finalreport

LIVE EXPORTS

Project code: **LIVE.224**

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Electrolyte supplementation of export cattle, and further investigations in the heat stress threshold of sheep and dairy cattle

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Abstract

This project involved three separate but linked experiments into the physiology of livestock heat stress. Experiments further investigated the supplementation of specific electrolytes in the water to cattle. In a summer feedlot, 30 animals received electrolytes over 18 days, and were compared with 30 unsupplemented animals. There was a live weight advantage of 2% after 12 days, during which time the supplemented cattle drank and ate more than the controls. This live weight difference was not sustained and the animals were not different at 18 days, nor after a further 7 days. A fluid balance experiment on six heifers held for seven days at high wet bulb temperatures in the climate controlled rooms did not produce any significant differences between supplemented and control groups. There was a significant increase in extracellular fluid volume in both groups after exposure to the heat. The heat stress thresholds for heavy rams, wethers, ram lambs and pregnant Friesian heifers were determined by logging core body temperature as they were exposed to increasing wet bulb temperatures over several days. The temperature at which they were 0.5°C above their normal core body temperatures was approximately 26°C wet bulb for the ram lambs, and 27°C wet bulb for the other classes of animals.

Executive summary

The first objective of experiments conducted in this project was to attempt to repeat previous work, done in LIVE.209, which showed a weight advantage of cattle after 18 days of supplementation with water based electrolytes on a commercial shipment to the Middle East. An experiment was conducted on land in a feedlot over summer, where greater monitoring and control was possible, compared to that on a ship. Sixty steers were used, divided into four pens of 15 animals each, of which two pens received plain water, and two pens received water supplemented with 1.8 g sodium bicarbonate and 3.5 g potassium chloride per litre of water. Animals were weighed before the start of supplementation and on days 6, 12, 18 and 25. Daily feed intake per pen was recorded, and total water intake for each of the treatment and control groups were measured. Urine was collected on the days of weighing and pH and specific gravity measured.

Environmental conditions were quite mild, but the supplemented animals did drink more than the control animals, and also ate more on several days. There was a weight advantage of the supplemented cattle of around 2% after 12 days of the experiment, but this was not sustained, and by 18 days, both groups weighed the same. After a further seven days of drinking plain water, there was no difference in live weight between the groups. Carcass weights after day 18 were not different between the groups, but there was a difference in colour of one muscle, with the treatment animals having a higher luminescence reading from the *longissimus dorsi*. Urine pH of the control animals was significantly below that of the treatment animals on days 6 and 12.

A fluid balance experiment was conducted using six heifers in the climate controlled rooms, in a cross-over replicated design, to determine what fluid shifts might be occurring in cattle supplemented with electrolytes. Three animals at each replicate received the water-based electrolyte supplement for nine days, of which seven days were spent at high wet bulb temperature, between 25.5 and 28.5°C wet bulb. During the intervening period of eight days between the replicates the cattle received plain water. As well as measuring live weight, individual feed and water intake, and physical variables such as rectal temperature and respiratory rate, the fluid volume of body compartments was assessed using indicator dilutions. Empty body water (EBW), extracellular fluid volume (ECF), and plasma volume (PV) were assessed before and after the heating in each replicate, using urea, sodium thiocyanate, and Evans Blue dye respectively. There were no measured differences between the groups in any of the variables. All animals ate less and drank more during exposure to the heat, and all animals lost weight during that time. Respiratory rate increased, and there were blood gas changes typical of panting. The percent of live weight measured as EBW in this experiment varied from 38 to 59 %, which is a greater range than recorded previously for normal animals, but does not indicate increased retention of body water in either group. ECF was significantly increased in both groups after exposure to the heat, indicating that there was a shift in fluid between body compartments.

It was not possible to arrange to do further electrolyte supplementation of cattle on a commercial shipment during the northern summer, and therefore the shipboard work was not done. Further consideration of this work, in light of all the preceding experiments, resulted in the shipboard work being cancelled at this time.

Several classes of livestock, identified by the live export industry as important, were used in work to determine their heat stress thresholds, so that this information could be included in the heat stress

risk assessment model, for optimum management of shipping these animals. The animals were subjected to periods of increasing heat and humidity, while their core body temperatures were recorded, so that heat stress thresholds for each class could be calculated. The existing definition of heat stress threshold is that wet bulb temperature at which the core body temperature is 0.5°C above normal, which we named HST2. We also identified the wet bulb temperature at which the core body temperature first significantly increased above normal (HST1), and when the core body temperature was 1°C above normal (HST3). HST3 was considered to be most closely related to the development of clinical signs of heat stress such as open mouthed panting.

Heavy Merino rams, wethers, and ram lambs were implanted with core temperature loggers, then housed individually within the climate controlled rooms, three of each class in each room, with a further two sheep of each class in a separate unheated control room. The rooms were gradually heated from 26°C wet bulb increasing every two days up to 32°C wet bulb. Feed and water intake, respiratory rate and character, and rectal temperature were recorded on each animal, and when a sheep had a rectal temperature above 40.5°C it was removed from the rooms on welfare grounds, with the expectation that it would have reached its HSTs. The loggers were retrieved from the animals and the data from each animal compared with the wet bulb temperatures recorded in the rooms, and the HSTs calculated. A similar protocol was used to determine the HSTs of Friesian heifers, three to five months pregnant. The heifers were too long to be penned individually, and so were housed three per room in group pens.

There was variation in the heat tolerance of the individual animals in each class, with some animals being particularly tolerant of the heat. Two different methods of calculation were used to determine the HSTs, and it was found that a method using confidence intervals (Method 1) was most successful in accounting for individual animal variation in the sheep, while a t-test method was suitable for the heifers. Ram lambs were less tolerant of the heat, which may be due to their immaturity, and also the slightly longer wool on those animals. In approximate wet bulb temperatures, the HST1 for the ram lambs was just under 25°C, and for the other classes of animals was 26°C. HST2 for the ram lambs was around 26°C, and for the other animals 27°C wet bulb, while HST3 for the ram lambs and heifers was just under 28°C and for the rams and wethers was approximately 29°C wet bulb. These figures give an indication of the relative susceptibility of the animals to heat and humidity, but also the variation that exists within any group of animal, and the almost infinite variations that can occur with size, weight, coat cover, and physiological status.

Contents

1 Background

1.1 Electrolytes

Project LIVE.224 was conducted to further investigate the effects of supplying cattle with supplemental electrolytes in the drinking water, during periods of high heat and humidity, with specific reference to the conditions experienced by cattle being exported by ship to the Middle East.

Work in LIVE.209A defined the physiological responses of cattle exposed to continuous prolonged periods (up to five days) of high heat and humidity. That work identified changes in blood gas variables, as the animals panted in the heat, and in particular there was a decrease in blood bicarbonate that persisted after the period of heat exposure. There was also a decrease in feed intake, and alterations in blood and urine concentrations of key electrolytes, indicating renal conservation of sodium, potassium and chloride after the period of heat exposure. The measured changes in those experiments led to the formulation and testing of a water-based electrolyte supplement, in both climate controlled rooms and on a commercial live export vessel.

In LIVE.209B there was a significant weight advantage in exported *Bos taurus* supplemented with electrolytes in the drinking water for the duration of a voyage from Western Australia to the Middle East, compared with unsupplemented cattle. This experiment was performed on a commercial ship, and the environmental conditions for those animals were not so severe as to cause clinical heat stress. Work in the climate controlled rooms with cattle exposed to more extreme heat did not result in any significant differences between supplemented and unsupplemented animals, although there was a trend for supplemented animals to lose less weight than the unsupplemented cattle. There was also an indication in both experiments that the supplement influenced acid-base balance, with significant effects on urine pH. There was no apparent effect of the supplement on body temperature, feed intake, respiratory rate or panting score. In neither situation could it be determined whether the supplement had a real benefit on health or welfare of the animals under conditions of heat stress, because the conditions on the ship were not extreme, and because the number of animals tested in the climate controlled rooms was too small for significance. However, the demonstration of a weight advantage in supplemented animals experiencing normal voyage conditions, even in the absence of clinical heat stress, could be of commercial significance.

Thus, further work was proposed to test the repeatability of the weight advantage, and to determine the nature of this weight, whether due to fluid retained in gut or tissue. The animals in previous experiments receiving the electrolyte supplement did drink more, while feed intake was apparently not different between the groups. Thus it was assumed that the increased live weight was due to the additional fluid intake, and retention of some of that fluid in the animal.

Fluid within an animal can be divided into several compartments. Fluid taken in by the oral route enters the gastrointestinal tract and is absorbed or passes through, with further absorption and secretion determining the final balance. Once in the body the total body water (TBW) is approximately 60% of body weight, and this can be divided into that water inside cells (intracellular fluid (ICF), 40% body weight), and that outside cells (extracellular fluid (ECF), 20% body weight). The ECF can be further divided into that in the blood stream (plasma, about 5% of body weight) and that around cells (interstitial fluid).

The amount of fluid in these compartments depends on fluid flowing from one to another in balance, largely determined by the relative concentrations of the different electrolytes, in particular sodium, potassium, and chloride, and whether the membranes separating the compartments are permeable. Hormones such as aldosterone, antidiuretic hormone, angiotensin and others all determine the body balance of these electrolytes and fluid, and exert their main control through renal excretion or conservation of fluids and electrolytes.

Determination of the amount of fluid in each of these compartments has routinely been done through the use of indicator dilution techniques (Guyton and Hall, 1996). Indicators that remain in the particular compartment are inserted into that compartment in known quantities, allowed to equilibrate, and then samples are withdrawn to determine the new concentration of the indicator and thus calculate the volume of fluid. Estimation of TBW has used tritium or deuterium, which distribute to all body water. Sodium thiocyanate, sodium bromide, and sodium thiosulfate have been used to measure the ECF, while Evans blue dye has been used to measure plasma volume.

These indicators have been evaluated for their use in ruminants. Deuterium oxide has been used to measure body composition including TBW in beef steers (Arnold et al., 1985; Arnold and Trenkle, 1986) and dairy cows (Odwongo et al., 1985; Martin and Ehle, 1986; Andrew at al., 1995), although there are problems with the equilibration of water between the blood and the gut. In cattle and sheep, urea has been used to measure empty body water, and both sodium thiosulfate and sodium thiocyanate have been used to estimate extracellular water (Rule et al., 1985; Hammond et al., 1988; Hammond et al., 1990; Gad and Preston, 1990; Ross et al., 1992). Evans blue dye has been used to determine plasma volume in calves (Wagstaff et al., 1992).

Use of electrolyte solution in transported livestock has resulted in differences in carcass weights (eg Schaefer et al, 1990; Gortel et al., 1992), which supports the concept that the weight advantage of the cattle in LIVE.209B was due to increases in TBW rather than simply gut fill.

1.2 Heat stress threshold

An additional benefit of the previously reported work in LIVE.209 has been the estimation of a heat stress threshold for the classes of animals examined. The heat stress threshold (HST) has been defined as, “the maximum ambient wet bulb temperature at which heat balance of the deep body temperature can be controlled using available mechanisms of heat loss such that at that local air wet bulb temperature the core temperature of the animal is 0.5^{o}C above what it otherwise would have been” (Maunsell Australia, 2003). The use of internal temperature sensors that record and transmit core body temperature have allowed accurate monitoring of the animal response to the conditions.

Previously, the term “upper critical temperature” has been used to describe the differing susceptibility of classes of animals to excessive heat load (Yousef 1985; Silanikove 2000). However, these previous studies determined upper critical temperature on the basis of dry bulb temperature only, and did not consider the effect of high humidity. During live shipment, measurement of wet bulb temperature is used to take into account the humidity as well as temperature, because high humidity is an important feature of the ship environment and can influence the ability of animals to lose heat, especially in situations where the ambient dry bulb temperature is around or greater than body temperature. The measurement of an upper critical temperature that uses wet bulb

temperature instead of dry bulb temperature is valuable during live export, to allow specific management decisions to be made about the transport of different classes of animals.

The animals so far examined in detail have been *Bos taurus* and *Bos indicus* heifers, Merino wethers, and Awassi rams. Other classes of stock are exported and data needed to be gathered about those animals so that more accurate information can be entered into the heat stress risk management software.

The classes of animals identified by exporters in 2005 as of high priority to determine HST were

a) ram lambs with medium coat
b) rams greater than 65 kg with medium coat
c) wethers greater than 65 kg with medium coat
d) Friesian heifers in the first third of pregnancy

Other classes of animals identified as of interest but a lower priority were British breed young bulls, Damara lambs, Boer goats, range nanny goats, and deer.

Of note in the original data for the risk management software was the very low heat stress threshold for lambs, which was at odds with anecdotal reports from exporters, and could make a large difference to the number of animals that could be carried on a journey. However, it has been found previously that younger sheep generally react more dramatically to high heat conditions than older sheep (Symington 1960; Thwaites 1967; Hahn 1985). Studies have found that lambs do not gain full adult heat tolerance until one year of age and have a lower upper critical temperature than adult sheep (Thwaites 1967; Hahn 1985). Of the lambs being live exported, most are rams, with ram lambs accounting for 10% of the total live export trade (Hassall and Associates Pty Ltd 2000). Rams have also been found to react more dramatically to hot conditions in terms of increased body temperature and respiratory rate than do ewes or wethers (Symington 1960). Thus, testing rams and ram lambs was of interest in the expectation that their heat stress threshold could be lower than that of wethers.

2 Project Objectives

2.1 Description

Experiments were conducted on land and in climate controlled rooms to improve the understanding of electrolyte replacement therapy in live export cattle. In addition, experiments in climate control rooms were conducted to validate and improve data on the heat stress threshold for a range of classes of sheep and cattle exposed to high wet bulb temperatures.

