liveexport

Physiology of heat stress in cattle and sheep

Project number LIVE.209

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Table of Contents

ABSTRACT

The physiology of clinical heat stress in cattle and sheep was defined under experimental animal house conditions. Changes in body temperature, feed and water intake, respiratory and heart rates, and acid-base and electrolyte balance were measured. This work has helped define the heat stress threshold for *Bos taurus* and *Bos indicus* heifers, Merino wethers and Awassi rams. An electrolyte supplement was proposed on the basis of measured changes in the cattle, and tested with positive results on urine pH indicating improved buffering capacity, and a body weight advantage. On the basis of these results, it is recommended that *Bos taurus* receive appropriate electrolyte supplements in the water when shipped on long haul voyages when they are exposed to high heat and humidity, for potential health and economic benefits. Future work could further clarify the nature and repeatability of these benefits.

EXECUTIVE SUMMARY

Experiments were conducted to define the physiology of heat stress in cattle and sheep considering particularly the physiological and biochemical changes that affect electrolyte balance in the animals, with a view to formulating appropriate supplementation of electrolytes. An experimental animal house facility with specifically modified climate controlled rooms was used to subject animals to sustained periods of high heat and humidity, as experienced by livestock exported by ship on long haul voyages to northern hemisphere destinations during the northern summer. Three baseline experiments were conducted with six cattle each (two with *Bos taurus* and one with *Bos indicus*) and one experiment was conducted with 18 sheep (12 Merino wethers and six Awassi rams).

These baseline experiments indicated the wet bulb temperatures at which the cattle were no longer able to maintain their normal body temperature ("heat stress threshold"). These data can be used in predictive models for heat stress management. The *Bos taurus* had a more pronounced panting response at lower wet bulb temperatures compared to the *Bos indicus*, which led to greater alterations in acid-base balance. However, once the *Bos indicus* passed their heat stress threshold, the responses and physiological changes were essentially the same as those measured in the *Bos taurus.*

There was a marked reduction in feed intake by the *Bos taurus* once their body temperature rose to the stage where the experimental animals were eating almost nothing. This severe inappetence has not been previously described, but has serious production and economic implications. The water intake of these heat stressed animals was very high, in some cases over 15% of body weight per day, indicating that animals will require good access to water at all times when exposed to hot, humid conditions.

From these intensive experiments, the alterations in electrolytes could be determined for animals subject to clinical heat stress, and a supplement was designed to replace the deficits. The electrolyte supplement was delivered in the drinking water, containing 1.8g/L sodium bicarbonate and 3.5g/L potassium chloride, and it was tested in three experiments on *Bos taurus*, two within the climate controlled rooms (12 animals in total), and one experiment on a commercial ship with 80 animals. All three experiments showed a similar result: that compared to control animals, supplemented animals drank more, had more alkaline urine, and had a weight advantage at the end of the experiment. For cattle experiencing clinical heat stress in the climate controlled rooms, treated animals lost 5.1% of their starting body weight, while control animals lost 7.9% of starting body weight. The cattle on the commercial shipment did not show clinical heat stress, and the treated animals gained 2.9 $\pm$ 1.7 % more weight than the control animals ($P<0.001$). The treatment was cost effective. The recommendation following these experiments is for *Bos taurus* to receive electrolyte supplementation in the water for long haul voyages, and such a practice can be implemented for the coming northern summer. The optimum nature and dose of the supplement has not been determined with only one type tested, but the supplement used in these experiments was more concentrated than many current commercial supplements. Given the weight advantage seen with these animals in the absence of clinical heat stress, the use of electrolyte supplements in *Bos indicus* cattle should be explored.

The electrolyte supplement did not improve feed intake, nor did it alter body temperatures of animals tested in the climate controlled rooms. An experiment investigating the effects of a pellet containing 80% good quality hay (60% IVD) compared to a standard straw-based shipper pellet did not show any differences in feed intake, nor any consistent pattern in rumen or core body temperature between the

diets in animals at moderate wet bulb temperatures. While there was no indication from this experiment that one of the pelleted diets was superior to the other, it did not answer the question of whether feeding hay or chaff may be useful for heat stressed animals, a practice used in feedlotting and occasionally in the export industry.

The heavy reliance of *Bos taurus* cattle on panting to dissipate heat, with the associated problems created with excessive expiration of carbon dioxide, also indicates that other methods of enhancing heat loss would be useful for these animals. Wetting and washing of cattle in times of heat stress is a technique used in the industry, and combined with good airflow, should improve evaporative heat loss from the skin, to reduce the reliance on respiratory heat loss.

The sheep experiment showed that inappetence was less related to the exposure to heat than to individual animal factors, with some animals eating poorly whether it was hot or not, and other animals eating well throughout the experiment. The heat stress thresholds of the animals could be determined from the core body temperature results, and the Awassi rams had a higher threshold than the Merino wethers. The Merino sheep panted extremely fast in response to the heat, but compared to the cattle, the sheep had less pronounced effects of panting on acid-base balance and electrolytes were not altered. This led to the recommendation not to proceed with testing an electrolyte supplement for sheep exposed to heat that continue to eat. The issue of shy feeding in sheep remains very important.

1 MAIN RESEARCH REPORT

1.1 Background

This project was conducted to determine the physiology of heat stress in cattle and sheep, considering particularly the physiological and biochemical changes that affect electrolyte balance in the animals, with a view to formulating appropriate supplementation of electrolytes. The project arose from the interest of livestock exporters in supplying electrolytes to cattle and sheep transported on ships, with the aim of reducing stress of those transported animals. This project follows the Desk Top Study LIVE.204 which reviewed the literature and surveyed exporters and experts on the "Use of electrolytes to reduce stress". LIVE.204 recommended further research to determine the most appropriate electrolyte supplement for cattle and sheep during shipping and the cost-benefit of supplementing cattle and sheep with electrolytes during shipping. The survey conducted by Alliance Consulting and Management determined that 50% of exporters felt that electrolytes should be included in the shipboard treatment regime for cattle, and 33% of exporters felt the same for sheep (Alliance, 2001). Further anecdotal reports from exporters have indicated a division of opinion about the use of supplements, which can be costly, and they wished for some guidance as to the benefits of the use of supplements.

