between PS at 8 am to 4 pm and rectal temperature were high ($r \geq 0.75$, see Table 18).

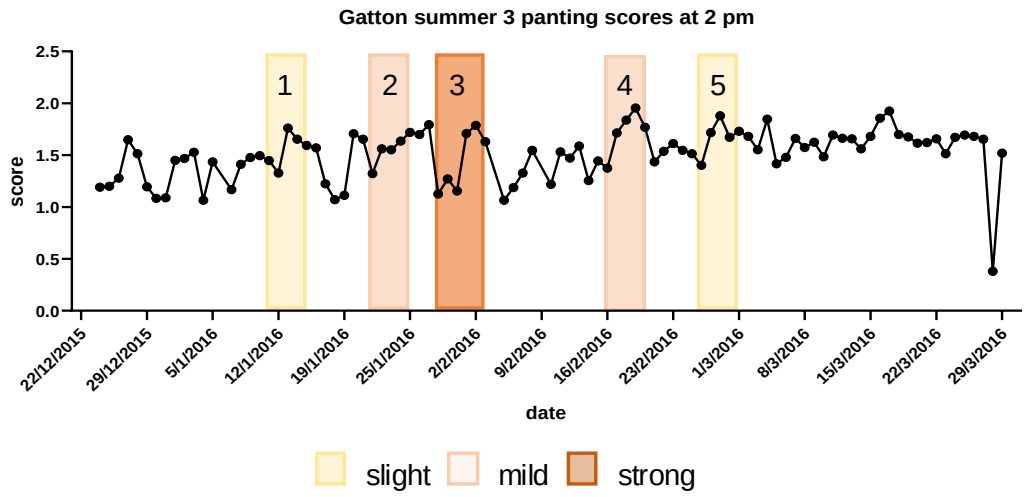

Fig. 127. Mean daily panting score at 2 pm temperatures during the Gatton summer 3 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks.

Mean DMI peaked at 15.4 kg/head/day prior to event 2. Reduction of mean DMI was not entirely consistent with onset of heatwave events but there was a seesaw pattern of recovery and drop in feed intake until early March (Fig 128). Events 2 and 4 produced textbook examples of sudden decrease in DMI in response to high heat load. There seemed to be some resistance to lowering DMI at the onset of event 3 possibly due to the sharp fall after recovery from event 2. The erratic feed intake observed 8 – 11 March was not related to a weather event of any nature. Its cause is unknown.

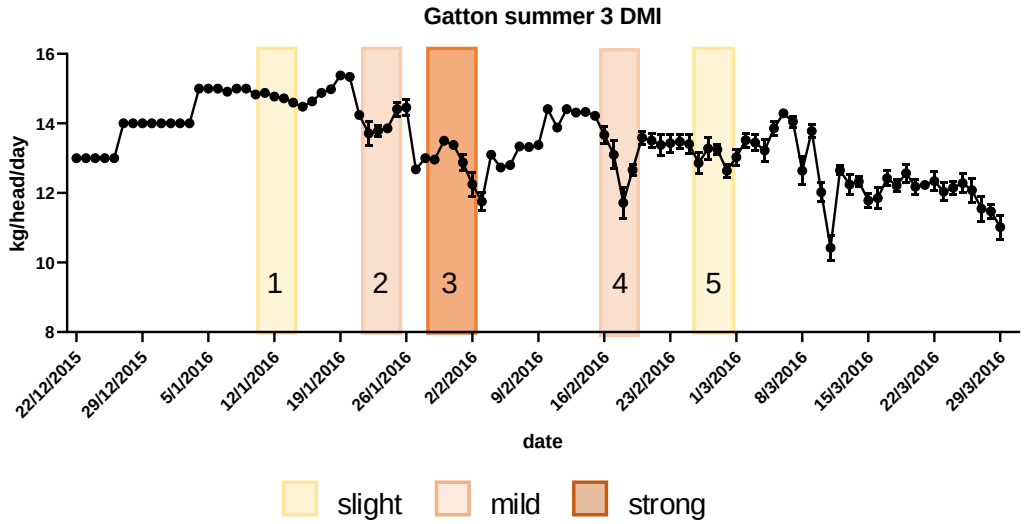

Fig. 128. Mean daily DMI during the Gatton summer 3 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Correlation between the mean DMI and mean PS at 10 am and 12 noon were negative but moderate (Table 18) There was no correlation between mean DMI and mean rectal temperature.

Table 18 Correlations of daytime panting scores with daily DMI and weekly rectal temperatures during the Gatton summer 3 feedlot trial.

Panting scores at	Daily mean DMI			Weekly mean rectal temperature		
	r	p-value	n	r	p-value	n
8 am				0.781	<<0.0001	17
10 am	-0.466	<<0.0001	96	0.865	<<0.0001	17
12 noon	-0.523	<<0.0001	77	0.760	0.001	16
2 pm				0.751	0.001	16
4 pm				0.753	<<0.0001	17

r: Pearson correlation regression; n: number of observations

Not surprisingly, the inconsistent mean DMI affected growth and weight gain. Live weight growth stalled on three occasions during Summer 3 (Fig. 129A). In all, Summer 3 animals experienced four falls of ≥ 1 kg/head/day in ADG during or following events 1, 3, 4 and 5. Events 3 and 4, classed as moderate-strong and mild respectively, were associated with falls of over 2 kg/head/day and ADG was close to zero for both events.

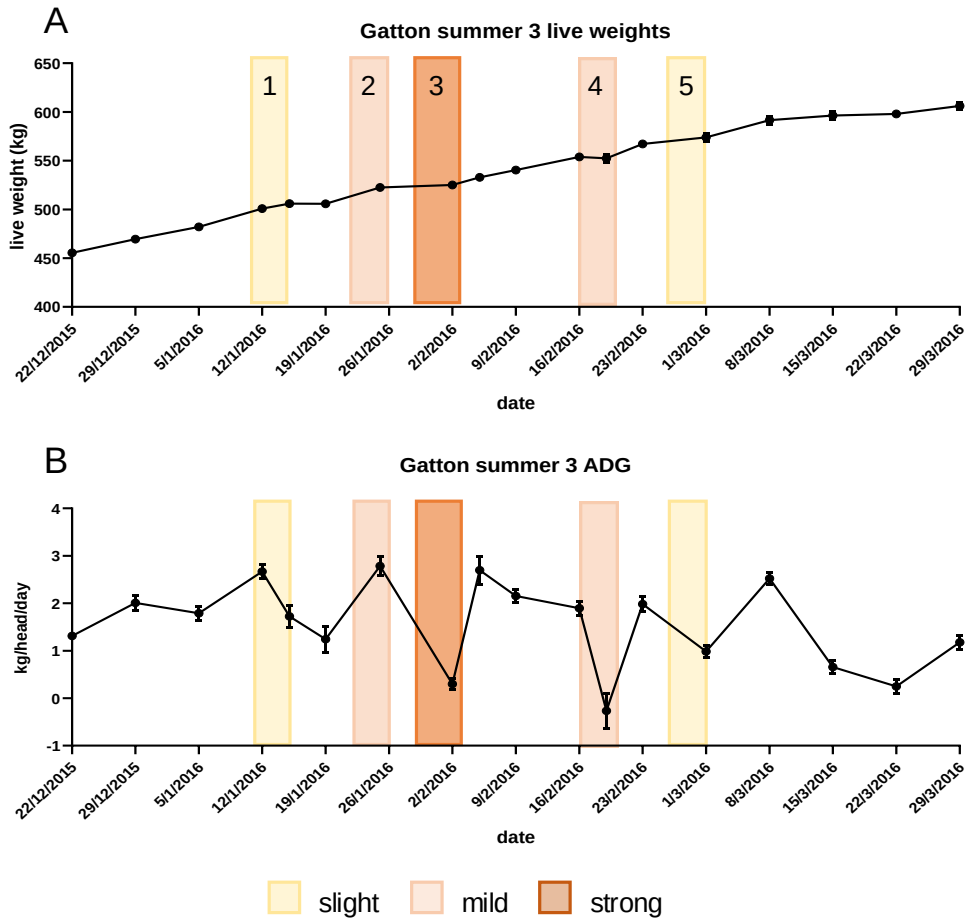

Fig. 129. Mean weekly live weights (A) and weekly ADG (B) during the Gatton summer 3 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

4.2.2 Nebraska summer feedlot trials

4.2.2.1 Weather conditions

The two summer feedlot trials conducted at the Mead site (UNL) were run over the summers of 2014 and 2015. The Nebraska summer 1 trial started in mid-May when the weather was still cool especially at night. The second summer trial commenced later, in mid-June 2015, and was completed by mid-August. On average, the conditions of both trials appeared similar. The mean maximum air temperatures, THI and HLI were not different (Table 19 and Table 20). However, summer 2 presented higher and more consistent minimum values and was significantly warmer than summer 1 on this basis.

Table 19 Nebraska summers 1 and 2 weather conditions (includes week prior to first bleed).