2.1.1 Primary objectives

By 30 December, 2005,

1. To determine if electrolyte replacement therapy on ship as demonstrated in LIVE.209B is repeatable so that industry is convinced of the live weight advantages.
2. Measure the live weight benefit of increasing the sodium bicarbonate content of the standard ship ration.

3. To confirm that the live weight advantage from electrolyte supplementation is due to additional water consumption and retention in the animal and not related to feed consumption.
4. Assuming water consumption and retention by the animal is the cause of the live weight gain, determine where this water is retained in the animal.
5. Measure any animal performance/welfare benefits during and post electrolyte replacement therapy, including the extent of the live weight advantage post supplementation
6. Using experiments in a climate room, measure the heat stress threshold (HST) in ram lambs, heavy rams and wethers and Friesian heifers.

3 Supplementation of electrolytes to cattle

3.1 Supplementation of electrolytes to cattle in the feedlot

3.1.1 Methodology

A line of 60 *Bos taurus* steers (Shorthorn and Shorthorn cross; 229 ± 5kg BW) were sourced from the south west of Western Australia. Animals were selected based on body weight and temperament and were from the same terminal sire. All experimental animals were treated for internal and external parasites with 0.5 mg/kg Cydectin pour-on (Fort Dodge, New South Wales, Australia) and vaccinated against leptospirosis with a 2 ml subcutaneous injection of Leptoshield vaccine (Commonwealth Serum Laboratories, Australia) prior to intensive handling.

Animals were placed in a paddock at the Murdoch University Farm and given 7 days gradual introduction onto a standard shipper cube; 9.5 MJ of ME and 8.9% crude protein per kg of DM (Appendix 1). Initially, all animals were supplemented with hay but this was reduced as the shipper cube ration increased.

At 1400 h on day 0, animals were randomly allocated into one of four pens in a feedlot on the farm so that each pen contained 15 animals. Each pen was 12.4 x 17 m in size (211m^2) and contained 2 concrete feed troughs (external dimensions 1250 x 400 x 280 mm and 2500 x 400 x 280 mm), 2 concrete water troughs (external dimension 1250 x 400 x 280 mm) and an area of shade in one corner.

The experiment began on day 1 and all animals were weighed at 0700 h (after 18 h off feed but not water). After weighing some rearranging of animals took place so that the total weight of animals in each pen was approximately the same. Animals were weighed again on days 6, 12, 18 and 25, again after 18 h off feed but not water. On days 6, 12, 18 and 25 the percent change in initial start weight (day 1 weight) was calculated by dividing the weight on that day by initial weight and then multiplying by 100.

Water was available *ad libitum* via two 1000L water tanks which gravity fed into the 2 water troughs per pen. Pens 1 and 2 were designated as treatment pens (n = 30) and pens 3 and 4 control (n = 30). After weighing on day 1, the treatment pens had electrolytes supplied in the drinking water at a rate of 1.8 g/L sodium bicarbonate and 3.5 g/L potassium chloride. Electrolytes were thoroughly mixed in the 1000L tank before dispensing. Both tanks were topped up as necessary so all animals had access to water *ad libitum* throughout the experiment. The total amount of water consumed for both treatment and control animals was calculated daily. The electrolyte supplementation continued

for 18 days. After weighing on day 18, electrolytes were removed from the treatment tank and both treatment and control animals had access to normal drinking water until the experiment ended on day 25.

Feed was supplied at or just above 2.5% BW divided into two feeds fed out at 0700 and 1300 h daily, so that there was feed available nearly all the time, similar to feeding practices on ships. Feed residues were removed at 0700 h each morning and the total amount of feed consumed for each pen was calculated daily. The total feed intake per pen per day was divided by the total BW of the pen and converted to grams consumed per kg of BW.

Urine was collected when animals were weighed on days 1, 6, 12, 18 and 25. Voided urine samples were collected as animals stood in a race prior to weighing. There was no control over which animals were sampled on any day. Urine samples were placed on ice and urine pH and specific gravity were measured within 1 h of collection.

After weighing on day 18, 2 animals from each pen (n = 4 each for treatment and control) were selected for slaughter. Selection for slaughter was based on BW so the weights of all selected animals were approximately the same. Carcass weight was measured after slaughter. After 24 h hanging in a chiller at 5°C, muscle pH and colour of the longissimus dorsi (LD) muscle were measured. At the same time samples were taken of the semimembranosus (SM), semitendinosus (SD) and LD muscles. Dry matter content of these muscles was calculated by weighing the sample and then drying it in a drying oven at 55°C for 3 days. After drying, the sample was weighed again and the dry matter content calculated.

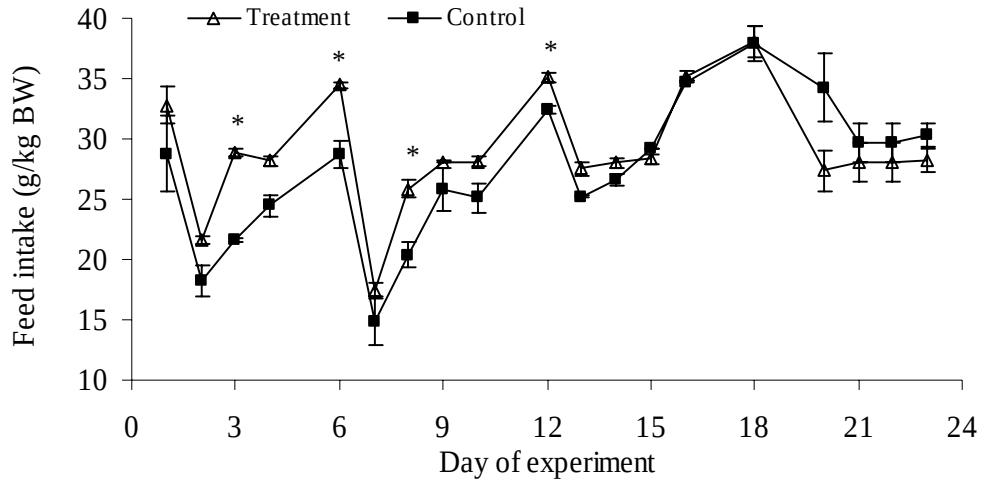
Environmental conditions were monitored by downloading dry bulb temperature (T_a °C), relative humidity (RH %), wind speed (WS m/s) and solar radiation (SR w/m^2) from the weather station located at Murdoch University. The weather station took measurements every 10 min and 2 h averages were calculated. Wet bulb temperature (WBT) was calculated from dry bulb temperature and relative humidity.

Throughout all experiments reported in this document, a 5% level of significance was used, unless specified otherwise.

3.1.2 Results

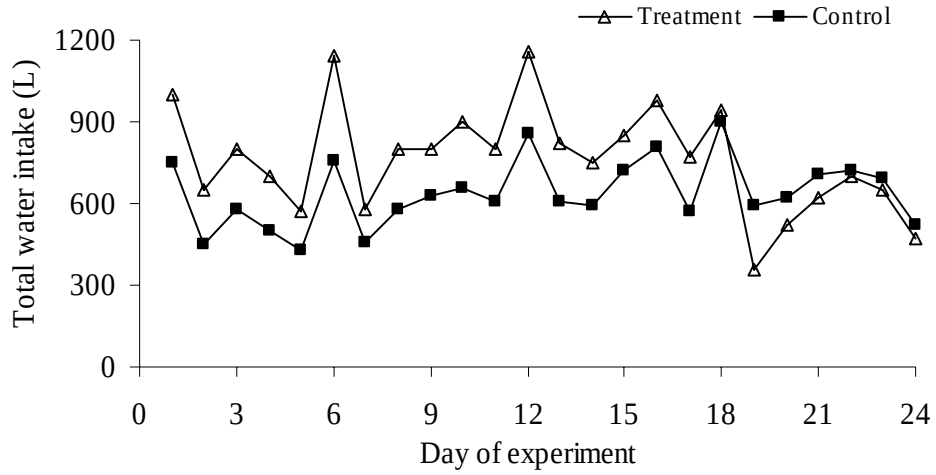
The mean daily feed intake of treatment pens (n = 2) was significantly greater than control pens (n = 2) (Figure 1) on days 3, 6, 8 and 12. The days before weighing (days 5, 11, 17 and 24) were not included in calculations, because on those days feed was removed at 1300 h so curfew weights could be obtained. In general, on the days after weighing, feed intakes for both treatment and control pens were increased. After day 12 there were no days on which feed intake was significantly different between treatment and control pens.

Figure 1: Daily feed intake for treatment and control pens over the duration of the experiment. Treatment animals were supplemented with electrolytes on days 1 to 18. Points show mean ± SEM. The subscript "*" indicates that on that particular day there was a significant difference ($P < 0.05$) in the mean between treatment and control groups.



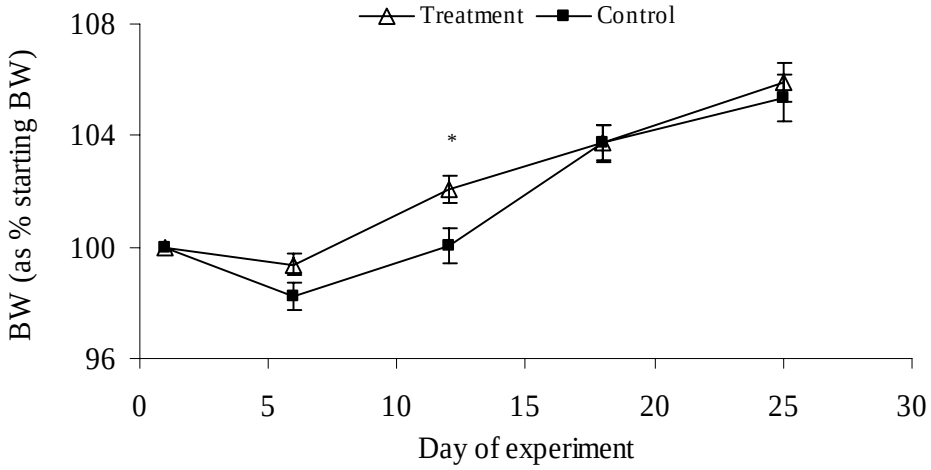
The daily total amount of water consumed for treatment animals appeared consistently greater than control animals between days 1 and 17 (Figure 2) or when treatment animals were drinking electrolyte supplemented water. Peaks in water intake for both treatment and control animals coincide with the day after weighing when animals were eating more. Due to the nature of the water delivery system, neither individual nor pen water intakes were calculated, therefore, it was not possible to analyse the difference in water intake between treatment and controls on any particular day. However, when the difference in the daily amount of water consumed between treatment and control animals was calculated for each day, the average difference on days 1 to 17 was significantly different ($P < 0.001$) compared to the average of days 18 to 24 (i.e. during and after supplementation). On average, when treatment animals were being supplemented with electrolytes (days 1 to 17), they were consuming 206 L/day (6.9 L/animal/day) more than control animals. On days 18 to 24 treatment animals were consuming on average 70 L (2.7 L/day/animals) less than control animals.

Figure 2: Total daily water intakes for treatment and control animals.



The mean BWs of treatment and control animals as a percent of their initial weight are shown in Figure 3. On day 6, the mean BW of treatment and control animals was 99.4 and 98.2% of initial weight respectively (P = 0.07). On day 12, the mean BW of treatment and control animals was 102.0 and 100.0% of initial weight respectively (P = 0.02). By day 18, both treatment and control animals were 103.7% of starting weight. On day 25, the mean BW of treatment and control animals was 105.8 and 105.4% of initial weight respectively.

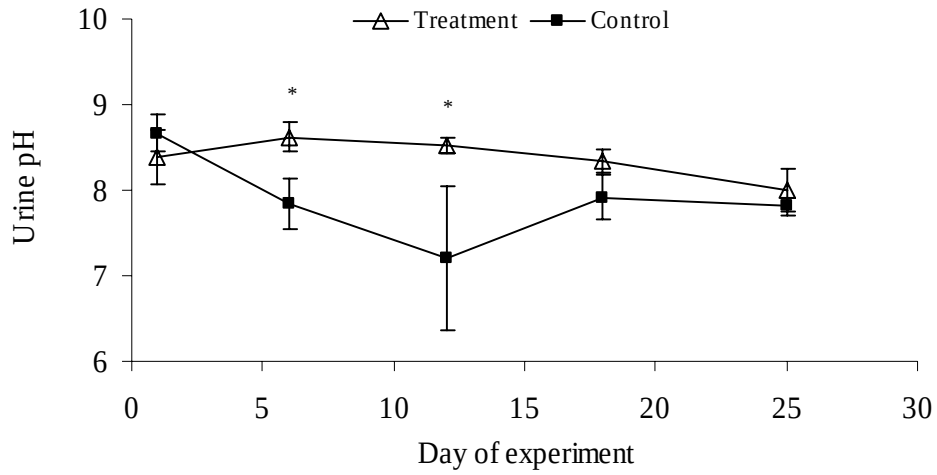
Figure 3: Mean body weights of treatment and control animals. Points show mean ± SEM. The symbol "*" indicates that on that particular day there was a significant difference (P < 0.05) between treatment and control groups.