1.2 Heat stress of exported animals

Heat stress is a term used to denote a state where an animal is responding to adverse hot conditions. Under such conditions an animal can respond to the heat by making physiological changes and adjustments within the body, so that it can survive in that environment. These changes will act to keep critical systems and mechanisms within the body functioning. However, if the heat load experienced by the animal becomes excessive, the critical functions may no longer be maintained, and clinical disease, collapse and even death can result. Such a situation may be described as severe or clinical heat stress.

Animals transported by ship can be exposed to hot and humid conditions. Voyage reports (eg MAMIC 2000a) indicated that animals travelling to the Middle East during the northern summer (May to October) experienced conditions over 30 °C wet bulb, often for sustained periods of several days, with nil or little diurnal respite. The air entering the decks can be hot and humid, and it can become worse with the addition of heat from the animals, so that animals further from the entry points can be subject to extreme heat and humidity. The continued generation of heat by the animals, and radiation of heat from the ship's surfaces can maintain these conditions day and night, especially in equatorial waters where there is little cooling at night.

These environmental conditions are different from those tested in many other situations (eg reviewed by Sparke *et al*., 2001). Animals kept outside may experience some relief from the heat at night, whereas there does not appear to be the opportunity for cooling at night on the ships travelling in equatorial regions. Animals outside may also receive the benefit of wind to enhance cooling, although in still conditions the forced ventilation that should occur on the ships will be an advantage. Animals kept below decks do not have the radiant heat gain from the sun, although there will be heat radiated from the ship surfaces. Humid conditions can hamper evaporative heat loss from animals. Thus, environmental conditions on the ships can be more extreme for the animals, which have little opportunity to escape the conditions. Depending on the stocking density, there may be limited scope for behavioural modifications to decrease heat gain or improve heat loss.

1.3 Physiological responses to heat stress

The type and magnitude of the physiological changes and adjustments to heat stress will influence how well the animal is able to respond to hot conditions, and there are species and breed differences in these factors. These responses work to both increase heat loss and decrease heat gain by the animal.

Heat is detected by thermoreceptors in the skin and buccal membranes, which detect ambient temperature, and also by central receptors in the hypothalamus and spinal cord that can detect changes in the blood temperature (Bligh, 1985).

There will be behavioural responses to the increased temperature. Animals can change posture, eg stand or spread out to increase surface area for heat loss, reduce activity, and seek shade if outside (Bligh, 1985).

The initial physiological responses initiated to increase heat loss redirect blood to the periphery, by vasodilation of skin blood vessels, and vasoconstriction of vessels supplying internal organs. Increased sweating rate then enhances heat loss from the skin. There will be loss of electrolytes in the sweat, with the amount lost differing between species, and perhaps between breeds, with other animal factors such as acclimatisation to heat also influencing the composition. Cattle sweat is hypotonic, containing mostly potassium (K^+), along with sodium (Na^+) and chloride (Cl^-) (Johnson, 1970; Beede and Collier, 1986; West, 1999).

If the loss of heat from the skin is not sufficient to maintain stable core temperature, additional heat can be lost from the respiratory membranes as the animal pants. Generally, for animals that both sweat and pant such as cattle, there will be a lag of two to three hours before the respiratory mechanisms for heat loss are initiated. These physiological responses are reviewed in Sparke *et al*. (2001). Evaporation from the skin provides the greater heat loss for cattle, up to about 80% of total evaporative heat loss (Robertshaw, 1985), but panting is an important mechanism for additional heat loss. Panting is the main method of evaporative heat loss for sheep (Thwaites, 1985).

Panting is of particular importance when the humidity increases along with the temperature, as experienced by animals shipped through equatorial regions during the summer. Evaporation of water requires a vapour pressure gradient for loss of heat energy as water evaporates to the surrounding air, but in very humid conditions this gradient is reduced, and therefore evaporative heat loss from the skin is reduced. Respiratory cooling can still occur under these conditions because inspired air is warmed to body temperature and therefore can take on more water vapour, which maintains the gradient. However, when the temperature of inspired air rises to near body temperature, this means of heat loss from panting also becomes limited (Sparke *et al*., 2001).

At first, with increasing environmental temperature, the respiratory rate increases, but the tidal volume decreases. This allows for increased heat loss from the respiratory membranes, but does not increase alveolar ventilation substantially, and therefore does not alter the total gas exchange for the animal. When heat stress becomes more severe, the depth of respiration increases back to near normal tidal volume while the respiratory rate remains elevated above normal (second stage panting). Overall, there is increased airflow over surfaces for evaporative heat loss, but there is also increased alveolar ventilation, up to five times normal in sheep and cattle. The increase in alveolar ventilation leads to excessive expiration of carbon dioxide and respiratory alkalosis (Hales, 1976; Sparke *et al*., 2001).

The change to second stage panting is thought to occur because there is continued heat stimulation of the respiratory centres via the hypothalamus, the carotid bodies, pulmonary receptors or medulla oblongata, along with a reversal of the effect of hypocapnia on respiration in hyperthermia (Hales, 1976). The stimulation will be of both rate and depth, but due to the interdependence of the two parameters, increasing the depth of respiration results in a slight decrease in rate.

The temperature at which panting is initiated to supplement the other heat loss mechanisms will depend on several factors, such as humidity, ventilation and airflow to the animal, and the animal factors mentioned above. Prior acclimatisation to the heat will also affect how well the animal is able to use means other than panting to maintain normothermia.