	Nebraska summer 1	Nebraska summer 2
Start date	15 May 2014	11 June 2015
Finish date	31 July 2014	13 Aug 2015
Maximum temperature (°C)		
Mean ± SD	27.8 ± 4.2	28.9 ± 3.6
Highest value	35.6	36.4
Lowest value	12.4	19.1
Minimum temperature (°C)		
Mean ± SD	15.9 ± 4.6	18.3 ± 3.0
Highest value	24.4	24.2
Lowest value	0.1	10.7
Maximum THI		
Mean ± SD	76.3 ± 6.2	78.8 ± 5.0
Highest value	88.9	89.0
Lowest value	55.6	65.8
Minimum THI		
Mean ± SD	60.4 ± 7.8	64.6 ± 5.0
Highest value	74.5	74.5
Lowest value	33.5	51.3
Maximum HLI		
Mean ± SD	86.5 ± 12.8	86.8 ± 15.6
Highest value	108.7	114.6
Lowest value	36.7	51.8
Minimum HLI		
Mean ± SD	51.3 ± 6.3	56.2 ± 3.7
Highest value	62.4	64.0
Lowest value	31.6	42.9

Table 20 Levels of significant difference amongst the overall weather conditions for the Nebraska summer 1 and 2 feedlot trials (determined by paired t-test). Values for the minima are given above the diagonal, and values for the maxima are below the diagonal.

p-values		minima	
	Air temperature	Summer 1	Summer 2
maxima	Summer 1		0.038
	Summer 2	n.s.	
	THI		
	Summer 1		0.045
	Summer 2	n.s.	
	HLI		
	Summer 1		0.0015
	Summer 2	0.075	

4.2.2.2 Nebraska heatwave events and animal performance and physiological responses

As for the Gatton summer trials, heatwave events were identified using the Hahn et al., (1999) descriptors. The two Nebraska summers delivered very different series of heatwave events.

Nebraska summer 1

The 2015 Nebraska summer produced a run of slight to mild-moderate heatwaves, five in all (Table 21).

Table 21 Nebraska summer 1 heatwave events (categorised according to the scale of Hahn et al., 1999).

Event number	dates	Heatwave category
1	16-20th June	mild
2	5-7th July	slight
3	10-13th July	slight
4	20-22nd July	mild-moderate
5	25-26th July	mild-moderate

There were rises in the mean maximum and minimum rumen temperatures associated with each heatwave event, except for event 4 (Fig. 130). Maximum rumen temperature responded strongly to the first event of the season, a mild event, and to the two slight heatwave events in early July. Surprisingly, or maybe as an indication of adaptation, there were muted rises in maximum rumen temperature in events 4 and 5, both mild to moderate. There was no change in minimum rumen temperature in response to these two late heatwaves. There were intermittent rises is rumen temperature that did not seem to be associated with heatwaves – 24 June and 17-18 July.

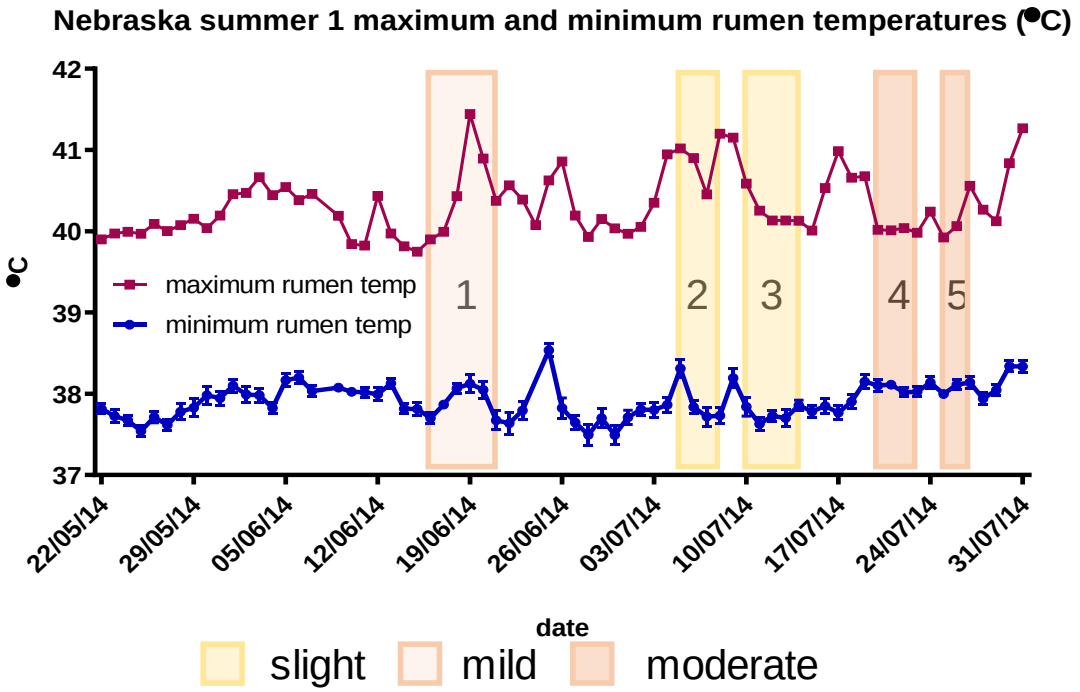

Fig. 130. Mean daily maximum and minimum rumen temperatures during the Nebraska summer 1 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

The mean panting scores (PS) rose and fell with the heatwave incidents for most of the trial period (Fig. 131). The slight heatwave events 2 and 3 achieved the highest mean PS. There was good correspondence with maximum and minimum rumen temperatures over this part of the summer e.g. mid-June to mid-July, but overall correlations with rumen temperature were not high (Table 22). In part, this is due to the lack of a rumen temperature response during heatwave events 4 and 5, and the high PS recorded during May into early June despite little change in the rumen.

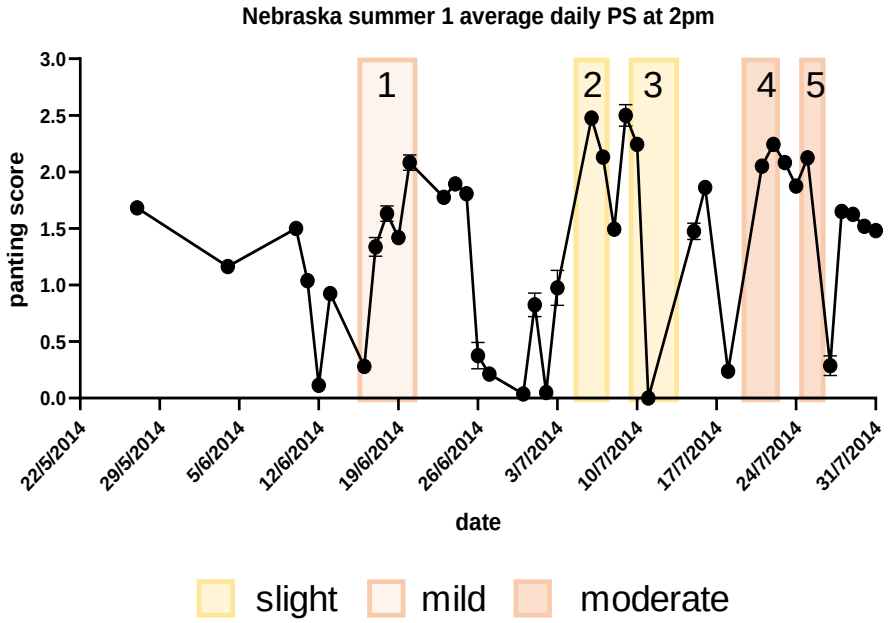

Fig. 131. Mean daily panting score at approximately 2 pm during the Nebraska summer 1 feedlot trial. Please note that the PS was not collected every day. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Table 22 Correlation of the mean 2 pm panting scores with mean daily maximum and minimum rumen temperatures and DMI during the Nebraska summer 1 feedlot trial.

	Daily panting score		
	r	P-value	n
Daily minimum rumen temperature	0.373	0.021	39
Daily maximum rumen temperature	0.176	n.s.	39
Daily DMI	-0.304	0.060	39

r: Pearson correlation regression; n: number of observations

Mean DMI over this 70 day trial exhibited pulsatile behaviour mostly triggered by heatwave events. Just prior to event 1, mean DMI peaked at 14.5 kg/head/day. It fell in response to events 1, 2 and 4 but recovered to just over 14 kg/head/day after events 1 and 3 (Fig. 132). The largest fall was 2.8 kg/head/day during event 2-3 (approximately 20% of the 2-3 July intake). Interestingly, the cattle managed to maintain intake during the whole of event 4, a mild-moderate heatwave, but finally succumbed prior to event 5. There were negative low correlations of mean DMI with mean PS (Table 22) and mean maximum rumen temperature ($r = 0.365$, $p = 0.027$).