Voided urine samples were collected on the days when animals were weighed and results are presented in Figure 4. The number of animals sampled varied between days and groups. There was also no control over which animals were sampled. On days 6 and 12 the control animals had

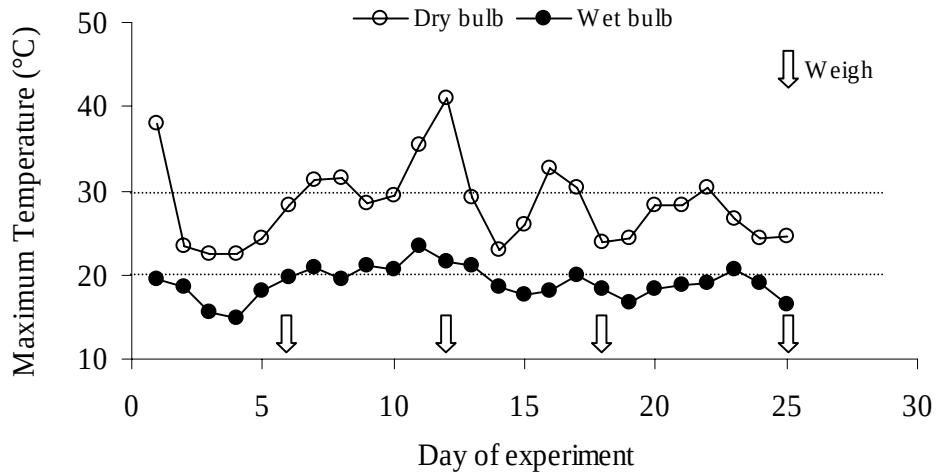
significantly lower urine pH than treatments. There were no significant differences in urine specific gravity between treatment and control groups (data not presented).

Figure 4: Urine pH for treatment and control animals. Points show mean ± SEM. The symbol "*" indicates that on that particular day there was a significant difference ($P < 0.05$) between treatment and control groups.



The maximum daily T_a and WBT were calculated and are shown in Figure 5. The hottest weather occurred between day 6 and 13 when the maximum WBT was consistently above 20°C. Dry bulb temperature peaked on day 12 and was consistently above 30°C between day 6 and 12.

Figure 5: Maximum daily dry bulb temperature and wet bulb temperature over the duration of the experiment.



The results from the animals slaughtered on day 19 (n = 4 for both treatment and control) are shown in Table 1. There were no significant differences detected in mean BW, carcass weight, muscle pH, or muscle dry matter between treatment and control animals. There was a significant difference in colour of the LD muscle between treatment and control animals with treatment animals having a

higher mean L colour reading of 37.6 and controls 35.38 (P = 0.02), meaning the muscle from the treatment animals was lighter.

Table 1: Mean body weight and muscle characteristics for electrolyte treated and control animals slaughtered on day 19. Values are means ± SEM, values in columns with different superscripts are significantly different

Treatment	Live weight (kg)	Hot carcass weight (Kg)	LD pH	LD Luminescence (L)	Muscle dry matter (%)		
					SM	ST	LD
Electrolyte	206.3±1.38	102.8±1.56	5.5±.02	37.60±0.38[a]	28.0±1.29	27.8±0.75	22.2±0.23
Control	205.8±1.60	102.8±3.01	5.6±.02	35.38±0.63[b]	27.7±1.57	26.0±2.49	23.0±0.49

3.1.3 Discussion

This experiment tested the effect of the electrolyte supplement on animals kept in a feedlot. Unlike cattle tested previously in the climate controlled rooms, and on board a live export ship (LIVE.209), the animals in this experiment were not subjected to particularly difficult environmental conditions; although the experiment was conducted during summer there were only a few days when the dry bulb temperature was over 30 degrees, and the wet bulb temperature remained low. There was also good night time cooling. In spite of there being no real environmental stress on the cattle, the treatment group appeared to drink more of the water containing the electrolyte supplement for the period of supplementation. Without individual water intakes it was not possible to calculate if the increases were statistically significant, but the average difference in water consumption between treatment and control groups on days 1 to 17 was significantly different to days 18 to 24. These results are consistent with previous work in the climate controlled rooms where cattle drank more when supplied with the same electrolyte supplement, and similar to the shipboard work, where again it was not possible to perform statistical analyses.

The feed intake by the animals in the treatment pens was greater during the period of electrolyte supplementation, compared to the control pens, and this was statistically significant on four days up to day 12. Whether the supplement had the same effect on feed intake in the previous shipboard experiment was not possible to measure, so this more intensively monitored experiment had the advantage of being able to confirm that there was a difference in feed intake between the groups.

The combined increase in feed and water intake of treatment animals resulted in a trend for those animals to lose less weight in the first 6 days of the experiment, and for there to be a significant weight advantage of 2% for treatment animals on day 12. This weight advantage was of similar magnitude to the weight advantage recorded in the ship board experiment (approximately 3%). However, by the weighing on day 18 in this feedlot experiment, both groups were similar in weight with that weight advantage of the treatment animals being lost. This contrasted with the ship experiment, where the weight advantage was evident at the day 18 weighing. The animals from the ship experiment were not followed after unloading from the ship, so there could be no indication how long the weight difference persisted. With this feedlot experiment, there was no difference between the weights of the groups one week after the supplementation ceased. It was likely that increased

feed and water intake played a role in the increased LW measured on day 12. It was unclear as to why the weight advantage of the treatment animals at day 12 was lost by day 18.

The electrolyte supplement appeared to have an effect on feed intake, and also on the acid-base balance of the animals, with the urinary pH of treatment animals remaining above 8, while the urinary pH of control animals dropped to be significantly lower on day 6 and day 12. The electrolyte supplement may have influenced dietary transition onto the pelleted feed, such that treatment animals were better able to buffer metabolic acidosis resulting from this transition. This may have resulted in the increased feed intake measured over the first 12 days of the experiment, after which the control animals had undergone adequate rumen flora changes to cope with the pellets, and so then ate similar amounts to the treatment animals. Increased dietary cation anion difference (DCAD) due to the electrolyte supplementation may also have improved feed intake in the treatment animals, as has been reported in dairy cows (Tucker *et al*., 1988).

Observations were recorded for both groups of animals that there was a high incidence of very loose faeces, especially in the first week, which supports the suggestion that the cattle had some problems adapting to the pellets. It was not possible to determine whether there were differences in the incidence or severity of diarrhoea between groups.

There was little apparent effect of the electrolyte supplement on the carcass by slaughter on day 19, apart from a difference in luminescence of the longissimus dorsi (LD) muscle, such that it was lighter in the treatment animals. Jacob *et al*. (2005) reported that dehydration can decrease luminescence, but there were no other indications that the carcasses differed in hydration.

3.2 Fluid balance experiment

3.2.1 Methodology

Six *Bos taurus* heifers (Angus and Murrey Grey cross; 410 ± 13 kg) from the Murdoch University breeding herd were selected based on suitable body weight and quiet temperament. All heifers were diagnosed as non pregnant and oestrus cycles were synchronised with two 25 mg injections of prostaglandin (Lutalyse®) 14 days apart.

Heifers were housed in the Murdoch University barn, two per pen, and given 7 days gradual introduction onto a standard shipper cube; 9.5 MJ of ME and 8.9% crude protein per kg of DM (Appendix 1). Initially, all animals were supplemented with hay but this was reduced as the shipper cube ration increased, until the animals were offered pellets only, at 3% of bodyweight.

The day before the experiment commenced (day 0), animals were weighed (18 hrs off feed but not water) and indwelling jugular catheters sutured in place. Animals were then randomly assigned to individual pens in either one of two climate controlled rooms (CCR; room 44 and 45) at Murdoch University. The six animals were then allocated to control and treatment groups (n = 3 for each), and received either plain or electrolyte supplemented water, respectively, from day 1 to day 9. To counter possible differences in rooms, two animals from room 44 and one from room 45 were designated as treatment, and one from room 44 and two from room 45 as control for the first replicate. Animals spent one day (day 1) at ambient conditions, and the CCR were turned on at 1600 h on day 2. The animals were then subjected to increased heat and humidity, ranging from 25.5 up to 28.5°C wet bulb temperature (WBT) with no night time respite (Figure 1). On day 9 animals were

removed from the rooms and weighed (after 18 hours off feed but not water). After removal from the CCR, the animals had an interim period of 8 days at thermoneutral conditions in the university barn, where the animals were housed in pens of two heifers and received pellets and plain water. This was followed by a second replicate experiment in the CCR with each animal returning to its original individual pen but assigned to the other group.

In the CCR, animals were fed at 3% of BW in two equally divided feeds at 0800 and 1300 h. Feed residues were removed and weighed daily before the morning feed. Water was available *ad libitum* in a 25 L bucket for each animal, topped up as necessary. Control animals received plain water, while treatment animals were supplemented with electrolytes added to the drinking water at a rate of 3.5 g/L KCl and 1.8 g/L $NaHCO_3^-$. Water residues were weighed and the total daily consumption was recorded at 0800 h.

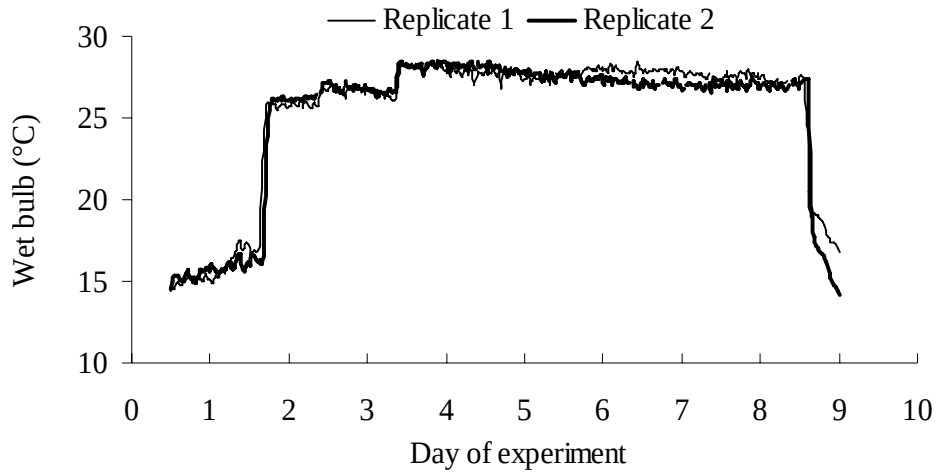
The animals were monitored carefully throughout for any signs of distress, and respiratory rate (RR), panting score (PS) and rectal temperature (T_r) were recorded three times daily, at 0800, 1300 and 1700 h. Data loggers (T-TEC Datalogger, South Australia) were used in each room to log dry bulb temperature and relative humidity every 10 minutes.

For both replicate experiments, empty body water (EBW), extracellular fluid (ECF), and plasma volume (PV) were measured on all animals, through indicator dilution of urea, sodium thiocyanate, and Evans Blue, respectively. This was done before (day 1) and after (day 9) the heating period. To calculate PV, 12 ml of a 1% solution of Evans Blue dye was infused via the jugular catheter (Gortel *et al.* 1992). This was immediately followed by an infusion of a solution containing 20% urea and 4% sodium thiocyanate at a rate of about 0.7 ml/kg over 2 min, which was used to calculate EBW and ECF (Preston and Kock, 1973; Gad and Preston, 1990). Three hours before infusion drinking water was removed from all animals. A venous blood sample was taken from the indwelling jugular catheter prior to infusion (T 0), and then at 6, 12, 18, 28, 40, 50 and 60 min from the mid point of the infusion. Interstitial fluid (IF) was calculated by subtracting PV from ECF. Packed cell volume (PCV), plasma protein (PP) and blood gas variables were obtained from the T0 sample.

3.2.2 Results

The 30 min mean WBT was calculated for both rooms during replicates. There was no difference between rooms during replicates and the average WBT for both rooms during replicates is shown in Figure 6. For all measured variables the trends in both experiments were similar; therefore, the two replicates were combined and analysed as one.

Figure 6: Wet bulb temperature in climate controlled rooms during replicate experiments.



Feed intakes decreased and water intakes increased with increasing WBT (Figure 7 and 8 respectively). Compared to ambient conditions (day 1) feed intake was significantly reduced on days 6 to 9 and 5 to 9 for treatment and control groups respectively. There was no significant difference in feed intake between treatment and control groups on any day.

There was no significant difference between groups in water consumption on any day, nor was there any difference in the increase in water consumption on any day. Compared to day 1, water intakes were significantly increased by days 3 and 4 for treatment and control groups respectively and remained increased over the duration of the experiment.

Figure 7: Mean daily feed intake (± SEM) for treatment and control groups (n = 6).