Sustained panting may be limited by the alkalosis that develops if there is a conflict between the drive to conserve expired carbon dioxide, and the continued elimination of heat through panting. However, buffering mechanisms will operate to maintain blood pH within the normal range (Whitehair *et al*., 1995), and this should allow panting to continue. Mechanisms to restore blood pH involve the immediate release of hydrogen ions (H^+) from intracellular locations, and renal mechanisms that conserve H^+ and excrete bicarbonate ions (HCO_3^-).

Alkalosis does have serious consequences for the well-being of the animal. It is associated with altered neurological function, because a lowered partial pressure of carbon dioxide in the cerebral blood vessels can cause vasoconstriction and reduce perfusion of the brain (Mitchell *et al*., 1972; Adrogue and Madias, 1998). Alkalosis can also decrease the availability of minerals such as magnesium and calcium, which can contribute to muscle tremors and tetany.

The impact of these physiological responses on electrolytes will be through the loss of fluid and electrolytes in sweat, and through interactions with the buffering of respiratory alkalosis. Potassium (K^+) and sodium (Na^+) ions are the major cations involved in maintaining acid-base status, and in alkalosis K^+ will exchange with H^+ and enter cells to maintain electroneutrality. Potassium ions will also exchange with H^+ in the renal tubules, and along with sweat losses, this urinary loss can lead to a total body deficit of K^+. Na^+ is conserved as much as possible in the animal, being the major cation involved in water balance, but when there is low body potassium, there is less aldosterone released, and the main drive for reabsorption of Na^+ from the urine is reduced. This can lead to further Na^+ losses. In situations of depletion of both K^+ and Na^+, there are less cations available for exchange with H^+ in the urine, and paradoxical aciduria can result, exacerbating the alkalosis.

An important aspect to maintaining normothermia is the reduction in heat production by the animal. This occurs with a decrease in metabolic rate (reviewed in Sparke *et al*., 2001). There will be behavioural responses, which decrease the activity and metabolic rate of the animal. In most animals, hot conditions result in a decreased feed intake, but the mechanism for this is unknown. It could be due to a reduction in the rate of passage of digesta, which increases gut fill for longer and depresses intake. There may also be a direct effect of the increased temperature on the feeding centre of the hypothalamus, resulting in a hormonal response, which could also decrease metabolic rate (Johnson, 1985). Thyroid activity is reduced in situations of heat stress, but the effect of heat on thyroid function takes at least 60 hours to be significant, so this is not an immediate response to acute heat stress, and instead can be involved in the acclimatisation of animals to sustained heat load. A decrease in thyroid hormones will act to decrease the metabolic rate, and reduce the amount of heat produced by the cells. There is some indication of intrinsic species and breed differences in resting metabolic rate that might account for different tolerance to heat.

Other hormonal responses to heat stress include a rise in plasma cortisol with short term exposure to heat, perhaps due to the ‘stress’ reaction, while in long term heat exposure there is a reduced cortisol turnover rate and a decreased plasma concentration. Growth hormone is also decreased in both short and long term exposure to heat, which will contribute to a lower metabolic rate, but also has implications for growth and production by animals (Johnson, 1985).

1.4 Implications for therapy of transported animals

The suggestions from previous research and the interpretation of basic physiological mechanisms indicate that there could be benefits of replacing those electrolytes that are lost. Provision of electrolyte supplements may benefit animals responding to heat stress. Electrolyte supplementation of heat stressed dairy cattle has revolved around supplying K^+, along with Na^+, which increases the strong ion difference and are essentially alkalising, providing ions which exchange with H^+. There is debate over the anion supplementation, whether this should be solely as Cl^-. Providing diets high in K^+ and Na^+ and normal for Cl^- resulted in improved feed intake and milk yield in dairy cows in hot weather, compared to diets high in Cl^- and normal for Na^+ and K^+ (Escobosa *et al*., 1984; Sanchez *et al*., 1994a). Whether a similar response to K^+ supplementation will occur in animals that do not have the same requirements as high producing lactating cows is uncertain (Beede and Collier, 1986; Stokka *et al*., 1996).

It is probable that heat stressed cattle in respiratory alkalosis will benefit from replacement of some carbonate, perhaps in the form of bicarbonate, to restore balance and improve their buffering capacity

(Sanchez et al., 1994b). Sodium bicarbonate has proved better than potassium bicarbonate in some experiments at improving intake and milk yield in dairy cattle in hot weather (Schneider *et al*., 1984), while potassium carbonate is a useful alternative (West *et al*., 1987). Chickens with respiratory alkalosis due to heat stress responded well to provision of extra carbon dioxide in the air they breathed, with increased partial pressure of arterial blood carbon dioxide and lowered blood pH (Koelkebeck and Odom, 1994).

Other options for dealing with high heat and humidity of transported animals involve selection of suitable animals for the conditions expected, strategic use of type and time of feeding, and the provisions of chilled water (Stermer *et al*., 1986; Baker *et al*., 1988; Wilks *et al*., 1990).

2 EXPERIMENTAL METHODS

2.1 Climate controlled rooms

Two existing rooms at Murdoch University were modified and set-up as climate controlled rooms, each capable of housing cattle or sheep, penned individually or together. Each room can hold three cattle or nine sheep in separate pens, each with individual feed and water for intensive monitoring. Each room is supplied with hot, humid air via a fan, heater and humidifier. The air fans were set to deliver 250 litres of air per second via electric duct heaters and Carel humidifiers with a steam capacity range of 10-33 kg/hr. The system is controlled external to the rooms, with electronic controls of heat and absolute moisture content of the air. These controls were set to deliver air at between 30-40 °C and containing 20-40 g moisture/kg, so that wet bulb temperature between 26 and 34 °C could be obtained within the rooms with the system operating. Exhaust air was set at 15 air changes per hour exhaust rate or 210 litres per second, with the slight positive pressure of the rooms intended to compensate because the rooms were not airtight, to prevent inflow of cold air from outside.