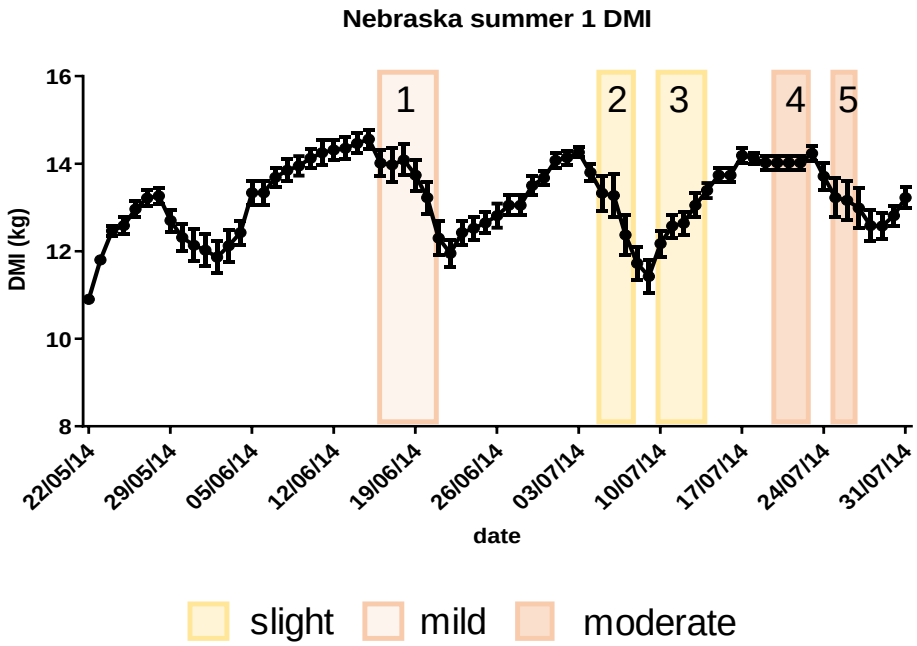

Fig. 132. Mean daily DMI during the Nebraska summer 1 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

At commencement of the trial, the average live weight was 487.8 ± 2.29 kg, and on completion, 70 days later, the mean live weight was 642.9 ± 8.60 kg. Overall, live weight gain appeared as very uniform during this summer feedlot trial (Fig. 133A), however, when presented as fortnightly mean ADG, there was a large but highly variable ADG at the second weigh-in, 19 June, which was conducted during the first heatwave event (Fig. 133B). The high ADG at this timepoint most likely reflected the reasonably consistent and high DMI of the week preceding the first heatwave event (Fig. 132). The substantial variability detected on the 19 June weigh-in may indicate high individual variability as rumen temperatures peaked (Fig. 130) and DMI fell (Fig. 132).

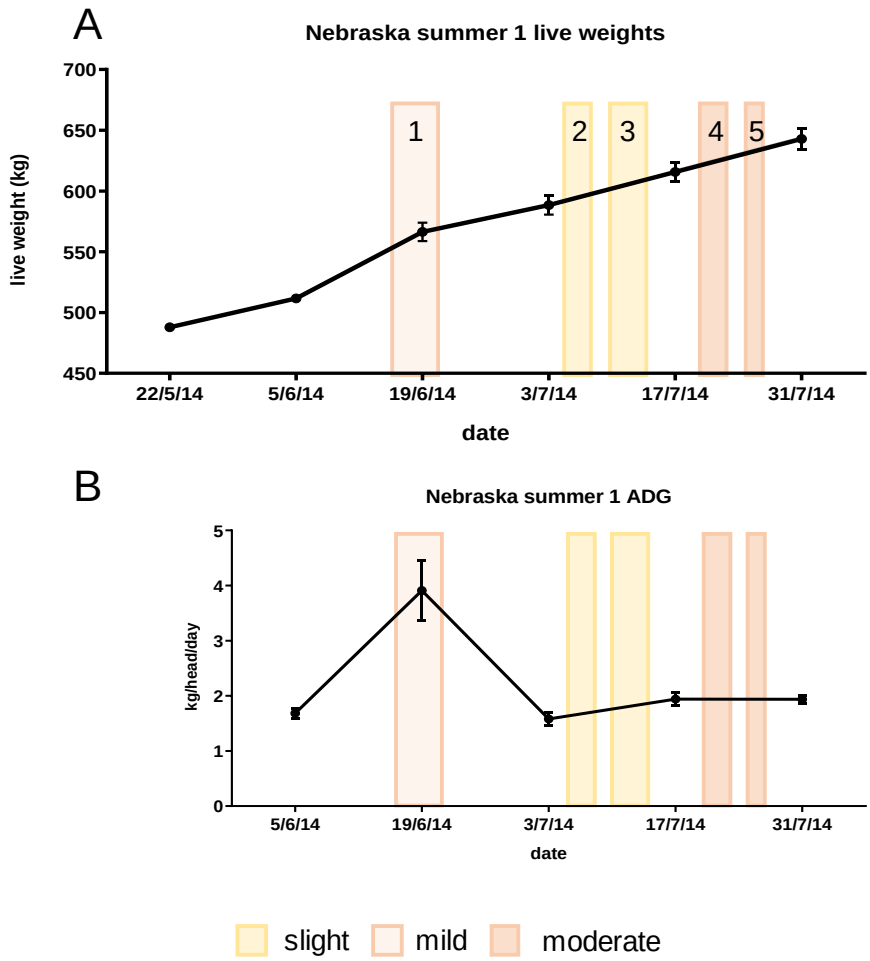

Fig. 133. Mean fortnightly live weights (A) and weekly ADG (B) during the Nebraska summer 1 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Nebraska summer 2

The summer of 2015 was characterised by an almost continuous 18 day heatwave in July. After a cool start to summer, there was a sudden onset of a strong to severe heatwave (event 2) followed by a two-day cooler interval before event 3, a moderate heatwave. Event 4, another strong heatwave, occurred three days after event 3 (Table 23).

Table 23 Nebraska summer 2 heatwave events (categorised according to the scale of Hahn et al., 1999).

Event number	dates	Heatwave category
1	20 - 22 June	slight
2	11-14 July	strong to severe
3	16-20 July	moderate
4	23 - 28 July	strong
5	7 - 9 August	slight

All heatwave events saw elevations of mean minimum and maximum rumen temperatures (Fig. 134). Events 2 and 4 induced 1.7°C rises in mean maximum rumen temperature, and event 3 was associated with a 1.2°C increase. The largest rises in minimum rumen temperature were recorded for events 2 and 4 (1.5 and 0.7°C respectively).

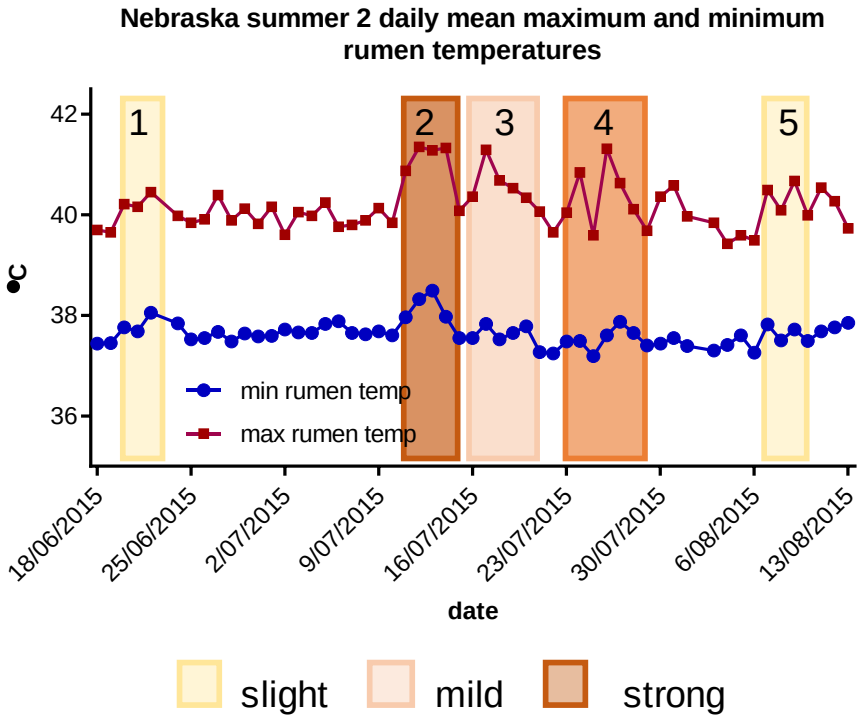

Fig. 134. Mean daily maximum and minimum rumen temperatures during the Nebraska summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks.

Panting scores were taken in the early afternoon on most but not all days. Mean PS at 2 pm rose with each heatwave event including the slight heatwave events (Fig. 135). The largest increase occurred for event 2. There was a strong correlation between the PS and maximum rumen temperatures (Table 24). The correlation was modest but significant with the minimum rumen temperatures.