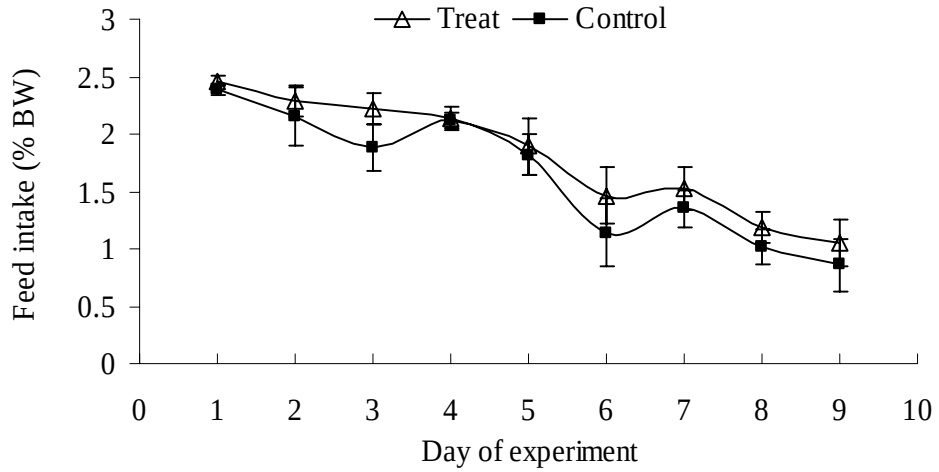
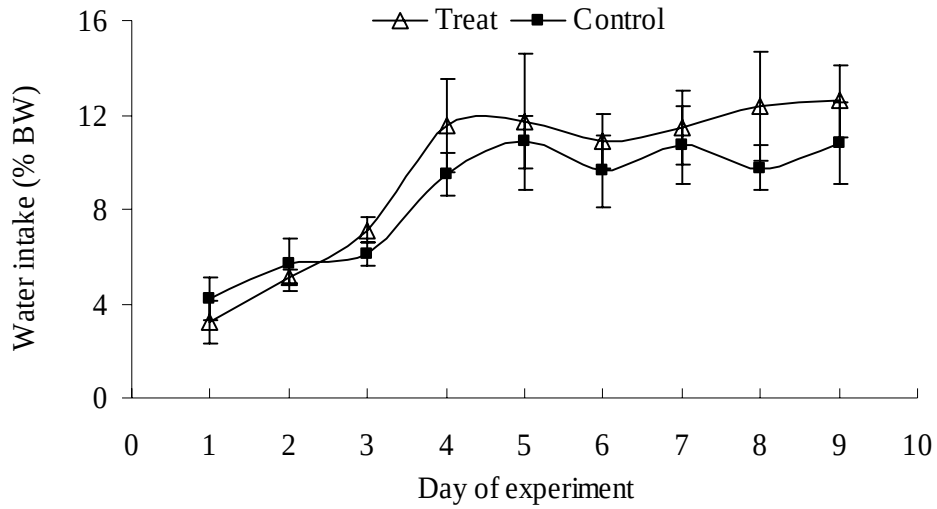
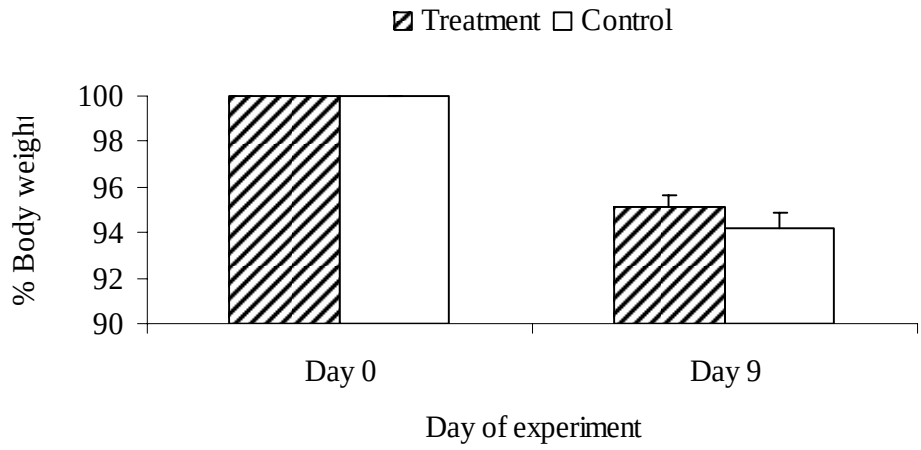


Figure 8: Mean daily water consumption (± SEM) for treatment and control groups (n = 6).



Body weights for treatment and control groups decreased significantly over the duration of each replicate experiment (Figure 9). There was a mean reduction in BW of 4.9 and 5.8% for treatment and control groups respectively, which was not significantly different between groups.

Figure 9: Change in body weight (as % of starting weight) over the duration of experiment for treatment and control groups.



RR and RT increased with increasing WBT and there was no difference between treatment and control groups (data not presented). Acid base variables and PCV and PP followed similar patterns to previous work (Beatty et al., 2006) (Table 2). The pCO_2 and HCO_3^- decreased as RR increased. There was no difference between treatment and control groups. PCV decreased for both treatment and control groups over the first replicate, then remained low for the rest of the experiment; there was no difference between treatment and control groups.

For empty body water, there was considerable between-animal variation, which meant there were no consistent patterns evident. There was no difference between before and after heating periods for either treatment or control animals (Table 2). Of some concern was the possibility that treatment animals might have lost greater amounts of fluid in the rest period between the two heating (and supplement) periods, but it is not possible to detect a significant difference between the groups. However, the treatment animals from the first replicate (Cows 15, 723, 791) that were control animals in the second replicate started the second replicate with significantly less EBW than when they started the first replicate (Figure 10), and while both groups increased EBW during the second replicate, that group of animals had significantly less EBW than the other three heifers.

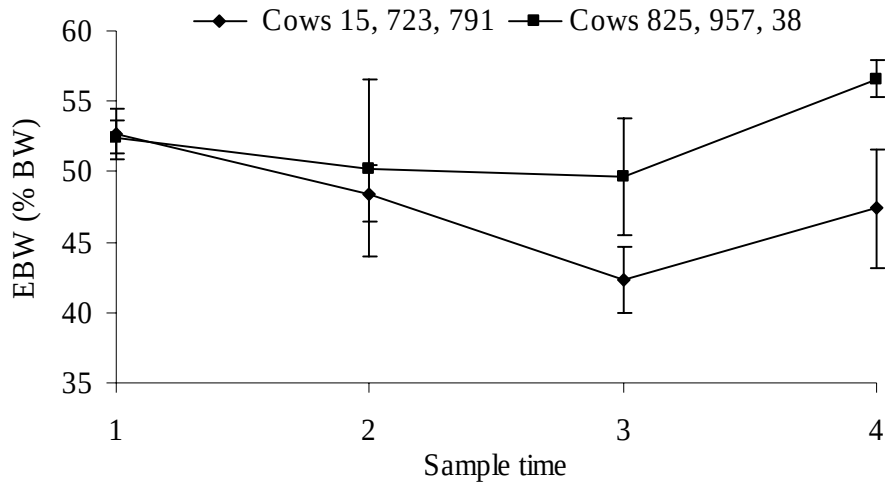
For both treatment and control groups, ECF increased over both heating periods (Table 2). However, there was no difference in the increase between treatment and control. Plasma volume was variable between animals and did not differ between treatment and control or before and after the heating period. Interstitial fluid (ECF minus PV) increased over the heating period. This increases appeared to be due to the increase in ECF. There was no difference between treatment and control.

Table 2: Mean ± SEM of measured variables for treatment and control groups combined over both replicate experiments. The combined column (n = 12) shows mean ± SEM for all animals before and after heat period.

Combined	**Before**			**After**		
	Treatment (n=6)	Control (n=6)	Combined (n=12)	Treatment (n=6)	Control (n=6)	Combined (n=12)
pH	7.40 ± 0.01	7.41 ± 0.00	7.40 ± 0.01	7.40 ± 0.00	7.37 ± 0.01	7.38 ± 0.01
pCO_2 (mmHg)	46.2 ± 0.01^a	44.5 ± 1.12^a	45.3 ± 0.85^a	36.8 ± 1.11^b	38.3 ± 1.50^b	37.6 ± 0.92^b
HCO_3^- (mmol/L)	27.7 ± 2.18^a	27.0 ± 0.58^a	27.3 ± 0.66^a	21.2 ± 0.48^b	21.2 ± 0.87^b	21.2 ± 0.47^b
PCV (%)	35.0 ± 1.30^a	33.7 ± 1.82^a	34.3 ± 1.18^a	30.0 ± 0.58^b	32.0 ± 1.29^b	31.0 ± 0.74^b
PP (g/L)	73.7 ± 1.63	73.5 ± 2.31	73.6 ± 1.28	76.0 ± 1.63	75.7 ± 1.69	75.8 ± 1.12
EBW (%)	51.2 ± 2.13	47.4 ± 2.55	49.28 ± 1.68	52.5 ± 2.11	48.8 ± 3.44	50.7 ± 2.00
ECF (%)	22.2 ± 0.62^a	22.6 ± 0.50^a	22.3 ± 0.38^a	24.5 ± 0.77^b	24.4 ± 0.50^b	24.4 ± 0.44^b
IF (%)	18.7 ± 0.61^a	18.3 ± 0.64	18.5 ± 0.42^a	21.0 ± 0.67^b	20.5 ± 0.49	20.8 ± 0.40^b
PV (%)	5.0 ± 0.45	4.7 ± 0.36	4.8 ± 0.37	4.3 ± 0.27	4.0 ± 0.19	4.2 ± 0.17

Differing superscripts indicate a difference ($P<0.05$).

Figure 10: Empty body water (EBW) calculated from urea space (% bodyweight, mean and SEM) of animals at sampling times 1 to 4 (before and after first replicate and before and after second replicate respectively). Cows 15, 723, and 791 were in the treatment and then control group for the first and second replicates respectively, while cows 38, 825 and 957 were in the control and then treatment groups for the first and second replicates.



3.2.3 Discussion

Changes in feed and water intake and acid base balance were predictable based on previous experiments conducted at Murdoch University (Beatty *et al.*, 2006). As with previous studies, the small numbers of animals tested and large between-animal variations meant that it was difficult to assess small changes in many variables. There was no difference in LW between treatment and control groups, with all animals losing weight during the heating period. This differs from previous ship board trials which showed a 3% difference in LW between treatment control animals after 18 days electrolyte supplementation (Beatty *et al.*, 2007), and from the feedlot experiment described above where there was a 2% difference in LW at 12 days. This may be explained firstly by the shipped and feedlot animals continuing to eat, compared to these animals decreasing feed intake and secondly by an assumed difference in fluid intake between treatment and control animals in those experiments, although no statistical analyses could be done on fluid intake in those experiments. It was hypothesised from previous experiments that the increase in LW was due to increased fluid intake; however, in the current experiment, there was no significant difference in water intake between the groups, unlike previous climate controlled experiments, and this could be explained by the large between-animal variation, with substantial consumption of plain water by one particular animal.

Without significant differences in bodyweight or water consumption in this experiment, it is not surprising that it proved difficult to detect any changes in body fluid compartments. It had been hypothesised that increased water intake would lead to alterations in body fluid compartments, and that these changes would be greater in those animals consuming larger quantities of fluid, which previously the treatment animals had done. However, because of the small number of animals used and the large between-animal variability, few results are statistically significant. The indicator dilution techniques used here to measure body fluid compartments have been successfully used by others to determine EBW, ECF (Gad and Preston, 1990) and PV (Gortel *et al.*, 1992) in cattle. The previous studies have shown a large variation in these fluid compartments in normal animals, and the larger

ranges in measures in our experiment may be due to some effects of the treatments imposed. Gad and Preston (1990) reported that EBW in steers ranged from 48.3 to 56.9% of bodyweight. The experiment described here showed a larger range in EBW, from 38 to 59% bodyweight, although the mean EBW of 50% is similar. ECF ranged from 19.6 to 23.3% in 6 normal steers measured by Gad and Preston (1990), while ECF in the experiment reported here ranged from 19.8 to 23.5% (before heat) and 22.2 to 27.2% (after heat). There was less between animal variation in ECF and this was reflected in the significant increase detected in ECF after the heat period, compared to the test taken before the animals were heated.

Plasma volume was even more variable in this experiment, ranging from 3.0 to 7.0% of body weight. This range was evident both before and after heating periods. Given that PCV decreased and ECF increased, it would be expected that PV would increase over the heating period, because plasma is part of the ECF, and an increase in PV would cause the volume of the blood occupied by cells (PCV) to decrease. This was not detected and may be a reflection of the low numbers of animals tested or variability within the Evans blue dye assay. There is evidence to suggest that usually an animal's response to excessive heat load results in a decrease in PCV and increase in PV (Van Beaumont *et al.*, 1981), although Gortel *et al*. (1992) did not find a difference in PV in electrolyte supplemented bulls. The other explanation for a decreased PCV in the absence of an increased PV is a reduction in the size of the red blood cells, and we have no data to confirm or refute that.

With the small numbers of cattle and large variability it was not possible to determine whether there was increased loss of EBW from the treatment animals in the period between the two replicates, but those animals did start the second replicate with significantly less EBW than the cattle at the beginning of the experiment. Both groups then had a similar increase in EBW. A potential complication of supplying additional electrolytes might be that after the period of supplementation, the animals then excrete these electrolytes in the urine along with additional fluid, which is consistent with the increased fractional excretion ratios of electrolytes measured in heat stressed cattle monitored post supplementation (Barnes *et al.* 2003).

4 Heat Stress Threshold Experiments

4.1 Sheep

4.1.1 Methodology

Twenty-four sheep typical of the live export trade were sourced from southern Western Australia: six four-year old Merino wethers (average weight 56 kg, condition score 2.5-3), six five-year old Merino rams (average weight 71 kg, condition score 3) and six eight-month old Merino ram lambs (average weight 58 kg, condition score 2.5). The wethers and rams were within two weeks off shears, while the ram lambs had 25 mm of wool.

One week before the start of the experiment, animals were surgically implanted with temperature loggers and telemeters for continuous monitoring of T_c. All sheep were housed in the Murdoch University barn and given 7 days gradual introduction onto a standard shipper cube; 9.5 MJ of ME and 8.9% crude protein per kg of DM (Appendix 1). Initially, all animals were supplemented with hay but this was reduced as the shipper cube ration increased. The experiment began on day 0 when sheep were weighed (after 15 h off feed and water) and then six of each class were strategically allocated to individual pens within the CCR, so that there were three animals of each class in each

room. A further two control animals from each class were individually penned in another room and not exposed to heat.

Animals were fed a standard sheep shipper pellet at 3% body weight (as fed), divided into two equal feeds offered at 0700 and 1300 h. Water was available *ad libitum* in buckets, topped up regularly. Daily feed and water intakes were recorded.

The sheep spent the initial days in the CCR at thermoneutral conditions, so that normal body temperature could be determined from core temperature loggers. The rooms were turned on and heat and humidity were increased to 26˚C wet bulb and the rooms held at that temperature for a period of 48 hours. Following this, the conditions were changed so there was a 2˚C wet bulb increase every 48 hours until the rooms reached 32˚C wet bulb. Sheep spent a maximum of twelve days in the rooms. Sheep were monitored closely, with measurements of RR and PS taken three times daily. Panting score was determined using the guidelines shown in Table 3, adapted from those developed for cattle (Mader *et al*., 2006). Rectal temperature was also taken three times daily, and sheep were removed from the rooms for welfare reasons when their T_c and T_r were consistently at or above 40.5˚C. This temperature was considered to give a reasonable expectation that the animals had T_c 0.5˚C above normal, as per the existing definition of heat stress threshold. Jugular blood samples were taken for blood gas analysis at the beginning of the study when sheep were unheated in the CCR and then once again immediately before the sheep were removed from the rooms.