The airflow rate was sufficient to study 1250 kg of beast, working on a fresh air inflow of 0.2 litres/second/kg body weight. The rooms were designed to provide a degree of control accuracy in the order of ± 1K temperature and ± 10% relative humidity.

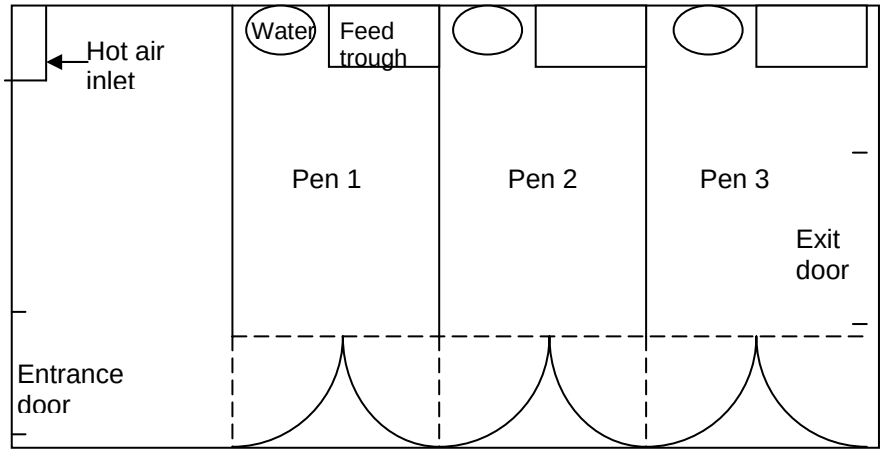
The rooms were modified in early 2002, and were operational by April 2002. Initial monitoring of the rooms was by manual recording of the displayed readouts on the control panels, while subsequently we obtained T-TEC Dataloggers which were suspended at a midpoint in the rooms for the duration of the experiments. Measurements of carbon dioxide, dry bulb temperature and relative humidity were taken at different points in the room, using a hand-held Testo 445 measuring instrument and ammonia levels were monitored using a Neotox MK5 ammonia meter.

For all of 2002 and the beginning of 2003, the rooms functioned well, running up to a maximum of 34 °C wet bulb. After the initial experiment, two additional insulating doors were inserted inside the exterior doors, to prevent cooling of that pen. Carbon dioxide concentrations remained within acceptable limits (recorded range 0.03 – 0.122), similar to those recorded on board ships, and the sawdust bedding was managed to keep ammonia also within acceptable low limits, never greater than 25 ppm, and generally less than 15 ppm.

After the second trial conducted in 2003, the humidifier bottles were changed, because the original bottles were developing large amounts of residue on the electrodes, which could compromise the function of the humidifier. The change to cleanable bottles resulted in problems, with the humidity never reaching the previous extreme, and with excessive build up of highly conductive material within the bottles causing arcing and shorting out of the humidifiers. After much discussion and consultation, a recommendation was received to supply treated water to the system, to alleviate these problems. The estimated cost of this modification is $15 000, and this has not been done. A two day test was run in March 2004 reverting to the original type of bottles, and the build up of highly conductive material did not occur. The humidity still did not reach as high as in 2002, but this may be a matter of recalibrating and servicing the system; there has not yet been any comment on this latest test from the manufacturers.

The individual pens for cattle measured 2.64 m^2, including space occupied by a 25 litre water bucket and a feed trough. This space was sufficient for *Bos taurus* up to 420 kg to turn around and lie down. *Bos indicus* tend to have longer bodies, and so the cattle chosen were smaller framed and therefore of lighter body weight. Water was available at all times for the animals, with buckets refilled manually from reservoirs kept on a platform above the pens. The water entering the buckets was kept around 26 °C, but then heated up to the room temperature in the buckets. Sawdust was used as bedding in the pens with the cattle and managed by removal of very wet, soiled bedding and addition of fresh sawdust. Cattle could be handled in these individual pens for measuring physical parameters and for the collection of samples. Figure 1 shows the outline of the pen design.

Figure 1. Layout of pens within the climate control room



The rooms were further modified by the addition of gates and pens so that nine sheep could be individually accommodated in each room, total space for each animal 0.88 m^2. Each sheep had access to its own water and feed bucket. As for the cattle, the water was kept topped up at all times.

2.1.1 Room conditions

The information from voyage reports was used to design a regime that would impose a cumulative heat insult on experimental animals within the rooms, followed by a cooling down period when the animals continued to be monitored. The initial pattern was to allow the animals a day or two to become accustomed to the rooms, followed by several days of gradual heating from ambient, then 5 days and nights at 32 °C wet bulb, and a final cool down period of three or four days. Later experiments also imposed a second short heat insult.

2.2 Measurements and monitoring

All experiments were approved by the Murdoch University Animal Ethics committee. The animals were monitored carefully for signs of excessive distress, and this intensive monitoring included staff staying overnight to check animals hourly during the hottest parts of the experiments.

Physiological parameters measured were heart/pulse rate, respiratory rate and character, rectal and core temperature. Skin temperature and sweating rate were also measured in some experiments. Jugular blood and mid-flow urine samples were collected for analysis of blood gas, haematology, biochemistry and electrolyte concentrations. Urine specific gravity and pH were also measured, and hormone and metabolite analyses were performed on some samples.