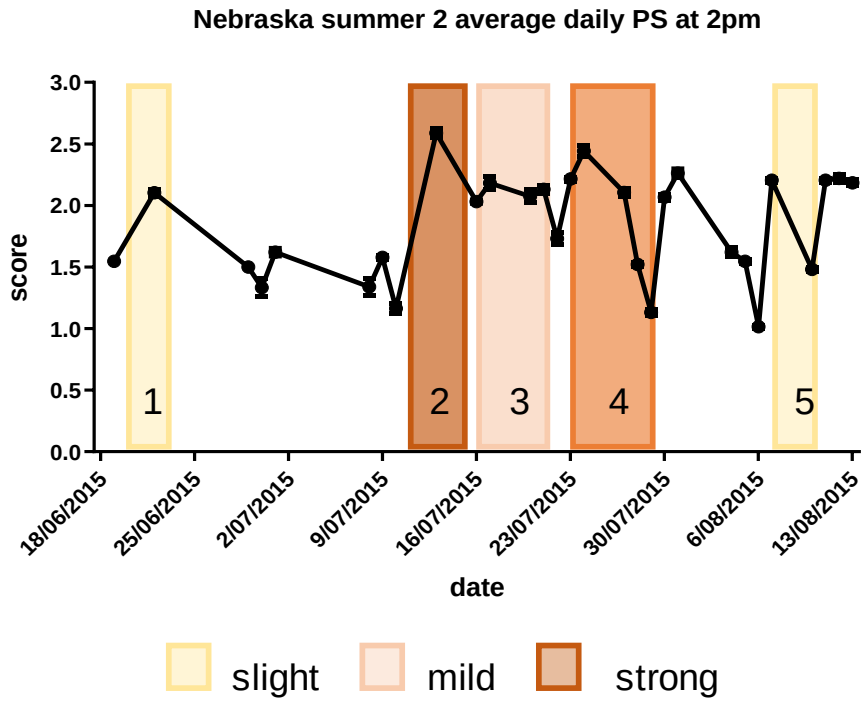

Fig. 135. Mean daily panting score at 2 pm temperatures during the Nebraska summer 2 feedlot trial. Please note that the PS was not collected every day. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Table 24 Correlation of the mean 2 pm panting scores with mean daily maximum and minimum rumen temperatures and DMI during the Nebraska summer 2 feedlot trial.

	Daily panting score		
	r	P-value	n
Daily minimum rumen temperature	0.495	0.006	29
Daily maximum rumen temperature	0.744	<<0.0001	29
Daily DMI	-0.416	0.025	29

r: Pearson correlation regression; n: number of observations

Prior to the summer 2 series of heatwaves, the cattle consistently consumed an average of 13.3 kg/head/day (Fig. 136). Daily mean DMI dropped by 50% (6.8 kg/head/day) during event 2, partially recovered for one day and dropped again with the onset of event 3 and came to a plateau at ~8.8 kg/head/day. It returned to 11.1 kg/head/day in the 3 day interval till the onset of event 4, when the DMI fell once more. The mean DMI returned to ~13 kg/head/day about 2 weeks after event 4. The mean PS had a moderate and negative correlation with DMI (Table 24).

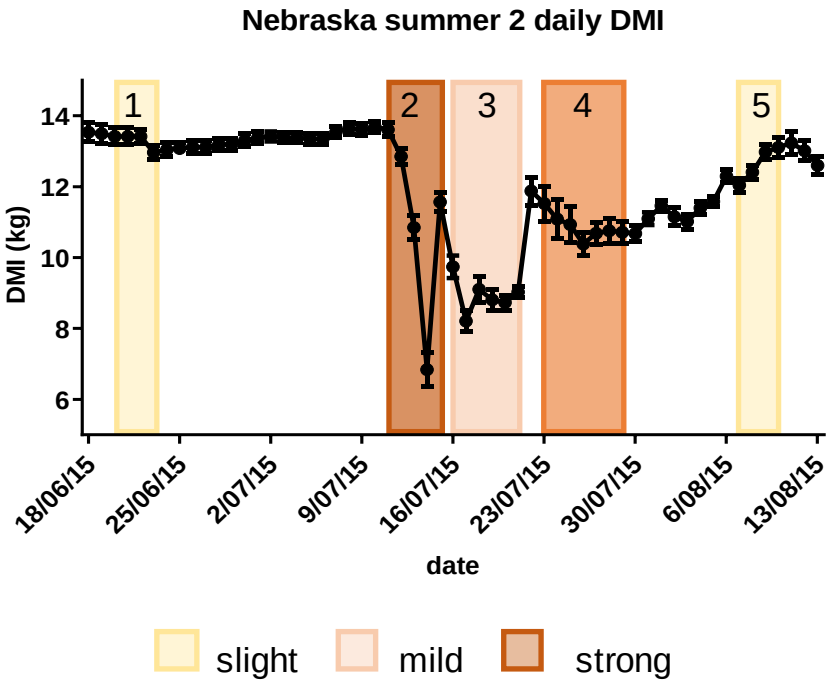

Fig. 136. Mean daily DMI during the Nebraska summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

Starting mean live weight was 535.3 ± 1.9 kg and mean finishing live weight was 635.3 ± 2.3 kg after 56 days on feed. Given the near continuous run of moderate to strong heatwaves in July, live weight gain was very low over that period. The average gain over 9 – 23 July was only 6 kg (Fig. 137A). The weekly mean ADG prior to the heatwaves was 2-2.5 kg/head/day. During events 2 and 3, it fell to 0.58 and 0.31 kg/head/day respectively but recovered strongly the following week despite the still reduced feed intake (Fig. 137B).

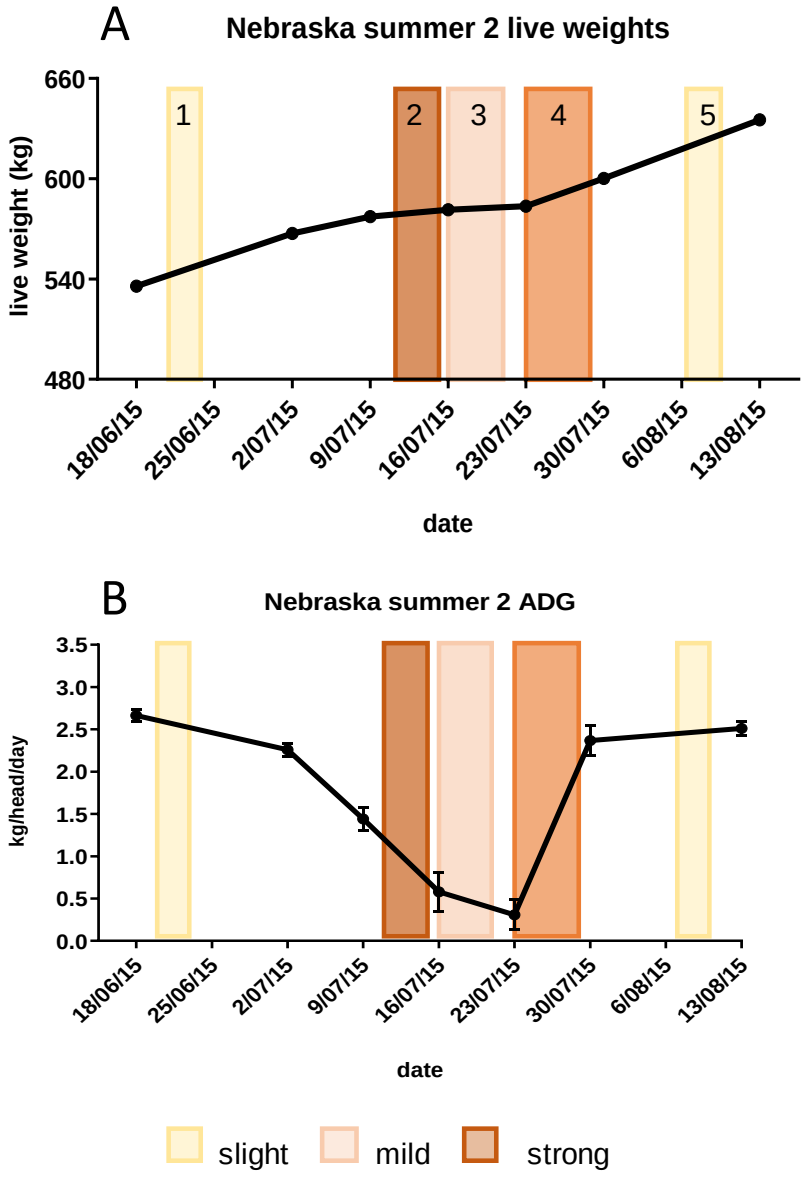

Fig. 137. Mean weekly live weights (A) and weekly ADG (B) during the Nebraska summer 2 feedlot trial. The numbered heatwave events and their classification are indicated by the solid coloured blocks. Bars indicate SEM.