Panting Score	Description	Respiratory rate
0	No panting	<60
1	Slight panting	60 - 130
2	Fast panting and open grin	130 - 180
3	Open mouth panting	> 180
4	Open mouth panting, tongue out	> 180

Table 3: Panting score used in the assessment of heat load in sheep

Room temperatures were monitored using temperature/ humidity data loggers (T-TEC Datalogger, South Australia). Two loggers were put into each room to determine accuracy of readings. A sling psychrometer was also used three times a day to check the wet bulb temperature and finetune the settings for the rooms.

Analysis of core temperature:

In this experiment three different heat stress thresholds were determined for each class of sheep, HST1, 2 and 3:

HST1: The daily mean wet bulb temperature on the day that daily mean core body temperature (CBT) first significantly increased above pre-heat values.
HST2: The daily mean wet bulb temperature on the day that daily mean CBT first significantly increased 0.5˚C above pre-heat values
HST3: The daily mean wet bulb temperature on the day that daily mean CBT first significantly increased 1˚C above pre-heat values.

The significant elevation of CBT and therefore the day and wet bulb temperature on that day, was determined using two methods, Method 1 (Confidence interval method) and Method 2 (t – test method), as described below:

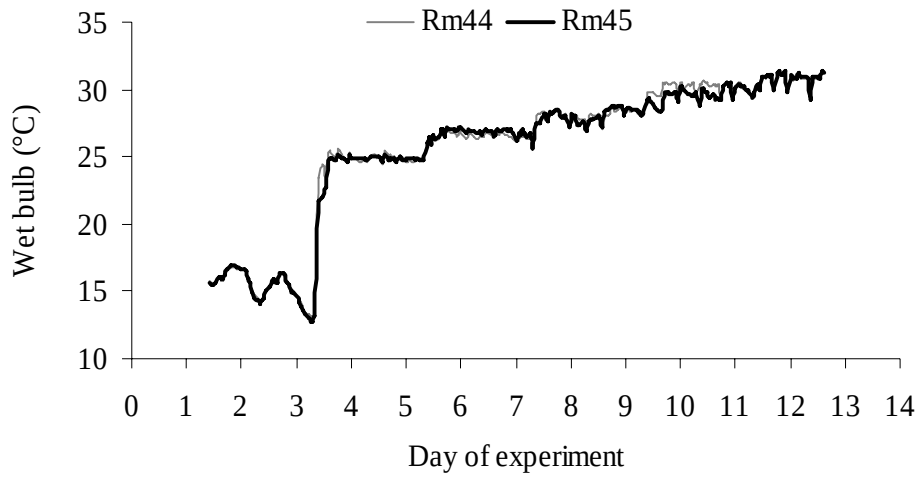
Method 1 (Confidence interval method):
Pre-heat core temperature for each sheep was determined by averaging the daily mean CBT during the pre-heat period of monitoring (d -7 to 2). The daily mean CBT for each sheep was then calculated while they were exposed to increasing wet bulb temperature. The day on which the mean CBT was above the 5% confidence interval of the pre-heat CBT was established as the day on which each sheep reached HST1. These days were averaged for each class of sheep, and the wet bulb temperature on that day was determined as the HST1 for that particular class of sheep. The cut-off CBT values for HST2 and HST3 were determined by adding 0.5 and 1 respectively to the pre-heat mean CBT and using the same method above to determine the average day and therefore the wet bulb temperature when CBT was above the 5% confidence interval of that value.

Method 2 (t-test method):
The pre-heat CBT for each sheep was determined as in Method 1 above. A two-way ANOVA, with animal and day as fixed factors and mean CBT as the dependant variable, was tested for an overall change over days from the pre-heat CBT. A Dunnett t-test compared the mean CBT for all sheep of each class on each day with the pre-heat mean CBT for that class of sheep, to determine the day on which there was significant elevation of CBT. HST2 and3 were determined in the same way with 0.5 and 1 added to the pre-heat mean CBT.

4.1.2 Results

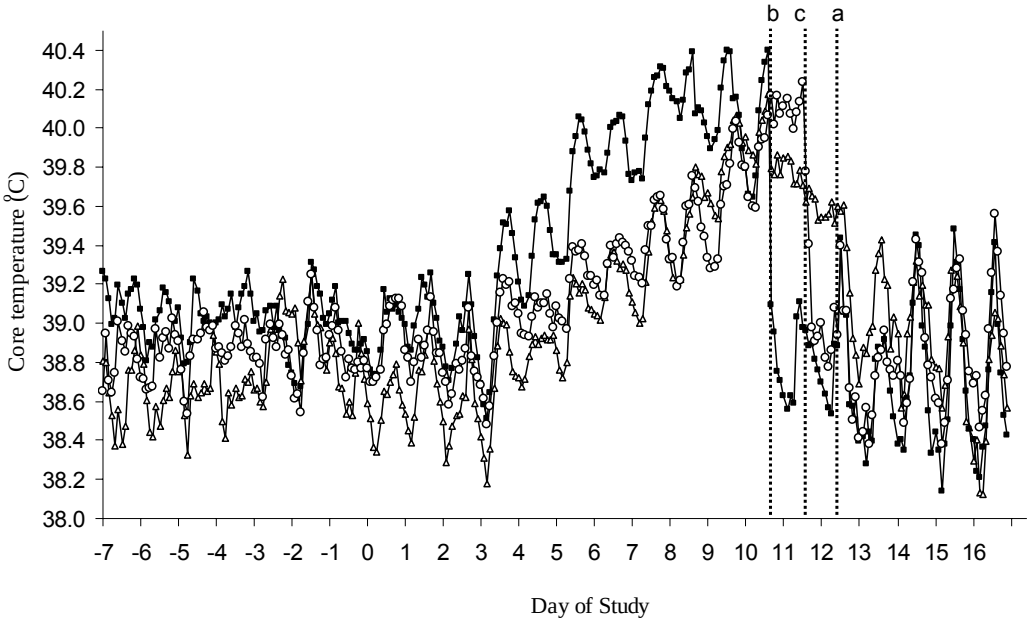
Environmental conditions were similar in each room and the calculated WBT from the data loggers stationed in each climate room is shown in Figure 11.

Figure 11: Wet bulb room temperature of climate rooms 44 and 45



Core temperature of the sheep significantly increased while sheep were in the climate rooms (Figure 12). There was diurnal variation of T_c of around 1°C while sheep were in the climate rooms, which is similar to that previously recorded for both sheep and cattle in LIVE.209, and occurred regardless of there being no daily variation in environmental temperature. This means that comparisons of temperature must take this diurnal variation into account. The following calculations have used daily T_c to account for this diurnal variation. Additionally, the calculations account for progressive removal of animals from the rooms for welfare reasons once the individuals reached 40.5˚C, which was used as an upper limit of CBT under the ethics approval for this study.

Figure 12: Two hourly mean core body temperature measurements of Merino adult rams (∆),Merino ram lambs (■) and Merino wethers (O) before entering the CCR (d -7 to -1), while in the CCR, and following when all adult rams (a), ram lambs (b) and wethers (c) had been removed from the CCR. For the periods outside the CCR, n = 6 for each class of sheep. For the period in the CCR (day -1 to when all animals removed) the data is based on decreasing numbers of animals as they were progressively removed for welfare reasons. Adult rams: d -1 to 10, n = 6; d 11 to 12, n = 2. Ram lambs: d -1 to 8, n = 6; d 9, n = 2; d 10, n = 1. Wethers: d -1 to 8, n = 6; d 9 to 10, n = 4; d 11, n = 3.



On day 12 of the study there were two rams remaining in the climate rooms. One of these rams reached 40.5˚C on day 12, and the decision was made to remove both animals, rather than leave one ram alone in the room.

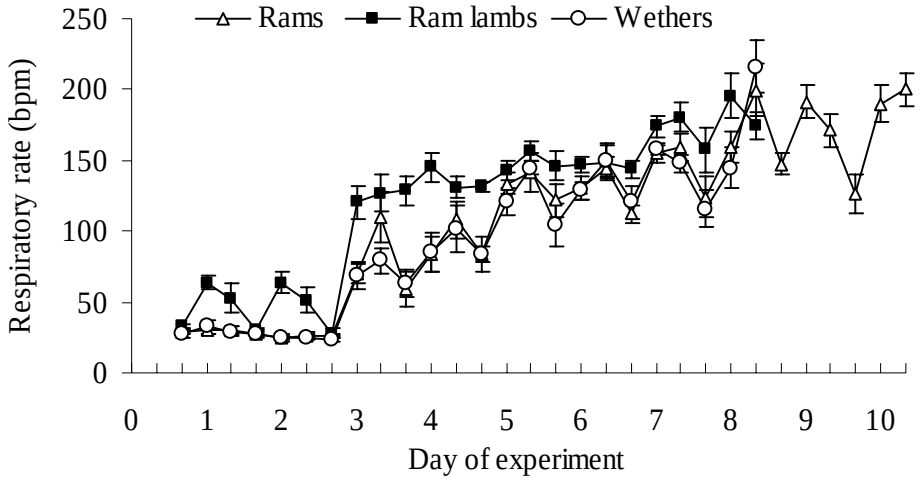
The HST1, 2, and 3 were calculated by both statistical methods, and there were slight differences in the results depending on the method of calculation (Table 4). By both methods of calculation, al sheep in the climate controlled rooms reached a CBT significantly above their pre-heat values (at HST1), while all wethers and rams lambs, and 5 of the rams, reached a CBT that was significantly 0.5˚C above pre-heat values (at HST2). Four sheep in each class reached a CBT significantly 1˚C above pre-heat values (at HST3).

Table 4. Heat stress thresholds (HST) 1, 2 and 3 (wet bulb temperature, °C) for the three classes of sheep, calculated by the Method 1 (Confidence Interval CI) or Method 2 (t-test).

Class of sheep	Normal Tc	HST1		HST2		HST3	
		CI	t-test	CI	t-test	CI	t-test
Ram lambs	39.0 ± 0.04	24.8	24.9	26	26.1	27.1	27.9
Rams	38.7 ± 0.09	25.5	26.7	27.1	26.7	28.9	28.9
Wethers	38.9 ± 0.08	25.3	26.1	27.2	26.1	28.2	30

Respiratory rates and panting score were the most useful and easily assessed indication that animals were affected by the increased heat and humidity (Figure 13). All of the sheep in the climate rooms did progress to open-mouthed panting, some with the additional feature of having their tongues out (Panting score 4), before being removed from the rooms. Using HSTs calculated by Method 1, the mean RR (breaths per minute) of the ram lambs once reaching their HST1 was 113 ± 9, while for the rams it was 104 ± 16 and for the wethers it was 91 ± 22. The mean RR of ram lambs once reaching their HST2 was 149 ± 5 while for the rams it was 133 ± 6 and for the wethers it was 140 ± 16.

Figure 13: Respiratory rate of sheep during the days all 6 sheep of each class were in the CCR (mean ± SEM).



The increased RR of the sheep in the heat did result in a drop in blood carbon dioxide with an associated drop in blood bicarbonate. Blood pH of Merino rams did significantly increase; however, there was no change in plasma pH for either the Merino ram lambs or wethers (data not presented).

Feed intake of rams and ram lambs, but not the wethers, decreased toward the end of the study (Figure 14). Water intake of all classes of sheep in the climate rooms increased in response to increased wet bulb temperature (Figure 15).

Figure 14: Feed intake of sheep during the period within the climate controlled rooms, as a percentage of body weight (mean ± SEM). n = 6 for each class of sheep; data for each class is only presented for the period that all sheep of that class were in the rooms.

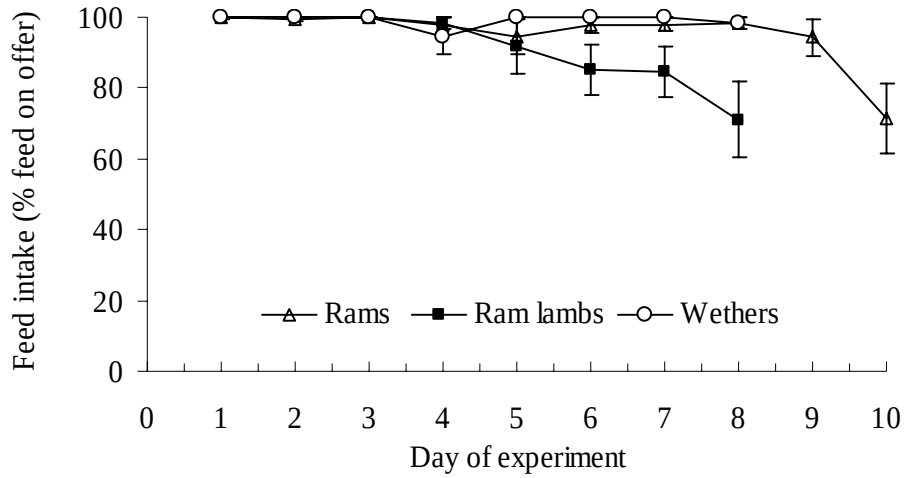
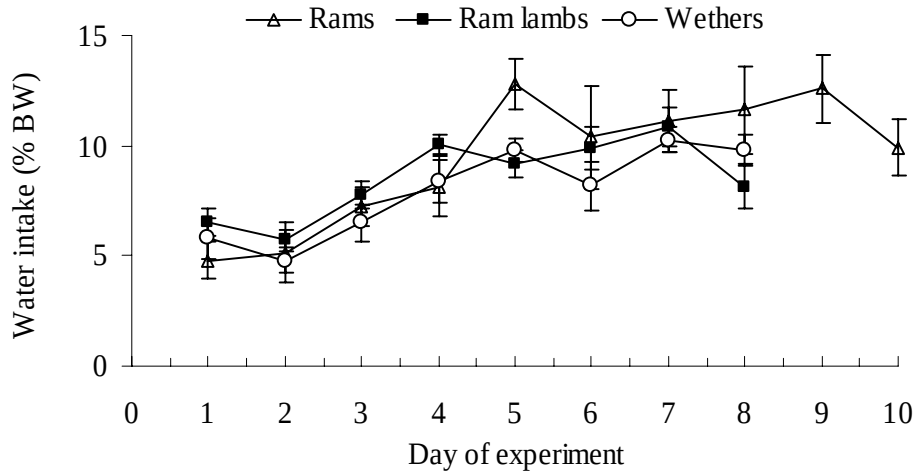


Figure 15: Water intake of sheep during the period within the climate controlled rooms, as a percentage of body weight (mean ± SEM). n = 6 for each class of sheep; data for each class is only presented for the period that all sheep of that class were in the rooms.