2.2.1 Core temperature

Core body temperature of animals in these experiments was monitored and recorded using temperature sensors (Datamet, Potchefstroom South Africa) with telemetric output received on a radio receiver (AR8000, AOR Japan) interfaced to a computer running dedicated software and/or temperature loggers (Stowaway XTI, Onset Computer Corp, Pocasset, Michigan, USA, specially modified with a range of 32-46°C and resolution of 0.04°C) that could be downloaded on removal of the device from the animal. In both cattle and sheep, the units were implanted into the abdominal cavity, via a surgical incision made in the right paralumbar fossa. Animals were sedated with xylazine, in the case of the cattle via a xylazine and lignocaine epidural (0.04mg/kg xylazine made up to 2ml with 2% plain lignocaine), and in the case of the sheep via intramuscular xylazine (0.05 mg/kg). Local anaesthesia of the region was obtained through paralumbar nerve blocks with lignocaine. A full surgical scrub of the area and sterile surgical procedures were used to minimize the chances of infection, and at the time of surgery the animals were also given intramuscular injections of long acting oxytetracyline antibiotic, and intravenous flunixin for post operative analgesia. The temperature sensors were sutured inside the peritoneum, and the abdominal cavity was

sutured closed. Recovery of the sensors required a similar surgical procedure. The animals were given at least a week after surgery to heal from the surgery, and were monitored for signs of infection or fever.

3 EXPERIMENTAL SCHEDULE

The following experiments were conducted and reported in detail in previous milestone reports:

1. First *Bos taurus* experiment (6 heifers), to determine the physiology of heat stress

2. Second *Bos taurus* experiment (6 heifers), to determine the physiology of heat stress

3. *Bos indicus* experiment (6 heifers), to determine the physiology of heat stress

4. First electrolyte intervention experiment (6 *Bos taurus* heifers), to determine the effects of the proposed electrolyte supplement

5. First sheep experiment (12 Merino wethers and 6 Awassi rams exposed to heat, 6 Merino wethers not exposed to heat), to determine the physiology of heat stress

6. Second electrolyte intervention experiment (6 *Bos taurus* heifers), to determine the effects of the proposed electrolyte supplement

7. Shipboard experiment (80 *Bos taurus* steers), to determine the effect of the electrolyte supplement

8. Feeding experiment (6 *Bos taurus* heifers), to determine the effects of different two pelleted feeds on animals exposed to heat.

3.1 The Physiology of Heat Stress in *Bos taurus* and *Bos indicus*: Experiments 1, 2 and 3

3.1.1 Materials and Methods

3.1.1.1 Animals

For Experiments 1 and 2, six *Bos taurus* heifers were chosen for reasonable temperament, and suitable bodyweight. The animals for Experiment 1 were Angus and Angus-cross heifers selected from the Murdoch University herd, and ranged in bodyweight from 336 to 408 kg, weighed after 12 hours off feed, with the total weight of animals in each room 1120 and 1148 kg. For Experiment 2 the Murray Grey-cross and Angus-cross heifers came from sale yard stock sourced from southern Western Australia. These animals ranged in bodyweight from 312 to 368 kg, weighed after 15 hours off feed, and the total weight of animal in each room was 992 and 986 kg. Experiment 1 ran from 27th April to 10th May 2002, and Experiment 2 ran from 13th June until 28th June 2002

Experiment 3 commenced on the 21st August and ran until the 5th September. Six non-pregnant pure bred Brahman heifers were selected, ranging from 289 to 386kg (after 15hrs off feed), and the total weight of animal in each room was 1022 and 1011kg. The cattle were sourced from Brahman studs below the 26th parallel and thus were not northern acclimatised. The need to choose appropriate cattle for the rooms meant that five of the animals were from the same stud, and the sixth animal was from a different property.

3.1.1.2 Preparation

All animals were treated for internal and external parasites and vaccinated against leptospirosis prior to intensive handling. They were checked for pregnancy and the oestrous cycles were synchronised with injections of prostaglandin. They were handled daily in a crush, to ensure they would tolerate the intensive sampling and monitoring they would receive in the rooms. Selection of animals with reasonable temperament was essential for operator safety within the confines of the individual pens, and further handling accustomed the animals to the procedures.

Ten to 14 days before the intended start of the experiment in the rooms, surgical implantation of the temperature sensors was performed. The Brahman heifers were also implanted with temperature loggers. After the surgery the animals were started on small amounts of pellets, to accustom them to the diet. The percentage of pellets was gradually increased so that they were eating only pellets by the time they entered the rooms.

The animals were held off feed for 12-15 hours before weighing on the day prior to entry to the rooms, day 0, and on that day, indwelling jugular catheters were inserted and fixed in place. The animals then entered the rooms on day 1.

3.1.1.3 Feed and water

Feeding in the rooms was with standard shipper pellets, at the rate of 2.25% dry matter of their curfew bodyweight. The composition of these pellets is shown in appendix 1. The feed was offered in two equal amounts at 7 am and 1 pm daily. The feeders were cleaned out before each morning feed, and the residues weighed. The water buckets were cleaned each morning or more frequently if the animal defaecated in the bucket; daily water intake was calculated each morning.

3.1.1.4 Room conditions

For the first one or two days in the climate controlled rooms the cattle were kept at ambient conditions. The system was turned on and the wet bulb temperature increased by 2 °C per day from 26 °C to 32 °C. For Experiments 1 and 2, the cattle were then kept at 32 °C for 5 days. The system remained on day and night, but in Experiment 1 there was some cooling through the external doors of each room, which was remedied in Experiment 2 by the use of insulated panels. In Experiment 3 the heat was increased up to 34 °C wet bulb by the 5^{th} day of the hottest period. The wet bulb temperature was then decreased to ambient conditions over the next three to four days, and then the animals were released from the rooms.