4.2.3 Are the blood changes seen in the acute heat load climate chamber experiments seen in the feedlot trials?

We have seen remarkable consistency in the response to high heat load in the climate chamber experiments by several blood biochemistry and haematology variables. These include ALP, bicarbonate, urea, creatinine, chloride and WBC counts. Are the climate chamber responses like those of the cattle in feedlots? The summer feedlot trials provided a more complex and changeable environment. In each summer trial, the animals experienced a series of heatwave events of differing intensity and duration as they gained weight and fat and may have on occasion competed for bunk space and shade.

The one major limitation to detecting these changes in blood variables during the feedlot trials was the weekly bleeding schedule. With few exceptions, many of the blood measures altered rapidly during acute heatwave events and recovered as soon as the heat load was reduced. As shown in the examples of plasma creatinine and bicarbonate given in Fig. 138, the weekly bleed schedule frequently and completely missed these changes.

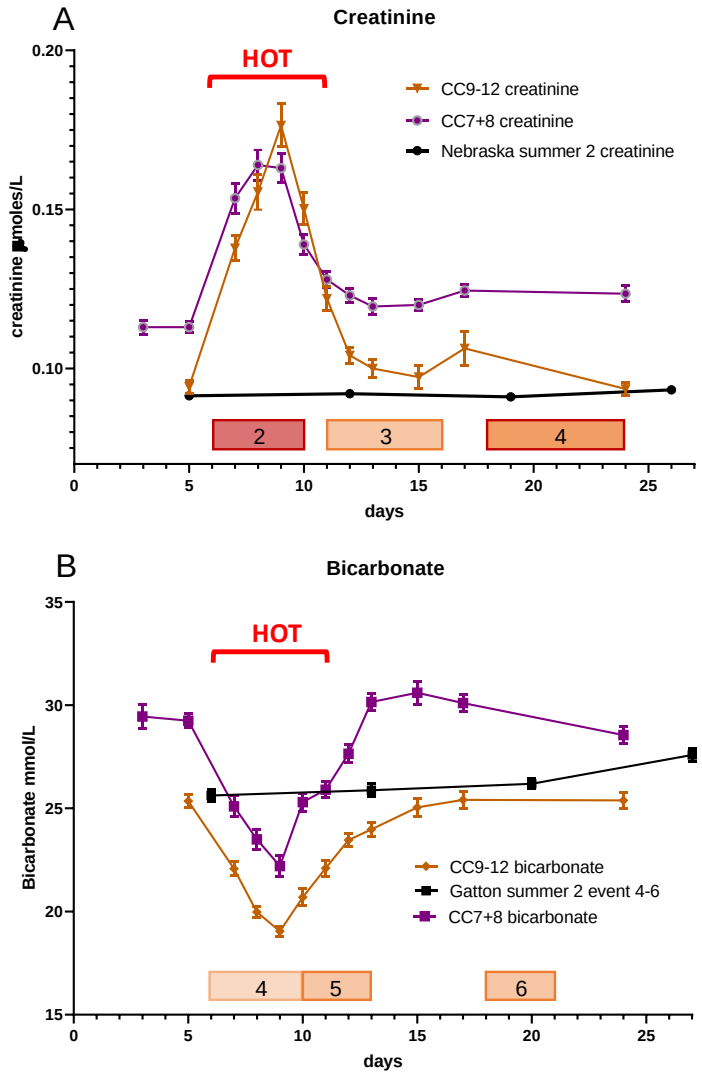

Fig. 138. Changes in plasma creatinine and bicarbonate concentrations during increased heat load. The first day of increased heat load has been standardised to day 6. Panel A. The daily mean creatinine concentrations from climate chamber experiments 7 and 8 (CC7+8), and 9-12 (CC9-12) are overlayed with the creatinine concentrations from the weekly bleeds taken during the Nebraska summer 2 trial events 2-4. Panel B. The daily mean bicarbonate concentrations from CC7+8 and CC9-12 are overlayed with the bicarbonate concentrations from the weekly bleeds taken during the Gatton summer 2 trial events 4 – 6. The numbered coloured boxes indicate the heatwave events and their intensity. The red bracket indicates the HOT period in the climate chamber experiments. Bars indicate SEM.

Thus, for blood variables with rapidly changing concentrations such as creatinine, urea, bicarbonate, potassium and glucose, there were only one or two heatwave events where these changes were captured. However, there were examples from the feedlot trials that the now anticipated changes were detected in association with moderate to strong heatwave events that occurred in these trials. Plasma ALP activity falls rapidly during high heat load and is slow to recover. Fig. 139 presents the ALP trajectories for three heatwave events which mirrored the climate chamber trajectories. Plasma cholesterol concentration behaves similarly to the ALP activity. The anticipated cholesterol response was seen for two heatwave events (Fig. 140). Plasma chloride concentration increases in high heat load. This rise was consistently observed in the feedlot trial heatwave events (Fig. 141).

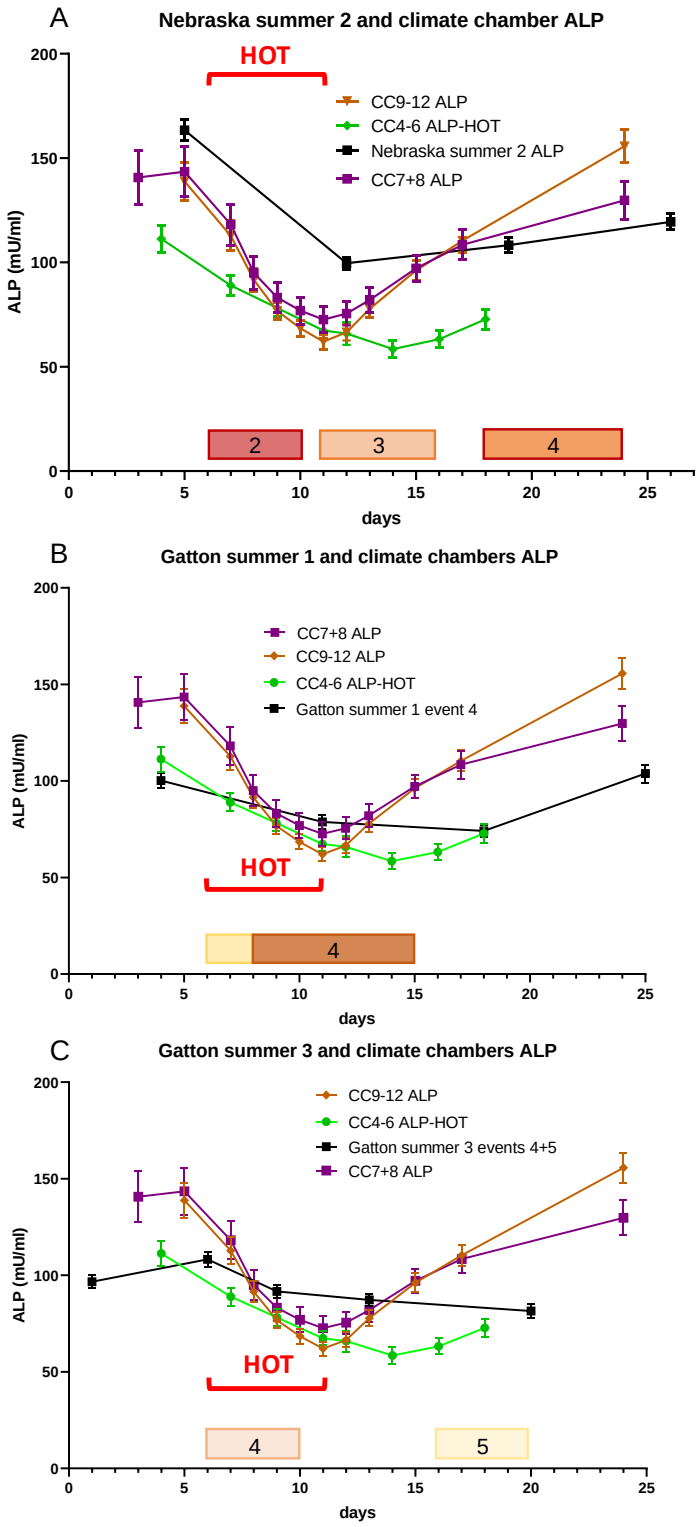

Fig. 139. Comparing the feedlot trial plasma ALP responses to those of the climate chamber experiments. The first day of increased heat load has been standardised to day 6. The daily mean ALP activities from CC7+8, CC9-12 and climate chamber experiments 4-6 (CC4-6) are overlayed with the plasma ALP activities from the weekly bleeds taken during (A) Nebraska summer 2 events 2-4; (B) Gatton summer 1 event 4; and (C) Gatton summer 3 events 4 and 5. The numbered coloured boxes indicate the heatwave events and their

intensity. The red bracket indicates the HOT period in the climate chamber experiments. Bars indicate SEM.