4.1.3 Discussion

The heat stress threshold of sheep can be measured in a number of ways, and it is very important to consider the effect of the normal diurnal variation in core temperature, which can be around 1°C. Heat stress threshold could be defined as the maximum ambient wet bulb temperature at which core body temperature no longer remains normal, which was determined here as HST1, when the mean daily T_c was significantly elevated above normal. A similar calculation can also be done for

maximum and minimum daily T_c, noting that in some animals the rise in minimum and maximum did not occur at the same time. HST1 does not take into account any other physiological changes of the animal. For example, although the core temperature may have significantly increased above normal, this occurs before other physiological changes take place, such as open mouth panting, decreased feed intake, or changes in acid base physiology. In this study the respiratory rate was still quite low when the HST 1 was reached. This suggests that although the sheep had a T_c significantly above normal, there was little other physiological response. .

The second heat stress threshold (HST2) was assessed as the wet bulb temperature at which average core temperature of a sheep was significantly increased 0.5°C above normal, consistent with that used previously in the Heat stress Risk Assessment Model software. Using this definition it was found that all wethers and ram lambs, and five out of six rams, reached this heat stress threshold. The respiratory rates of the sheep were somewhat higher once HST2 was reached.

A further calculation was done to determine the environmental wet bulb temperature at which there were obvious clinical signs of heat stress; the most easily observed sign is open mouthed panting, which occurs at around a mean T_c of 40°C, at which time there were significant changes in blood gas measurements. In hindsight, additional blood samples taken at other points may have proved useful in further identifying the cut-off point between normal and abnormal physiology; however, from the previous sheep experiment it was determined that there were not marked alterations in acid-base physiology until the sheep progressed to open mouthed panting. There are variations in the actual figures for HST depending on the method of calculation, in large part due to the variation between animal responses, because there were only six animals of each class tested in the heat, and the animals were removed once they had reached a cut-off temperature, for reasons of animal welfare. The t-test method may slightly over-estimate the HST, while especially for HST3, the confidence interval method may result in an underestimate.

The lower HST for the ram lambs may be due to an effect of their physiological status, younger and growing, but may also be due to their greater wool coverage, at 25 mm in length. Wool is hygroscopic-endothermic in nature, and a thick fleece (7 - 8 cm) interferes with evaporative water loss from the skin (Klemm, 1962), and may therefore interfere with heat loss under hot, humid conditions (Lee, 1950; Klemm, 1962; Thwaites, 1985). Experiments by Hofman and Riegle (1977) found that when sheep were exposed to 40˚C and 40% humidity, unshorn sheep had a thermoneutral zone that was approximately 5 to 8˚C lower than the shorn sheep. However, the fleece can confer some protection from radiant heat. Thwaites (1967) found that when exposing Merino wethers to high radiant heat as well as hot humid conditions, the tolerance of the sheep progressively increased as the animals fleece increases in length, with maximum tolerance obtained at a fleece length of 40 mm. The ram lambs with 25 mm of wool were considered typical of sheep that are exported, and the LEAP guidelines (2001) only specify increased pen area per head for animals with greater than 25 mm of wool. There was no opportunity in this experiment to investigate the effect of wool length on heat tolerance of sheep of the same class.

Work has also been done comparing heat tolerance of younger, growing sheep to mature sheep. Hahn (1985) found that a growing lamb had a lower upper critical temperature (25˚C) than a shorn ewe (29˚C). Thwaites (1967) found that sheep do not gain full adult heat tolerance until about 1 year of age.

Previous studies have found that rams react more dramatically to hot conditions in terms of body temperature and behaviour than do ewes (Hafez *et al.,* 1956; Thwaites, 1985). This is thought to be in part due to the higher metabolic rate of rams than ewes or wethers (Lee, 1950). However, no research has been found to compare heat tolerance of rams versus wethers. All wethers and rams reached HST1 and 2 and four of the six sheep from each class reached HST3. There were no obvious outliers in the study. Therefore this study suggests that both wethers and rams have similar heat stress thresholds.

4.2 Friesian heifers

4.2.1 Methodology

Six Friesian heifers from the same breeding herd were sourced from the south west region of Western Australia based on weight, temperament and stage of gestation. They averaged 420 $\pm$ 19 kg liveweight, and were 3 to 5 months pregnant based on artificial insemination dates and confirmed by manual palpation. Eight days before the commencement of the experiment animals were surgically implanted with temperature telemeters (Datamet, Potchefstroom, South Africa) and temperature loggers (ibutton DS1922L, Maximum Dallas, California, USA). Loggers were set to log core body temperature (T_c) every 15 minutes.

Heifers were housed in the Murdoch University barn and given 7 days gradual introduction onto a standard shipper cube; 9.5 MJ of ME and 8.9% crude protein per kg of DM (Appendix 1). Initially, all animals were supplemented with hay but this was reduced as the shipper cube ration increased.

Animals were randomly allocated to individual pens in either one of two CCR at Murdoch University at 1000 h on day 0. It became clear during the first 24 h that the larger heifers were unable to lie down. Therefore, at 0900 h on day 1 individual pens were removed and the three heifers in each room were housed as one group.

Heifers spent days 1 and 2 at the prevailing environmental conditions, then the CCRs were turned on at 0900 h on day 3 and set for 22°C WB. The set WBT was increased by approximately 1°C each day at 0900 h. The heifers were removed from the rooms for welfare reasons when their rectal and core body temperatures reached 40.5°C. Data loggers (T-TEC Datalogger, South Australia) were used in each room to log dry bulb temperature and relative humidity every 10 minutes. Each day of the experiment ran from 0800 h to 0759 h.

Feed in the CCR was fed at 3% of BW in two equally divided feeds at 0800 and 1300 h. Feed residues were weighed and daily feed intake for each group was recorded at 0800 h. Water was available *ad libitum* via 3 x 20 L buckets in each room. Water residues were weighed and the previous total daily consumption for each group was recorded at 0800 h.

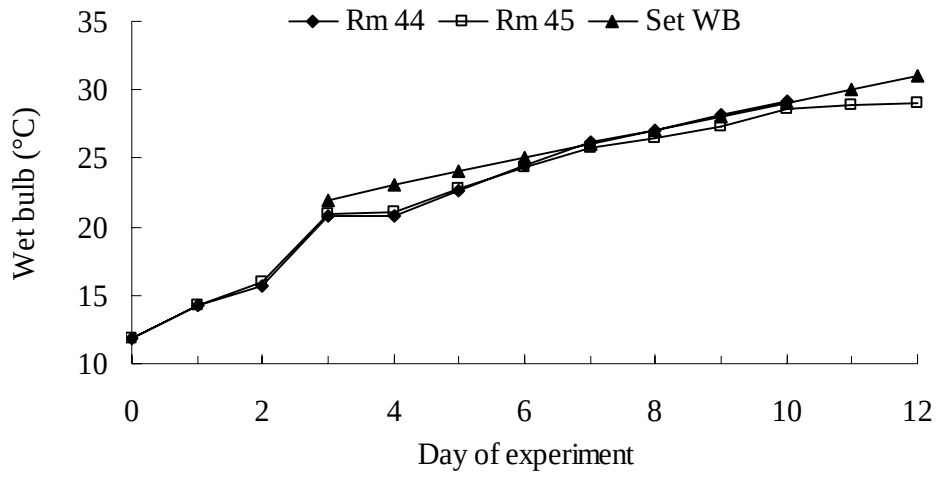
Heifers were weighed (after 18 h off feed but not water) on day 0 and when they exited rooms. Respiratory rates and PS were recorded 4 times daily at 0800, 1300, 1700 and 2200 h. Blood for blood gas analysis was taken via coccygeal venipuncture on day 0 and when the animals exited the rooms.
The heat stress thresholds for the heifers were all determined using only the Method 2 (t-test method) as described for the sheep. ‘Normal’ core body temperature (CBT) was determined by averaging the CBT of heifers over 4 days at thermoneutral temperature (d-1 to d 2). The daily mean

T_c for each heifer was calculated, and a two way ANOVA, with animal and day as fixed factors and mean T_c as the dependant variable, was used to test for an overall change over days. A Dunnett t-test was used to compare the mean T_c of each day with the "normal" day. HST1, 2 and 3 were determined, as described above for the sheep (Section 4.1.1).

4.2.2 Results

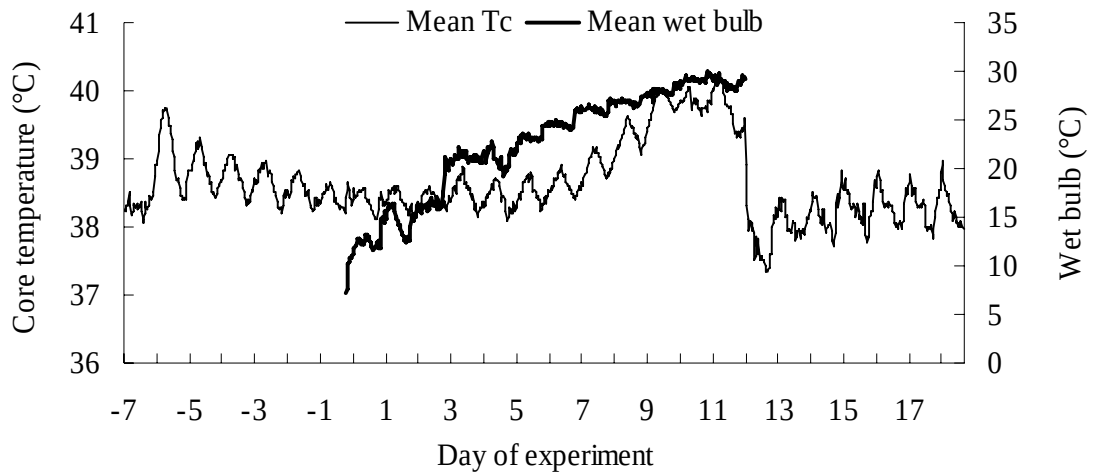
The daily mean WBT for each room is shown in Figure 16. There was no difference between climate rooms. The three heifers in room 44 all had rectal temperatures greater than 40.5°C on day 10 and were removed from the heat.. The three heifers in room 45 had rectal temperatures greater than 40.5°C by 1300 h by day 12 and they were all then removed from the heat.

Figure 16: Comparison between the set bulb temperature and the recorded mean daily wet bulb temperature for both climate controlled rooms.



For each heifer, half hourly averages of T_c were calculated from the recordings made every 15 minutes (Figure 17). Between 0900 h on day 10 and 1330 h on day 12, n = 3 as the 3 heifers from room 44 were removed from the experiment. After 1330 h on day 12, n=6 with all heifers removed from the rooms and subject to the prevailing environmental conditions outside. The maximum mean T_c recorded was 40.2°C at 1800 h on day 11. The maximum individual T_c recorded was 41.2°C at 0030 h on day 10.

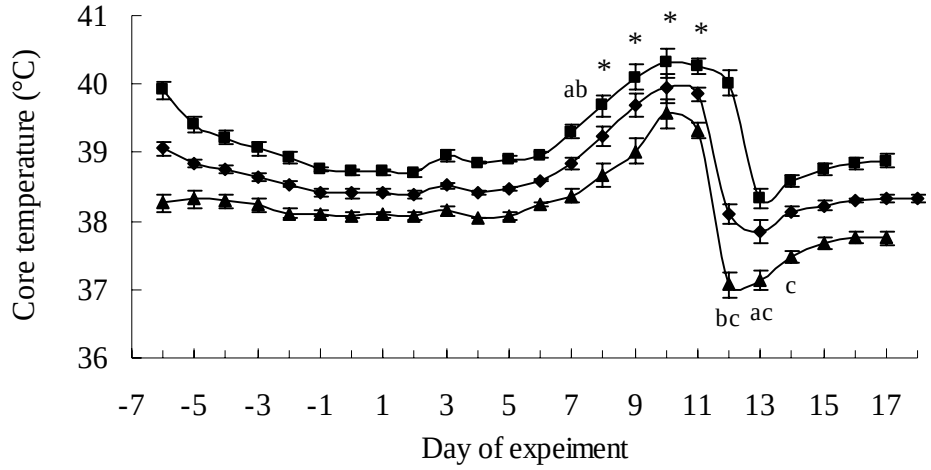
Figure 17: The mean core body temperature of heifers compared to the mean recorded wet bulb temperature of climate controlled rooms (half hourly averages)



The normal daily mean T_c for these pregnant Friesian heifers was 38.4°C, within the normal range for dairy cattle. Their HST1 was 26.0°C WB (day 7), HST2 was 26.8°C WB (day 8), and HST3 was 27.9°C WB (day 9).