3.1.1.5 Body weight

Animals were weighed before entering the rooms, after the hottest period, at the end of the trial and then weekly for 3 weeks. For the weights before and at the end of the experiment, the animals were restricted from feeding for 12-15 hours; during the trial the animals were eating very little and gut fill would have been similar to the pre- and post-trial weights. The final weight was measured 30-60 minutes after the animals had been brought in from the paddock, and so included gut fill.

3.1.1.6 Monitoring

The animals were monitored closely, and behavioural and clinical signs of heat stress such as drooling, panting, ability to stand, and neurological depression were used to determine their response to the high heat and humidity. Physical parameters were monitored on the animals throughout.

Panting was scored based on the guide developed by J.B. Gaughan, S.M. Holt and T.L. Mader as follows:

Breathing condition	Panting score
No panting – normal.	0
Slight panting, mouth closed, no drool or foam.	1
Fast panting, drool or foam present.	2
As for 2 but with occasional open mouth.	2.5
Open mouth and some drooling. Neck extended and head usually up.	3
As for 3 but with tongue out slightly.	3.5
Open mouth tongue out and drooling. Neck extended and head up.	4
As for 4 but with head down.	4.5

Core body temperature was recorded nearly every minute per animal via the implanted temperature sensors, and rectal temperatures, respiratory and heart rates were recorded two to three times per day, before samples were collected. Blood and urine samples were collected one to three times per day, with the more frequent sampling during the hottest period. These samples were collected at 6am, 12 noon and 10 pm, to give a picture of changes throughout the day.

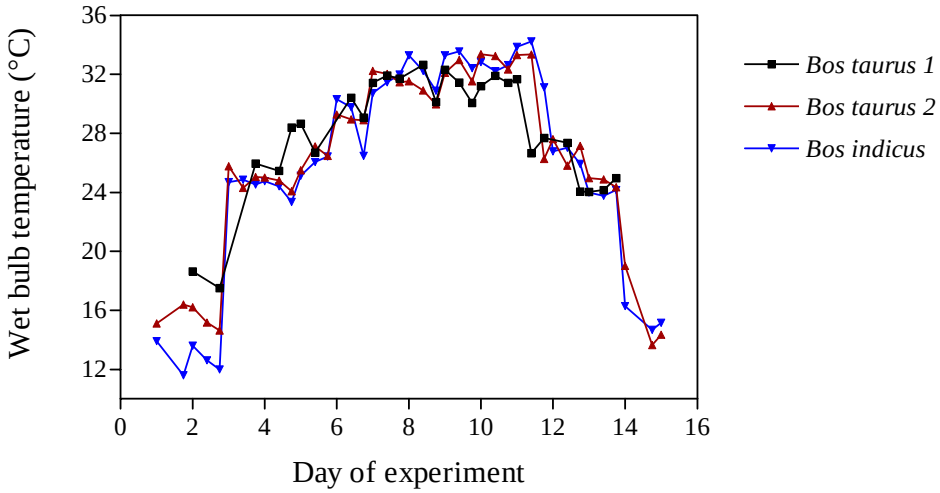
Environmental measurements were made once to three times daily, when the blood and urine samples were collected. These measurements were of ammonia, carbon dioxide and temperature and humidity, taken adjacent to the output of the humidifier, at the other side of the room, and also at the vent for the outflowing air from the room.

3.1.2 Results

3.1.2.1 Room conditions

The climate controlled rooms operated adequately, to provide the required wet bulb temperatures at around 80% relative humidity. Figure 2 shows the wet bulb temperatures measured in the rooms each day. In Experiment 1, there was one less day without any heat at the beginning of the experiment, so for comparison all the graphs for that experiment start later than for Experiments 2 and 3.

Figure 2. Wet bulb temperatures for the climate controlled rooms for the three heat experiments. Each value is the mean of three measurements in the two rooms.



3.1.2.2 Clinical signs

The *Bos taurus* in Experiments 1 and 2 showed clinical signs of heat stress, with drooling, increased respiratory rates, up to the point of open mouthed panting in some animals. As the hottest period progressed, the animals became depressed and “doughy”, reluctant to rise, uninterested in food, and some showed slight neurological signs with staring, glazed eyes. The *Bos indicus* in Experiment 3 showed very few of these clinical signs. They did not pant to the same extent, and never showed open-mouthed breathing. Five of the six *Bos indicus* remained alert and could get up and move around; at the very hottest temperatures, the one animal from a different stud lost interest in food and was reluctant to rise or move around.

3.1.2.3 Core and rectal temperatures

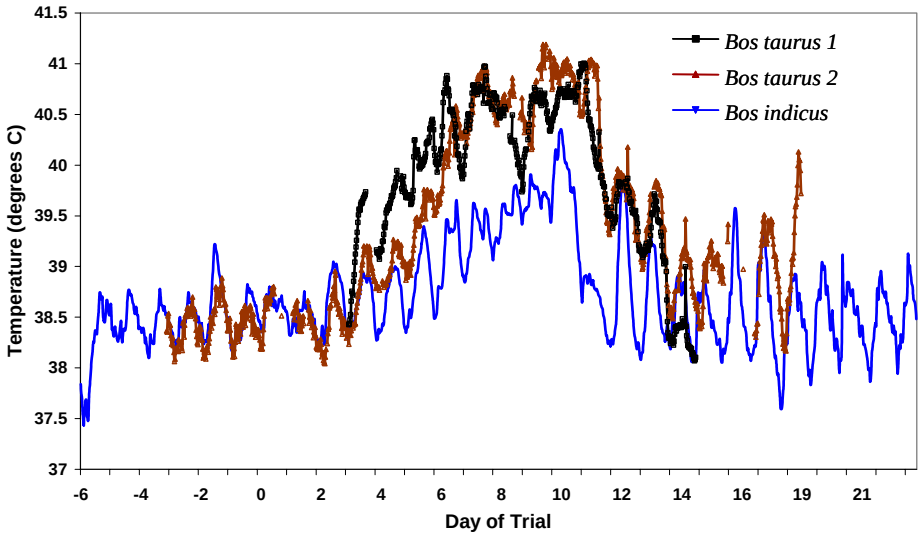
Figure 3 compares the mean core body temperatures for the three heat experiments, showing that the core temperatures of the *Bos taurus* increased earlier and went higher than the core temperatures of the *Bos indicus*, who were exposed to hotter conditions. There was good correlation between core and rectal temperatures ($P<0.001$) indicating the usefulness of rectal temperature in clinical monitoring of animals subjected to high heat load.