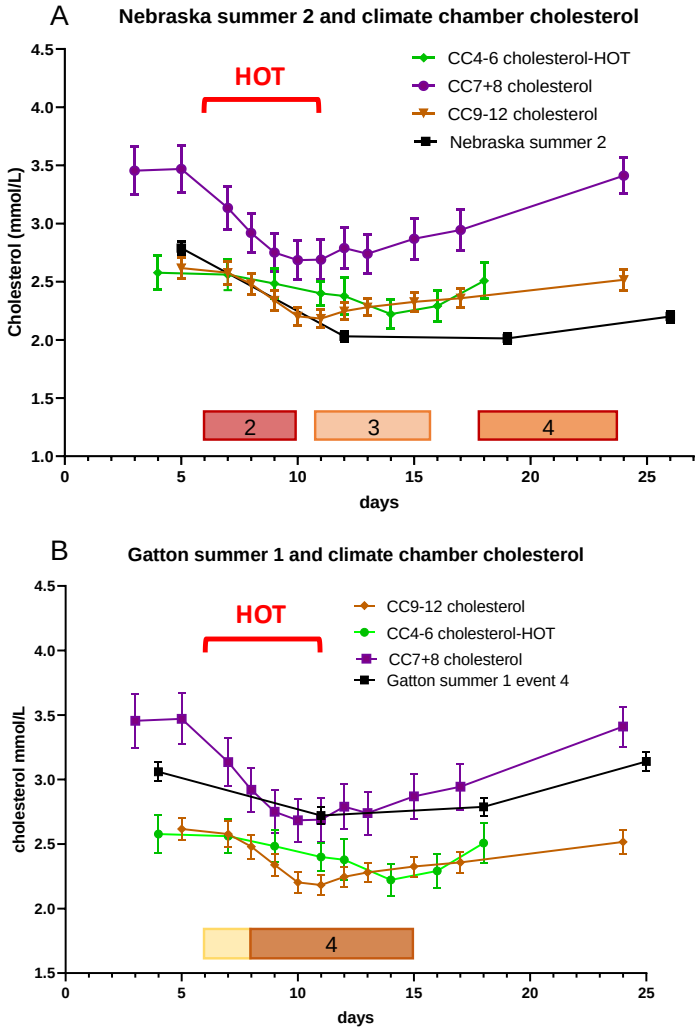

Fig. 140. Comparing the feedlot trial plasma cholesterol concentration changes to those of the climate chamber experiments. The first day of increased heat load has been standardised to day 6. The daily mean cholesterol concentration from CC7+8, CC9-12 and CC4-6, are overlayed with the plasma cholesterol concentration from the weekly bleeds taken during (A) Nebraska summer 2 events 2-4; and (B) Gatton summer 1 event 4. The numbered coloured boxes indicate the heatwave events and their intensity. The red bracket indicates the HOT period in the climate chamber experiments. Bars indicate SEM.

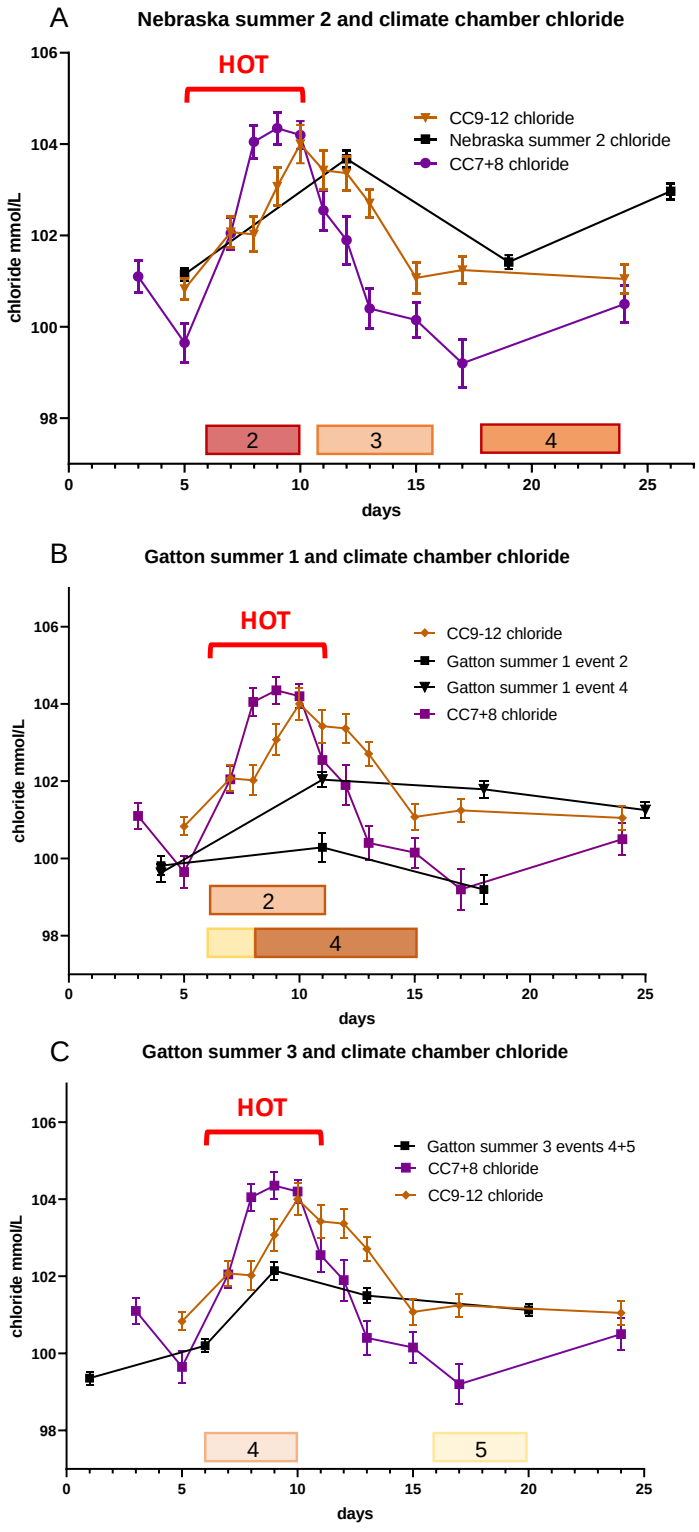

Fig. 141. Comparing the feedlot trial plasma chloride concentration changes to those of the climate chamber experiments. The first day of increased heat load has been standardised to day 6. The daily mean chloride concentration from CC7+8 and CC9-12 are overlayed with the plasma chloride concentration from the weekly bleeds taken during (A) Nebraska summer 2 events 2-4; (B) Gatton summer 1 events 2 and 4; and (C) Gatton summer 3 events 4 and 5. The numbered coloured boxes indicate the heatwave events and their

intensity. The red bracket indicates the HOT period in the climate chamber experiments. Bars indicate SEM.

Table 25 below summarises when responses in blood variables were detected in the major heatwave events during the summer feedlot trials. Gatton summer 3 event 3, was associated with the most blood variable changes that corresponded to changes in the climate chamber studies. Gatton summer 1 event 4 was a strong heatwave event also that coincided with changes in several of the blood variables. These two events were classified as strong heatwaves, and of 4-5 day duration, similar to the high heat load climate chamber experiments.

Surprisingly, Gatton summer 3, event 4, which was classified as a mild event, appeared to induce the appropriate responses. The physiological data for this event (Fig. 126-129) indicated a comprehensive high heat load response in the cattle despite the low intensity heatwave classification. The bleeds from Nebraska summer 2 picked up several blood changes, but the results for some parameters may have been more difficult to interpret due to the ongoing nature of the series of heatwaves experienced that summer.

Table 25 Comparison of blood variables between Gatton and Nebraska summer feedlot trials.

	Neb2	GS1(2)	GS1(4)	GS2(3)	GS2(4-6)	GS3(3)	GS3(4+5)
ALP	✓	✓	✓	✓	✓	?	?
creatinine	×	×	✓	×	×	?	✓
urea	×	?	?	×	×	×	✓
bicarbonate	?	×	×	✓	×	✓	✓
chloride	✓	✓	✓	✓	✓	✓	?
cholesterol	✓	?	✓	×	×	?	×
potassium	?	×	×	×	×	✓	×
glucose	?	×	×	×	×	✓	?
CK	×	×	×	×	×	✓	×
WBCs	×	×	×	×	×	✓	✓
neutrophils	×	×	×	×	×	✓	×
eosinophils	×	×	×	×	✓	×	×

Neb2: Nebraska summer 2; GS = Gatton summer.

4.2.4 Discussion and conclusion

Overall, typical high heat load responses (reduced DMI, increased PS, elevated maximum and minimum rumen and rectal temperatures) were associated with mild-strong heatwave events. However, there were some interesting inconsistencies amongst those measures and the intensity of the heatwave events. Two examples can be found with Gatton summer 1 event 4, a strong late season heatwave, and Gatton summer 2 events 4-6, a series of mid-season mild-moderate heatwaves. The former event induced large changes in maximum and minimum rumen temperatures, DMI was reduced and ADG was zero, and yet the anticipated large increase in PS did not occur. The Gatton summer 2 events 4-6, were characterised by varying degrees of changes in maximum and minimum rumen temperatures, increased PS but no proportionate reduction in feed intake.