The daily mean, maximum and minimum core body temperatures are shown in Figure 18. The values are averages of all six heifers, either in the CCR or out, except for day 11, where the values are calculated from the three heifers remaining subject to the hot conditions. Compared to unheated environmental conditions (days -1 to 2), the mean T_c was increased on days 7 to 11 and decreased on day 13. The mean maximum T_c was increased on days 7 to 12 and the mean minimum T_c increased on days 8 to 11 and decreased on days 12 to 14. The mean daily T_c was 1.0°C above normal on days 9, 10 and 11.

Figure 18: Daily maximum, mean and minimum core body temperatures (mean ± SEM) for heifers. Climate controlled rooms were operating between days 3 and 12. Days on which temperatures differed ($P < 0.05$) from the mean of days -1 to 2 (unheated conditions) are represented by the symbol "a" for mean, "b" for maximum, "c" for minimum and "*" for mean, maximum and minimum. n = 6, except for day 11 when n = 3.



The daily range in T_c (i.e. daily maximum minus minimum) did not change whilst climate control rooms were operating and remained approximately 1°C (data not presented). The daily range in T_c was only increased on days 12 and 13 ($P < 0.05$) when compared to ambient conditions on days 0, 1 and 2.

Due to the heifers being housed in groups of 3, individual feed and water intakes could not be measured, resulting in n = 2 groups. This still allowed statistical comparisons to be made. As WBT increased, feed intakes decreased (Figure 19). On days 9 and 10 feed intake was decreased compared to day 2, the day chosen as representative of normal feed intake after allowing the animals a day to settle into the rooms. The animals did drink more at higher WBT, and water consumption was significantly increased on days 4, 5 and 9 compared to day 2 (Figure 20).

Figure 19: Mean daily feed intake (n = 2 groups) of heifers during the experiment plotted with mean daily wet bulb temperature in climate controlled rooms. Days on which mean feed intake differed ($P < 0.05$) from day 2 are represented by the symbol "*".

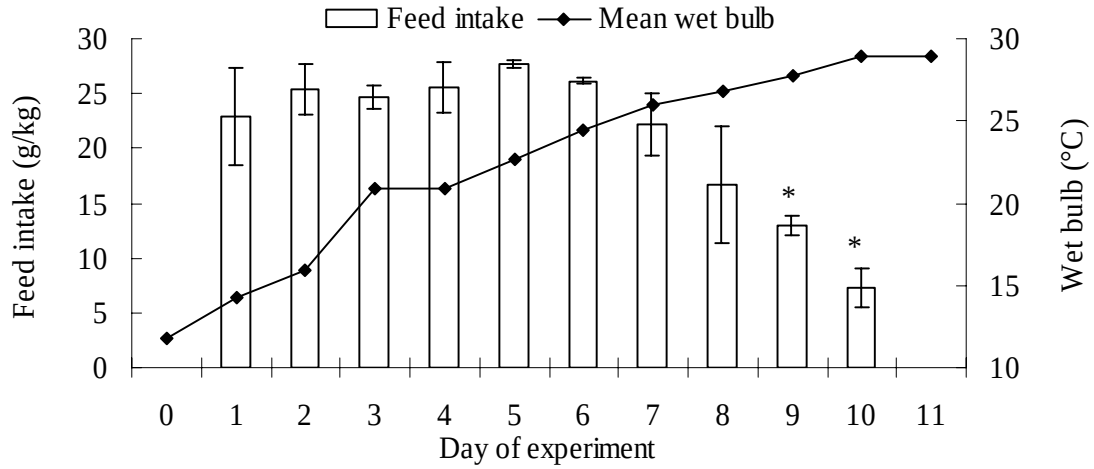
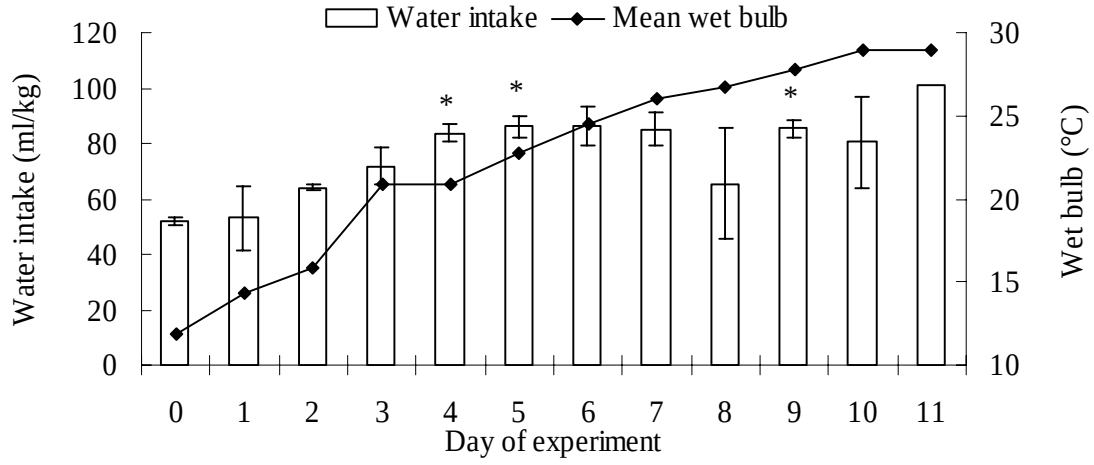


Figure 20: Mean daily water intake (n = 2 groups, except on day 11 n = 1) of heifers during the experiment plotted with mean daily wet bulb temperature in climate controlled rooms. Days on which mean water intake differed ($P < 0.05$) from day 2 are represented by the symbol "*".



Increases in WBT were highly correlated with increased RR ($r^2 = 0.91$) and panting score (Mader et al., 2006; PS) (data not presented). The mean maximum RR recorded was 122 bpm at 2200 h on day 9. By day 1700 h on day 6 (WBT = 24.7°C), all heifers had a PS of 1. Between day 6 and 9, PS varied between 1 and 2, but by 2200 h on day 9 (WBT = 28°C), all heifers had a PS of 2

Blood gas parameters were measured before heifers entered CCR (ambient environmental conditions) and when they exited rooms (i.e. when rectal temperatures were approximately 40.5°C). Mean bicarbonate concentration decreased from 26.2 to 19.8 mmol/L (P = 0.002). Mean pCO_2 decreased from 44.3 $\pm$ 5.9 to 33.5 $\pm$ 1.75 mmol/L (P < 0.05). Blood pH concentration did not change (data not presented).

4.2.3 Discussion

The heifers adapted to the conditions and diets readily, and were more comfortable in group pens, which were more representative of shipboard conditions. As with previous studies, feed intakes decreased, and water intakes increased, with increasing WBT. The animals were removed from the CCRs when they had reached the cut-off core temperature, so the depression in feed intake was not as pronounced as for animals that continue to be subjected to environmental conditions above their HSTs. Water intake was similar to that recorded in previous cattle experiments in the CCRs.

For the Friesian heifers tested in this experiment, a mean daily T_c of 1°C above normal (HST3 = 27.9°C WB) resulted in heifers showing clinical signs of heat stress such as open mouth panting, and there were acid base changes measured on blood samples taken at exit from the rooms. Unlike the sheep lines described above, there was less variability in how the heifers responded to the heat, and thus Method 2 (t-test method) was appropriate for calculating the HSTs, although HST2 and 3 were actually the same using both methods of calculation (calculations not shown).

The heifers continued to have a similar daily range in T_c of around 1°C despite their exposure to increasing environmental temperature, similar to the results of previous experiments (Beatty et al., 2006), and the diurnal range in T_c is very important when considering the heat stress thresholds. For these heifers to reach their HST3, their mean daily T_c had to be over 39.4°C. This would equate to maximum core body temperatures on that day being around 40°C. Core body temperatures at or above 40°C may result in open mouth panting and clinical signs of heat stress; thus HST3 would appear to give a good indication of when the animals were clinically heat stressed.

Mean, maximum and minimum core temperatures did not all increase significantly at the same environmental temperature. The daily mean and maximum T_c were significantly increased on day 7 when the environmental WBT was 26°C. On day 8 the mean minimum T_c was also increased (WBT = 26.8°C). This indicates that maximum CBT can rise significantly in response to a particular environmental temperature, but the minimum may be unchanged if the animal is still able to dissipate heat successfully. As the environmental temperature continues to rise, the animal is no longer able to dissipate all the heat, and the minimum CBT becomes elevated above normal.

As discussed in previous reports, increases in RR due to prolonged and continuous high WBT can lead to acid base disturbances. This was the case in this experiment with bicarbonate and tCO_2 concentrations decreasing during the hottest period, indicating respiratory alkalosis. All heifers were manually palpated to confirm pregnancy at the time of removal of temperature loggers. The heat exposure and subsequent increase in core body temperature did not appear to affect pregnancy status.

5 Success in Achieving Objectives

5.1 Objectives

The objectives listed for LIVE.224 were as follows:

1. To determine if electrolyte replacement therapy on ship as demonstrated in LIVE.209B is repeatable so that industry is convinced of the live weight advantages.
2. Measure the live weight benefit of increasing the sodium bicarbonate content of the standard ship ration.
3. To confirm that the live weight advantage from electrolyte supplementation is due to additional water consumption and retention in the animal and not related to feed consumption.
4. Assuming water consumption and retention by the animal is the cause of the live weight gain, determine where this water is retained in the animal.
5. Measure any animal performance/welfare benefits during and post electrolyte replacement therapy, including the extent of the live weight advantage post supplementation
6. Using experiments in a climate room, measure the heat stress threshold (HST) in ram lambs, heavy rams and wethers and Friesian heifers.

5.1.1 Objective one

This objective was divided into two parts. First, we tested the supplement in a feedlot where we could have much greater access and control over what happened with the animals, and were able to monitor more closely. The second part was to be the replication of LIVE.209B on board a live export ship. The replication at the feedlot was successfully conducted at Murdoch University during the summer months. Unfortunately, mild environmental conditions were experienced during the experiment. A LW advantage of 2% for treated animals was measured on day 12 but not by day 18. This differed from the ship board experiment where a 3% LW advantage was evident for animals receiving 18 days of electrolytes in drinking water. We were able to confirm a difference in urine pH indicating an effect of the supplement on acid-base balance, and were also able to show that there was a significant effect of the supplement on feed intake of the cattle on several days during the first 12 days of the experiment.

We were unable to get on board a live stock vessel at the appropriate time of year to conduct the replication on board ship. This was due to a combination of factors, but primarily due to reduced export of cattle to the Middle East during the northern hemisphere summer. The few export companies that were shipping cattle to the Middle East during the northern hemisphere summer months were not inclined to have research conducted on board.

5.1.2 Objective two

This objective was not met as we were unable to conduct an experiment on board a ship to the Middle East for the same reasons as stated above. Without an obvious benefit of supplementation on land under mild conditions, there was no attempt made to test this objective under other conditions.

5.1.3 Objective three

We can confirm that electrolyte supplemented animals drank more than control animals, although the methodology in the feedlot proved to be somewhat flawed, because using only one water tank each for either treatment or control pens meant that daily comparisons could not be statistically tested. Additionally, while the treatment animals in the fluid balance experiment appeared to drink more than the control animals, the large variability meant that again this daily difference could not be proved statistically.
However, we cannot confirm that the LW advantage was due to increased water consumption alone and not feed intake. Results from the feedlot experiment indicated that, on some days, treatment animals had significantly higher dry matter intake compared to control animals. It is possible that the supplement aided the treatment animals as they became adapted to the pelleted feed over the first 12 days, after which time there was no difference in feed intake.

The fluid balance experiment did not show a difference in LW between the groups with no significant differences in fluid intake, and therefore could not confirm or refute the hypothesis that retention of fluid within animal contributed to a LW change.

5.1.4 Objective four

The fluid balance experiment, conducted in the climate controlled rooms, was somewhat successful in measuring body fluid compartments in individual animals. The experiment highlighted large between animal variation and potential problems with some of the techniques involved in measuring body fluid compartments. Given there was no significant difference in fluid intake or LW between the groups, and the small differences in body fluid compartments expected between treatment and control animals, no differences in fluid volumes of the various body compartments were detected between treatment and control groups. However, we can say that for both groups there was a significant increase in fluid intake in the heat and this resulted in an increase in extracellular fluid.

5.1.5 Objective five

Based on the feedlot electrolyte supplementation experiment, there did not appear to be any performance/welfare benefit post electrolyte therapy. Under those specific environmental conditions there was no weight advantage after 18 days of electrolyte supplementation, nor any difference between groups after a further 7 days without electrolytes. This would seem to indicate no detrimental effect of the supplement on LW.

5.1.6 Objective six

This objective was achieved with successful calculations of heat stress thresholds for ram lambs, heavy rams and wethers and pregnant Friesian heifers. There was further consideration of the changes in core body temperature under regimes of continuous heating, and the relationship of body temperature with clinical signs as the animals responded to the heat.

6 Impact on Meat and Livestock Industry – now & in five years time

6.1 Electrolyte supplementation

6.1.1 Now

This research on the use of electrolyte supplementation for cattle exposed to conditions such as would be experienced on board a live export vessel has investigated the repeatability of a weight advantage of supplemented animals and the nature of fluid shifts in heated and supplemented animals. In responding to industry interest on this area, the projects have made advances in understanding requirements of and responses to electrolytes under hot conditions, so that more informed decisions can be made about their use.

6.1.2 In five years time

Only one formulation of electrolytes was tested. Further work using other additives that improve hydration may useful. Continued research into the effects of altered hydration of the carcass, such as meat quality and the capacity to withstand various stressors would be applicable to both export systems and land based production.