3.1.2.4 Feed and water intake, body weight

The feed intake of the *Bos taurus* decreased dramatically as the heat continued, so that they were eating virtually nothing by the second day of the hottest period. Feed intake of the *Bos taurus* slowly rose again in the cool down period, but they were not eating their full feed by the time they were removed from the rooms. Most of the *Bos indicus* continued to eat well throughout. Water intake was increased for all three experiments as wet bulb temperature increased, with very large intakes recorded for the animals in Experiment 1. There were no indications of dehydration in these animals from the samples measured, with plasma protein and packed cell volumes remaining the same or decreasing which indicated haemodiltution, and the production of very dilute urine with lowered specific gravity.

Bos taurus lost significant body weight during the period within the rooms, and the weight had not been regained one week after removal from the rooms. *Bos indicus* lost less weight during the period within the rooms, and gained weight in the week afterwards.

Figure 3. Mean core body temperature of animals in three heat experiments. Hottest period was from days 7-11.



3.1.2.5 Respiratory rate, blood gas

As noted above, the animals increased their respiratory rates in response to the heat. The initial response of the *Bos taurus* occurred at lower wet bulb temperatures than that of the *Bos indicus*, but by the end of the hottest period, all animals had similarly high respiratory rates.

Blood gas responses were consistent with the increased respiratory rates, and similar in all experiments, although responses were less marked in the *Bos indicus*. Blood carbon dioxide and bicarbonate concentrations were reduced during the hottest period, while blood pH was maintained at a normal level in the lead up to and during the hot period. Once the heat was turned off, the blood carbon dioxide concentration quickly rose to pre-heat values, but bicarbonate was much slower to return to those values, and thus blood pH dropped. Urine pH followed a similar pattern to blood pH, becoming acidic after day 11 when panting ceased.

3.1.2.6 Electrolytes

Plasma concentrations of electrolytes were generally within what is considered a normal range. However, there were decreases in plasma concentrations of sodium and calcium, while potassium remained reasonably constant. There were no consistent alterations in plasma magnesium or chloride.

The urine concentration of these electrolytes was confounded by dilution effects, and the fractional excretion ratio (FER) was calculated for each. This compares the percentage of the electrolyte excreted with the excretion of creatinine, which is not reabsorbed from renal tubules after filtration. Thus, the FER

gives an indication of whether the electrolyte is being conserved or excreted. The FER of sodium decreased over the duration of all three experiments, and was very low after the heat period. The FER of potassium also decreased over the heat period. For both electrolytes, this change was less marked for the *Bos indicus*.

3.1.3 Discussion and proposed electrolyte supplement

The cattle tested in these three experiments did experience physiological changes associated with exposure to high heat and humidity. The nature of the responses appeared similar between *Bos taurus* and *Bos indicus*, but the magnitude of the changes was less in the *Bos indicus*, and occurred at higher wet bulb temperatures, indicating that they had a higher heat stress threshold, that is, the wet bulb temperature above which the animal is not able to maintain a normal body temperature. The panting was associated with decreases in blood carbon dioxide concentrations as the heat continued, indicating there was excessive alveolar ventilation. During the hottest time the animals appeared able to compensate for the tendency to respiratory alkalosis, maintaining blood pH, presumably via renal compensation and excretion of bases such as bicarbonate, and blood bicarbonate decreased. The rapid return of blood carbon dioxide concentration after cessation of the high temperatures was not matched by a return in blood bicarbonate concentrations, leading to acidosis, with reduced blood and urine pH. We hypothesized that this reduced body buffering capacity could lead to further compromise of animals exposed to a second heat insult, or other perturbations of acid base status such as feed related acidosis.

Plasma concentrations of electrolytes are not necessarily good indicators of electrolyte deficiencies (NRC, 2000), so that while plasma electrolytes remained within a normal range, the decrease in plasma sodium was significant. The decrease in plasma calcium was more related to the decrease in feed intake than the effects of heat, determined by a pair feeding experiment. Reductions in the fractional excretion ratios of sodium and potassium indicated conservation of these electrolytes. The drastic reduction in feed intake by the *Bos taurus* during the hottest period meant that they were unable to replenish lost electrolytes.

Electrolyte supplementation was proposed to remedy these measured changes. It was considered that *Bos taurus* would benefit most from the supplement, because they showed the greatest alteration in the measured parameters, whereas *Bos indicus*, with a higher heat stress threshold, would be much less likely to be affected during voyages if the temperatures experienced were similar to the experimental conditions, and thus the supplement was tested in *Bos taurus* only. The proposed electrolyte supplement contained 1.8g $NaHCO_3$ and 3.5g KCl per litre, giving a total of 5.3g/L or 0.53% salt, which is much higher than many of the commercial products. This formulation was based on:

1. Physiological changes seen in *Bos taurus* in Experiments 1 and 2.
2. NRC (2000) recommendations for young stressed cattle.
3. Dietary cation anion difference (DCAD).
4. Commercially available electrolyte products used in the live export industry.