There were examples of strong responses to the first mild or moderate heatwave of the season in Gatton summer 1 event 2 and Gatton summer 2 event 3. These saw sharp rises in PS and rumen temperatures, and reduced DMI and ADG. On the other hand, Gatton summer 2 events 7 and 8, two back-to-back late season events after some weeks of very stable weather conditions and rising feed intake, were associated with profound changes in PS, rumen temperatures, rectal temperature and feed intake. Given these apparently inconsistent interactions between conditions, physiological responses and feed intake, it is not surprising that those managing cattle at feedlot operation can be caught unprepared. Acclimation, intake, days-on-feed and weight (fat deposition), “wash-out” of the implant actives can all affect the ability to control and dissipate accumulated heat load.

Over the ~100-day period in the feedlot pen, there were clear metabolic changes in plasma insulin (increasing), cholesterol decreasing, creatinine increasing etc. These were normal changes to be expected with increasing size for animals on a finisher diet. Another learning garnered from the feedlot trials was the strong effects of seasonality on the steers’ growth rate and intake, and metabolic, endocrine and inflammatory status. Two winter feedlot trials were conducted, one each in Gatton and Mead (Nebraska). While not presented in this report, and not fully analysed yet, there were clear differences between the ‘summer’ and ‘winter’ animal. In fact, we think some of the differences in responses between the sequential cohorts entering in the climate chamber experiments (about 4 weeks apart) may be due to these seasonal changes. The CC4-6 cohorts that entered the chambers over May, June and early August seem to exhibit this signal.

It was very reassuring to see that some of the metabolic responses observed in the high head load climate chamber experiments were captured during or after the more intensive heatwave events in the feedlot trials. As discussed earlier in this section, the data collected from the weekly measures of the blood variables were more limited in their information due to the rapid dynamics of the metabolic parameters during and after high heat load. The industry can be confident that the physiological responses observed in the climate chambers largely reflect high heat load responses in the feedlot pen.

5 Discussion

5.1 Synthesis

At the outset of this research program, we sought evidence that gut integrity was compromised under heat stress and this provoked an unregulated systemic inflammatory response. We considered that this was the underlying mechanism of the poor performance and welfare of heat stressed feedlot cattle. The expectation was that we would be investigating means to avoid loss of gut integrity and reduce the inflammatory response to improve outcomes.

At the time, the results of the chronic and acute moderate heat load climate chamber experiments (CC1-3 and CC4-6), and the feedlot trials that were being run concurrently, were disappointing. However, the results of the two high heat load climate chamber experiments (CC7 and 8, and CC9-12) have been highly informative. As seen from the physiological data collected from these trials, and presented in this report, the animals accumulated very high heat loads under these protocols. This was especially so for CC9-12, where the animals were in average Dalby summer conditions prior to and after the thermal challenge.

Integrating the physiological, metabolic, endocrine and inflammatory data has been a challenge. This is our interpretation at this stage.

During high heat load:

- There is no systemic inflammation and no evidence for loss of gut integrity in healthy feedlot steers coping with high heat load.
- We can dismiss other theories of rumen acidosis and lactic acidosis as major contributors to the symptoms of heat stress.
- The brain and organs are committed to reducing endogenous heat load by all possible mechanisms
    - Behavioural
        - rapid reduction in feed take to limit substrate for rumen fermentation
        - increased water usage to ensure evaporative cooling from the skin
        - seek shade but reduce movement
    - Increase heat dissipation
        - Blood flow
            - Diversion to the skin to allow direct heat loss and loss through evaporative cooling
            - Reduced blood flow to all organs (except brain and lungs)
                - As a consequence, the organs reduce metabolic activity (but are not hypoxic) and the output of these organs is reduced
            - Increased respiration rate – allowing increased heat exchange deep into the viscera through the very large lung internal surface area
    - All other functions, for example, growth and reproduction are placed on hold.

But there are consequences to invoking these mechanisms.

- Acid-base balance
  - Blood bicarbonate is rapidly removed by the increased respiration rate. This induces a respiratory alkalosis.
  - The kidneys are working hard to maintain acid-base balance. Chloride is retained leading to a hyperchloremia.
- Energy metabolism
  - Reduced feed intake and a reduced blood flow to the rumen
    - limited acetate and propionate to the liver to supply glucose
    - reduced urea 'dumping' into the rumen
    - rumen
      - altered rumen microbiota (increase in methanogens)
      - loss of diurnal pH cycling with pH range restricted to 6.5-6.7
  - liver glycogen consumed in the early stages to defend glucose concentration
  - probably limited mobilisation of fatty acids from fat deposits and limited oxidation of the fatty acid in the liver and kidney
  - there appears to be limited release of lactic acid and amino acids by skeletal muscle to enable gluconeogenesis in the liver
- reduced blood flow
  - reduced processing and secretion of bilirubin into the bile, symptomatic of a cholestasis
  - reduced glomerular filtration by the kidneys leading to retention of creatinine and urea in the blood. High blood levels of urea are neurotoxic.
  - reduced hepatic function – reduced cholesterol synthesis and ALP
  - reduced bone growth evidenced by actual bone resorption
  - reduced production of hormones from fat and the thyroid
- increased white blood cell numbers especially the neutrophils. This may be due to vascular changes restricting migration in and out of the tissues. Eosinophil numbers are markedly decreased.

However, blood volume is maintained, and the inflammatory system is controlled. In many animals, the plasma cytokine concentrations decreased markedly during high heat load.

The CC9-12 climate chamber protocol is likely to be the limit of an in-chamber thermal challenge. All metabolite and enzymes reporting organ damage were on a firm upward trajectory on the third and last day of maximally hot conditions. A fourth day at this level of thermal challenge might have had severe consequences for many more animals in this trial.

In Recovery

The comprehensive study of the recovery phase confirmed known responses but revealed many unanticipated findings. In the climate chamber experiments, the steers were highly sensitive to any improvement of conditions and responded accordingly. As soon as conditions moderated, feed intake tentatively resumed. Blood glucose was lowest at this point. This might be an indication of the time taken to re-established rumen production and transport of VFAs, and restart gluconeogenesis in the liver using these substrates. Blood glucose then rapidly returns to normal levels despite feed intake improving more slowly

demonstrating the high level of cooperation between the liver and rumen to return this ‘vital service’.

New findings include

- Rumen temperature and diurnal amplitude are reduced compared to prior to the thermal challenge implying a negative thermal balance
- Blood pH is mildly acidotic on average. Some animals are clearly acidotic
    - Respiration rate is low relative to the pre-thermal challenge, resulting in high CO_2 relative to blood bicarbonate and contributing to a respiratory acidosis
    - Increased production of lactate and blood lactate contributing to a metabolic acidosis
- Increased liver enzymes in the blood as a possible indication of liver damage but may be reporting the consequences of a hepatic ischemia as blood flow fully returns to liver (similar to a reperfusion injury).
- Liver and bone ALP and cholesterol are slow to recover to normal levels. This has implication for liver function, bone growth and production of hormones derived from cholesterol including the mineralocorticoids (e.g. aldosterone) and glucocorticoids (e.g. cortisol).

The varying dynamics of the appearance of increased or decreased amounts of these ‘liver function’ enzymes are likely to be informative as to the nature of the cellular events and affected zones within liver during heat stress. AST and GLDH simultaneously and immediately rise in Recovery, whereas GGT lags by a few days. Clearly, the appearance of ALP into plasma is under a different mechanism from the other liver enzymes. Interestingly, the cholesterol trajectory is very similar to ALP.

- Blood urea is low relative to the pre-thermal challenge, and for some time. Regulation of blood urea is a function of the kidney and liver. The low concentration may reflect decreased production of urea by the liver as N is retained to produce amino acids for tissue repair, increased urea being returned to rumen and the kidney attempting to secrete any excess.
- The reduction in circulating white blood cells of all lineages except eosinophils.

The acute moderate heat load climate chamber experiment was not a just a milder version of the high heat load experiments. The thermally challenged animals were taken overnight from thermoneutral conditions to a daily maximum temperature of 35°C (THI of 86) at which they were kept for five whole days. Also, there was no step to recovery conditions. Some of the physiological parameters showed clear signs of adaptive responses in day 4-5 of this moderate heat load period.