With risk management procedures in place within the live export industry the potential for heat stress incidents and therefore the need for intervention strategies such as electrolyte supplementation should be reduced.

Development of systems that can measure the impacts of various procedures and management regimes on the welfare and behaviour of animals will allow testing of the effects of these procedures. Thus, the welfare benefits of supplementation could be assessed, as a separate issue from production or health.

6.2 Heat stress thresholds

6.2.1 Now

The heat stress threshold results can be incorporated directly into the Heat Stress Risk Assessment Model (HSRAM), so that more accurate predictions can be made and therefore different classes of animals can be managed appropriately.

The proactive nature of this work should be promoted, as the live export industry seeks to make knowledgeable decisions about best practice management of animals.

6.2.2 In five years time

The climate controlled rooms at Murdoch University can be used to further define heat stress thresholds of other lines of livestock, for further refinement of HSRAM. Any new data that can be incorporated into the model will be beneficial to the industry both economically and in terms of animal welfare, and again shows the proactive work of the industry.

Other situations can also be tested and modelled, such as conditions experienced in feedlots and extensive grazing in summer.

7 Conclusions and Recommendations

7.1 Electrolytes

The electrolyte mix that has been tested in these experiments was formulated in response to changes measured in animals under pronounced clinical heat stress, and tested to discover if there were health, welfare or production benefits to supplementation of these specific electrolytes in the water to animals exposed to hot conditions.

Testing on limited numbers of animals experiencing clinical heat stress within climate controlled rooms has not resulted in any difference in core body temperature, respiratory rates or panting response, so there is no evidence that giving these electrolytes in the water alters the way the cattle respond to high heat and humidity. Under situations causing clinical heat stress, and also milder conditions, the urine pH of the supplemented cattle was higher than that of unsupplemented cattle, which is presumed to mean that the supplemented cattle have a better capacity for buffering, which may prove important post heating, when animals tend to develop an acidosis. However, we have no evidence to show whether this maintains or improves health of the cattle. There has been limited opportunity to test the supplement on large numbers of animals experiencing such severe environmental conditions, and therefore we cannot say whether the supplement would improve the health of such animals, in terms of measures such as mortality or morbidity.

An unintended result in LIVE.209 was the approximately 3% weight advantage of supplemented cattle after 18 days ship board journey, compared to the unsupplemented animals. This occurred in the absence of severe clinical heat stress. Such a weight difference would be of economic benefit if it was carcass weight, and sustained for a period after supplementation. A subsequent experiment conducted in a feedlot on land also showed a weight advantage of supplemented cattle, but this was not sustained. While there was a difference in feed intake of the feedlot cattle, the overall impression from all experiments conducted is that the majority of the weight advantage is due to increased fluid in the animal, and while some may be gut fill, it appears this may be also be in the extracellular space. Whether this is of benefit in helping animals cope with excessive heat load, or whether it has positive effects on meat quality has not been determined. At this stage, it is difficult to recommend using the electrolyte supplement in the hope of a weight advantage, because of variable results. However, there is no indication that the supplement has a negative effect on either health or weight.

The cattle appeared to drink the supplemented water readily, and in most experiments treated animals drank significantly more than the control cattle. No direct behavioural testing was done to determine if the cattle actually had a preference for the supplemented water, or to detect any welfare benefits of supplying the supplemented water. Therefore we cannot make any conclusions about a welfare benefit.

Thus, we have no evidence that conclusively supports supplementing electrolytes in the drinking water on board live stock vessels.

Additional management factors that influence this decision include the difficulties in supplying a constant concentration through the water lines to the cattle, issues with increased urination and wet bedding, and the effectiveness of the risk management software that limits the chance of cattle being exposed to environmental conditions that would result in clinical heat stress.

7.2 Heat stress thresholds

Several classes of livestock identified by exporters as of importance – heavy rams, wethers, ram lambs, and pregnant Friesian heifers – were subjected to periods of sustained heat and humidity and their core temperatures recorded over those periods, and related to the wet bulb temperature of the climate controlled rooms in which they were housed. Heat stress thresholds (HST) were calculated for these livestock, and the raw data and our calculations from that work have been passed on for use in the HSRAM. We recorded further the nature of core temperature responses to high heat and humidity, and identified three thresholds of significance as animals were exposed to sustained periods of high wet bulb temperature. HST1 was the wet bulb temperature at which the mean daily core temperature of the animals significantly increased above its 'normal' core temperature. HST2 was the wet bulb temperature at which mean core temperature was significantly increased 0.5°C above 'normal'. Once mean core temperature increased above the 5% confidence interval of normal mean temperature plus 0.5 °C it was said to have reached HST 2. HST3 was the wet bulb temperature at which mean core temperature was significantly increased 1 °C above normal. This was observed to correspond more closely to other clinical indicators of heat stress, such as open mouthed panting. Also of importance in these observations was the effect of sustained exposure to high wet bulb temperatures, as distinct from acute exposure, and this is a factor that does not appear yet to be fully incorporated into the HSRAM. Further work could explore this.

The use of these figures for classes of livestock that had not previously been studied intensively means that the predictive modelling for risk management can be more accurate, and therefore management of these classes of livestock more tailored to their needs. It was also obvious from this work that there was a range of tolerance to the conditions, and further work could test individuals and identify genetic lines of animals that are more heat tolerant.

8 Bibliography

Andrews, F.M., Nadeau, J.A., Saabye, L. and Saxton, A.M. (1997). Measurement of total body water content in horses, using deuterium oxide dilution. *American J. Vet. Res*. 58: 1060-4.

Andrew, S.M., Erdman, R.A. and Waldo, D.R. (1995). Prediction of body composition of dairy cows at three physiological stages from deuterium oxide and urea dilution. *J. Diary Sci.* 78: 1083-95.

Arnold, R.N., Hentges, E.J. and Trenkle, A. (1985). Evaluation of the use of deuterium oxide dilution techniques for determination of body composition of beef steers. *Journal of Animal Science*. 60: 1188-1200.

Arnold, R.N. and Trenkle, A. (1986). Equilibration and passage of water in the gastrointestinal tract of cattle in relation to estimating body water by compartmental kinetic models. *Journal of Animal Science*. 63: 1400-9.

Barnes, A., D.T. Beatty, E. Taylor, S.K. Maloney, C. Stockman, and D. Pethick. (2003). LIVE.209: The physiology of heat stress in cattle and sheep. *Final Report*. Meat and Livestock Australia. Sydney, Australia.

Beatty, D.T., A. Barnes, E. Taylor, D. Pethick, M. McCarthy and S.M. Maloney. (2006a). Physiological responses of *Bos taurus* and *Bos indicus* to prolonged, continuous heat and humidity. *Journal of Animal Science.* 84: 972-985.

Beatty, D.T., A. Barnes, R. Taplin and S.K. Maloney. (2007). Electrolyte supplementation of live export cattle. *Australian Journal of Experimental Agriculture*: 47: 119-24.

Fielding, C.L., Magdesian, K.G., Elliot, D.A, Cowgill, L.D. and Carlson, G.P. (2004). Use of multifrequency bioelectrical impedance analysis for estimation of total body water and extracellular and intracellular fluid volumes in horses. *American J. Vet. Res*. 65: 320-6.

Forro, M., Ciesler, S., Ecker, G.L., Walzak, A., Hahn, J. and Lindinger, M.I. (2000). Total body water and ECFV measured using bioelectrical impedance analysis and indicator dilution in horses. *Journal of Applied Physiology*. 89: 663-71.

Gad, S.M., and R. L. Preston. (1990). In vivo prediction of extracellular and intracellular water in cattle and sheep using thiocyanate and urea. *Journal of Animal Science* 68: 3649-3653.

Gortel, K., A. L. Schaefer, B. A. Young, and S. C. Kawamoto. (1992). Effects of stress and electrolyte supplementation on body fluids and weights of bulls. *Canadian Journal of Animal Science* 72: 547-553.

Guyton, A.C. and Hall, J.E. (1996). Textbook of medical Physiology. Ninth Edition. W.B. Saunders Company. USA.

Hassall and Associates Pty Ltd (2000). Economic contribution of the live export industry, Sydney Australia

Hafez, E. S. E., Badreldin, A. L. & Sharafeldin, M. A. (1956). Heat tolerance studies of fat-tailed sheep in the subtropics. *Journal of Agriculture Science*. 47: 280-286

Hahn, G. L. (1985). Management and housing of farm animals in hot environments in *Stress Physiology in Livestock, Vol. 2*, Ed. Yousef, M. K., CRC Press, Boca Raton, Fla

Hammond, A.C., Rumsey, T.S. and Haaland, G.L. (1988). Prediction of empty body components in steers by urea dilution. *Journal of Animal Science*. 66: 354-60.

Hammond, A.C., Waldo, D.R. and Rumsey. T.S. (1990). Prediction of body composition in Holstein steers using urea space. *Journal of Diary Science*. 73: 3141-5.

Hofman, W. F. & Riegle, G. D. (1977). Thermorespiratory responses of shorn and unshorn sheep to mild heat stress. *Respiration Physiology*. 30: 327-338

Klemm, G. H. (1962). The reactions of unshorn and shorn sheep to hot wet and hot dry atmospheres. *Australian Journal of Agricultural Research*. 13: 472 – 478

Lee, D. H. K. (1950). Studies of heat regulation in the sheep, with special reference to the merino. *Australian Journal of Agricultural Research*.1: 200 – 216

Mader, T. L., M. S. Davis, and T. Brown-Brandl. (2006). Environmental factors influencing heat stress in cattle. *Journal of Animal Science* 84: 712-719.

Martin, R.A. and Ehle, F.R. (1986). Body composition of lactating and dry Holstein cows estimated by deuterium oxide. *Journal of Diary Science*. 69: 88-98.

Maunsell Australia Pty Ltd (2003). Development of a heat stress risk management model. Project LIVE.116. Meat and Livestock Australia Limited.

Odwongo, W.O., Conrad, H.R., Staubus, A.E. and Harrison, J.H. (1985). Measurement of water kinetics with deuterium oxide in lactating dairy cows. *Journal of Diary Science*. 68: 1155-64.

Preston, R. L., and S. W. Kock. (1973). In vivo prediction of body composition in cattle from urea space measurements (37470). In: Proceedings of Society of Experimental Biology Medicine. p 1057-1061.

Ross, J.G., Preston, R.L.and bartle, S.J. (1992). Evaluation of sodium thiosulfate as an extracelluar water marker in cattle. *Journal of Animal Science*. 70: 434-8.

Rule, D.C., Arnold, R.N., Hentges, E.J. and Beitz, D.C. (1986). Evaluation of urea dilution as a technique for estimating body composition of beef steers in vivo: validation of published equations and comparison with chemical composition. *Journal of Animal Science*. 63: 1936-48.

Schaefer, A.L., Jones, S.D.M., Tong, A.K.W., Young, B.A., Murray, N.L. and Lepage, P. (1992). Effects of post-transport electrolyte supplementation on tissue electrolytes, haematology, urine osmolality and weight loss in beef bulls. *Livestock Production Science*. 30: 333-46.

Schaefer A.L., Jones, S.D.M. and Stanley, R.W. (1997). The use of electrolyte solutions for reducing transport stress. *Journal of Animal Science*. 75: 258-65.

Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock production science.* 67: 1-18

Spurlock, G.H., Landry, S.L., Sams, R., McGuirk, S. and Muir, W.W. (1985). Effect of endotoxin administration on body fluid compartments in the horse. *American Journal of Veterinary Research*. 46:1117-20.

Symington, R. B. (1960). Studies on the adaptability of three breeds of sheep to a tropical environment modified by latitude – The response of mature and young rams to a thermal burden induced by exercise. *Journal of Agricultural Science*. 55: 303 – 310

Thwaites, C. J. (1967). Fleece length and the reactions of sheep to elevated humidity and radiant heating at high ambient temperatures. *Research in Veterinary Science*. 8: 463 – 466

Thwaites, C. J. (1985). 'Physiological responses and productivity in sheep', in *Stress physiology in livestock volume 1 Basic Principals*, eds. M. K. Yousef, CRC Press, Florida, pp. 47 – 55

Van Beaumont, W., S. Underkofler, and S. Van Beaumont. (1981). Erythrocyte volume, plasma volume, and acid-base changes in exercise and heat dehydration. *Journal of Applied Physiology* 50: 1255-1262.

Wagstaff, A.J., Maclean, I., Michell, A.R. and Holmes, P.H. (1992). Plasma and extracelluar volume in calves: comparison between isotopic and 'cold' techniques. *Research in Veterinary Science*. 53: 271-3.

Woodford, S.T, Murphy, M.R. and Davis, C.L (1984). Water dynamics of dairy cattle as affected by initiation of lactation and feed intake. *Journal of Diary Science*. 76: 2336-43.

Yousef, M. K. (1985). 'Thermoneutral zone', in *Stress physiology in livestock volume 1 Basic Principals*, eds. M. K. Yousef, CRC Press, Florida, pp. 67 – 75.

9 Appendices

9.1 Appendix 1

Results of feed analysis:

Test	Unit	Result
Moisture	%	10.2
Dry matter	%	89.8
Ash	%	7.1
Crude Protein (N x 6.25)	% of DM	8.9
Acid Detergent Fibre	% of DM	22.7
DM Digestibility	% of DM	65.6
Metabolisable energy	MJ / kg DM	9.5
Phosphorus	%	0.220
Potassium	%	0.697
Sulphur	%	0.244
Sodium	%	0.200
Calcium	%	1.509
Magnesium	%	0.206
Chloride	%	
Copper	mg / kg	4.22
Zinc	mg / kg	43.30
Manganese	mg / kg	80.5
Iron	mg / kg	173.6
Nitrate	mg / kg	
Boron	mg / kg	7.81