On the whole, the responses of the thermally challenged steers in the acute moderate heat load climate chamber experiment (CC4-6) were similar to those of the high load experiments. As might be expected, the magnitude of the change was reduced. This occurred for DMI, rumen and rectal temperatures, and the plasma parameters, bicarbonate, creatinine, chloride, ALP and triglycerides, and the white blood cell populations. The thyroid hormones, and adiponectin also decreased in concentration, and there was no indication of an inflammatory response. Closely controlled metabolites, such as glucose, urea and bilirubin did not change under this level of heat load i.e. the liver and kidneys were at work

maintaining their appropriate concentrations. In recovery too, there were similarities with the changes seen after thermal challenge in high heat load experiments. Rumen temperatures were lower in recovery, along with plasma urea and ALP. Plasma AST and GGT, and the hormones all rose in recovery.

The chronic moderate heat load experiment (CC1-3) saw the steers incrementally stepped up from thermoneutral conditions over six days to their hot conditions of 32°C daily maximum temperature. They remained in these conditions for ten days before returning to thermoneutral conditions. Given the six days to initiate adaptive mechanisms and the far milder thermal challenge conditions, the physiological and metabolic responses are invoked but subtle, but more or less consistent with the later experiments with much higher heat load.

The overall consistency of the responses through the levels of escalating heat load imposed by these experiments is formidable. The reproducibility between the high heat load trials also inspires confidence in having a robust methodology and consistent understanding of the impact of high heat load on the feedlot steer.

5.2 Objectives met

5.2.1 Provide recommendations for nutritional management of feedlot cattle during summer, and/or during periods of high heat load, to improve productivity and welfare of feedlot cattle, through:

5.2.1.1 Establish the need for nutritional strategies to manage heat load through the high risk periods of summer, or all of summer feedlot studies.

The five summer feedlot trials presented varying conditions, and frequency and intensity of heatwaves. A number of moderate to strong heatwave events act as case studies for characterising the actual in-pen responses of feedlot steers to conditions that cannot be completely replicated in climate chambers. Notwithstanding, with the limited data collection and blood sampling of these larger cohorts of steers, we were able to identify physiological, metabolic and endocrine changes in some of these events similar to those observed in the high heat load experiments. The rumen temperature data was critical to verifying heatwave responses.

It was notable that while there were ‘textbook’ physiological responses for many of the heatwave events, the magnitude of the animals’ responses were not always proportionate to the intensity or duration of the heatwaves. Undoubtedly there is much more at play than just THI.

5.2.1.2 Understand the impact of heat load conditions on immune status, and the altered metabolic and inflammatory responses induced by these conditions.

We can now describe the physiological, metabolic and endocrine consequences of increasing heat load on feedlot steers on a finisher grain diet. In fact, we know more about the heat load responses on multiple dimensions in steers than any other species or cohort including humans. We have collected a globally unique sample and data set.

The incremental elevation of heat load through our series of climate chamber experiments have shown that adaptative responses are invoked as soon as heat load is increased. The feedlot steer is very sensitive to weather conditions and responds rapidly and appropriately. The high heat load experiments have shown the limits of adaptation and homeostatic mechanisms at work to ensure the animal copes and survives. While we did not encounter the inflammatory responses that we initially proposed, we suspect that if coping mechanisms had collapsed completely, we could have seen the initiation of organ failure, and loss of gut integrity.

5.2.2 Develop new nutritional interventions and management strategies before, during and after heat load events.

Having optimised the use of the climate chambers in previous trials, the final climate chamber trial (CC9-12) was designed to investigate the effects of the heat load ration on cattle under high heat load and thereby identifying this as a nutritional intervention for heat load events. This experiment introduced an industry standard heat load ration to the animals either two days before or on the day of the high heat event. The control group received the standard finisher ration throughout the trial.

This very successful trial has produced some strong leads for the livestock industry on the impact of heat stress. The most obvious observation was that animals that are on full finisher ration should not be changed to a heat load ration once the heat load event has begun. This diet (diet 2 in the trial) proved to be the most injurious to the steers. It was definitely worth noting that those animals who were introduced to the finisher ration two days prior to the heat event had no fatalities or removals from the chambers.

This climate chamber protocol and its results now provide a platform to investigate other potential interventions in heat stressed animals. For example, reducing DMI prior to heat stress should ameliorate the more severe responses to increased heat load as endogenous heat production in the rumen would be reduced. Additionally, more data could be obtained for proactive diets such as diet 3.

Other interventions could also be tested. The blood gas analyses obtained in this trial provided an insight into possible acidosis in the animals during recovery, so possible supplements could be tested to alleviate this problem and help with recovery. Similarly, rumen probiotics could also be considered to aid restoration of rumen function.

The dramatic drop in feed intake at the onset of a heat load event suggests that any interventions in diet must occur either before or after the event. If anything is to be administered during the event, it should be deliverable by water as water intake increases under hot conditions, whereas DMI falls markedly.

5.2.3 Increased ongoing nutritional research capability for industry through training and mentoring of a postdoctoral fellow at the University of Queensland

This project has supported a number of staff and students at the University of Queensland to ensure an ongoing capability in the livestock industry.

- Dr Megan Sullivan was appointed as a post-doctoral fellow at the start of the project and has been pivotal to its success. She was fully funded by the project throughout. She is now employed by DAF Qld.
- Miss Stephanie Sammes worked on both feedlot trials and climate chamber experiments and has almost completed a PhD through UQ on results from these heat stress trials.
- Miss Alex Gloria has just completed her Honours year in the Centre for Advanced Imaging, UQ on the metabolomic data from the plasma samples collected in CC5, CC6, CC7 and 8 climate chamber experiments. She was awarded a first class Honours.
- Miss Audrey McInnerney was employed on the project as a CSIRO Indigenous cadet as she completed her undergraduate studies at UQ. She was involved in the cytokine assays of the blood samples from several trials. Audrey has now begun her PhD studies with the U University of Queensland.

Other students and volunteers engaged through CSIRO have also benefitted from being involved with this project including

- Miss Hyab Mehari (QLD) – developed and performed plasma assays and is now enrolled in a PhD at the University of Queensland
- Miss Elle Stephenson (QLD) – developed and performed plasma assays and is also now doing a PhD at Griffith University.
- Miss Mara Macs (VIC), an undergraduate in biomedical sciences, developed the adiponectin assay and assayed plasma samples to gain further laboratory skills. She has graduated and is now employed as a medical laboratory scientist in rural Victoria.
- Mr Pedro Ratto – a Brazilian PhD student who used plasma samples to investigate proteomic detection methods for cytokines. Pedro has graduated, completed a post-doctoral position in Denmark, and now returned to Brazil.
- Miss Cintia Carol – a Brazilian PhD student helped develop and perform the thyroid hormone assays on plasma samples. Cintia has graduated from UNESP (Jaboticabal).

6 Conclusions/Recommendations

- The rumen temperature data is a very rich source of information on cattle response to diet and the environment. What is the relationship between rumen temperatures and time at high temperatures with PS, HLI and metabolic variables? How much heat load can be accumulated/experienced before there are deleterious changes during heat stress and/or recovery? It should be possible to develop a physiological and metabolic model of response to heat load based on the rumen temperature. Currently the heat load models for industry are based on PS and the environmental conditions. PS measures can be subjected to bias and error and may not be performed consistently by different operators and at different times of the day. The conceivable future could be based on rumen temperature boluses placed in a small proportion of head/pen to act as sentinel animals. This would require an HLI model

based on rumen temperature. Such a fully automated system with alerts is feasible in the not too distant future.

- The industry now has access to a robust and reliable protocol for imposing high heat load on feedlot steers under climate chamber conditions. We know a lot about how these animals cope under these conditions; we can discriminate coping responses from not-coping. This offers the opportunity to scientifically test and validate any number of interventions and altered rations or ration additives. Possible candidates for assessment are
    - Reducing intake prior to thermal challenge to lower the rumen fermentation rate thus heat of fermentation and hepatic metabolic heat production
    - Targeting the systemic acidosis during recovery. Would delivery of a bicarbonate salt in water or feed improve blood pH and recovery of feed intake?
    - What drugs or metabolites would assist the liver during and after heat stress? Can we reduce the indicators of liver damage that we see during recovery?

7 Key Messages

7.1 Heat stress in the feedlot steer

The feedlot steer is very adept at invoking mechanisms to adapt to increased heat load. However, sudden intense heat wave events have serious physiological and metabolic consequences for the heavy feedlot steer (i.e. Black Angus). The immediate response is to reduce internal heat production by markedly reducing feed intake and metabolic rate, and increase cooling by increased respiration and shunting blood to the skin. Following the heat wave, the liver and the rumen, and possibly the kidneys, take time to recover. However, there is no evidence for gut damage and uncontrolled inflammatory responses in the healthy animal coping with heat stress.

7.2 Ameliorating high heat load

We have developed a robust and reproducible methodology to test a wide variety of interventions to ameliorate heat stress in the feedlot steer. For example, we investigated the timing of introduction of the ‘heat load’ ration and showed that changing the ration at the same time as the onset of the heat wave resulted in poor outcomes the animals and productivity; and worse than no change to ration at all. Introduction of the ‘heat load’ ration two days prior to a heat wave could be more effective.

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