Suggested daily intake of sodium and potassium for stressed young cattle (250kg) is 10-14g and 57-67g respectively or 0.2-0.3% and 1.2-1.4% of ration (NRC, 2000). The concentrations of Na and K in the standard shipper pellet that we used were 0.065 and 0.486% respectively. Assuming there would be little or no feed intake during the time of greatest heat stress, electrolytes needed to be supplemented via the drinking water. Based on the measurements in the above experiments, heat stressed animals could drink 10% body weight (40L for a 400kg animal), so that a 400kg animal would receive 20g of sodium, 52g of bicarbonate and 70g each of potassium and chloride from the supplemented water, even if not eating at all.

Studies in the dairy (West *et al*, 1991; West *et al*, 1992; Sanchez *et al*, 1994) and swine (Haydon *et al*, 1990) industries have shown that increasing the dietary cation anion difference (DCAD) increases dry matter intake. These studies define DCAD as milliequivalents of Na + K – Cl per kilogram of feed DM. West *et al* (1992) found that increasing the DCAD of the diet from 120.4 to over 400 meq/kg of feed dry matter also provided greater blood buffering capacity of the blood, indicated by increased blood pH, bicarbonate and blood base excess. Thus providing additional sodium and potassium would improve the DCAD for these heat stressed cattle, and using bicarbonate rather than chloride alone as the anion keeps the DCAD high.

The decision was made to also increase the amount of sodium bicarbonate in the feed by 1%, and to provide the supplemented water and feed throughout the whole of the experimental period within the rooms, rather than at any particular period of heat or recovery.

3.2 The Efficacy of an Electrolyte Replacement Therapy : Experiments 4 and 6

3.2.1 Materials and Methods

3.2.1.1 Animals

Two replicate experiments (A and B) were conducted, with six *Bos taurus* heifers (Angus and Murray Grey cross) in each. The animals were selected from Murdoch University stock, and so had the same sire, and the same previous management history. They ranged in weight from 310 – 400 kg. The animals were randomly allocated to an individual pen and to treatment or control group for the first replicate. In the second replicate, to control for any pen and room effects, assignment of treatment and control animals to pens within each room was the opposite to that in the first replicate.

3.2.1.2 Preparation

Preparation of the animals was as for the previous experiments. They were accustomed to handling, and surgically implanted with temperature sensor telemeters and loggers ten days before entry into the climate controlled rooms. The heifers were kept off feed and water for 15 hours and weighed, before jugular catheters were inserted and fixed in place, and the animals entered the rooms.

3.2.1.3 Feed and water

Feed was offered at 2.5% of the curfew bodyweight (as fed), split into two equal feeds, given at 7 am and 1 pm daily. A standard shipper pellet was used (composition appendix 2), and the treated animals received pellets containing an additional 1% sodium bicarbonate. Feed residues were removed and weighed daily, before the morning feed.

Water was available *ad libitum* in the 25 litre buckets, filled manually as necessary from reservoirs above the pens. The treatment animals received supplementation with 1.8g/L sodium bicarbonate and 3.5g/L potassium chloride per litre of water. This meant that each litre of water contained 0.5 g sodium, 1.3g bicarbonate, 1.75 g potassium and 1.75 g chloride. Water intake was calculated daily before the morning feed, when the buckets were washed out and refilled.

3.2.1.4 Measurements

Each replicate lasted 18 days in the climate controlled rooms, and the animals were monitored and samples taken in a similar manner to the first experiments, but on a less intense schedule. Blood and urine were collected 4 times a day on days 2, 11, 15 and 18, and once daily on all other days. Heart rates, respiratory rates, panting scores and rectal temperatures were recorded 3 times daily; core temperatures were recorded every minute from the internal sensors.

3.2.1.5 Room conditions

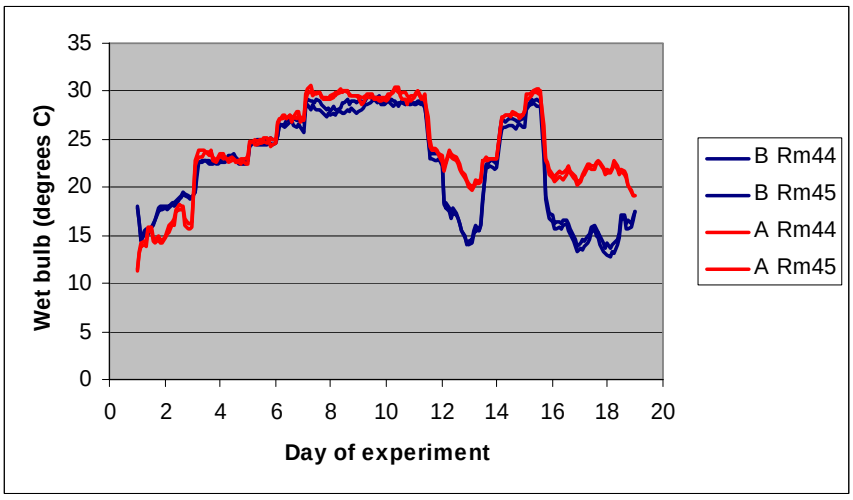
Temperature and humidity in the rooms followed a similar pattern to previous experiments, with several days warm-up then a heat period of 5 days set at 32 °C wet bulb. After two days at cooler temperatures, the animals were subjected to a second shorter heat insult, then monitored over the final cool down period. Temperature and humidity data loggers (T-TEC Datalogger, South Australia) were used to measure the wet bulb of each room during the replicates.

3.2.2 Results

3.2.2.1 Room conditions

During these replicates, the humidifiers were not as effective as in previous experiments, especially in the second replicate, and the wet bulb temperatures did not quite reach the set values. Figure 4 shows the mean wet bulb temperatures for the two rooms and the two replicates, as recorded on the Dataloggers.

Figure 4 Wet bulb temperature (°C) in rooms 44 and 45 during replicate experiments A and B



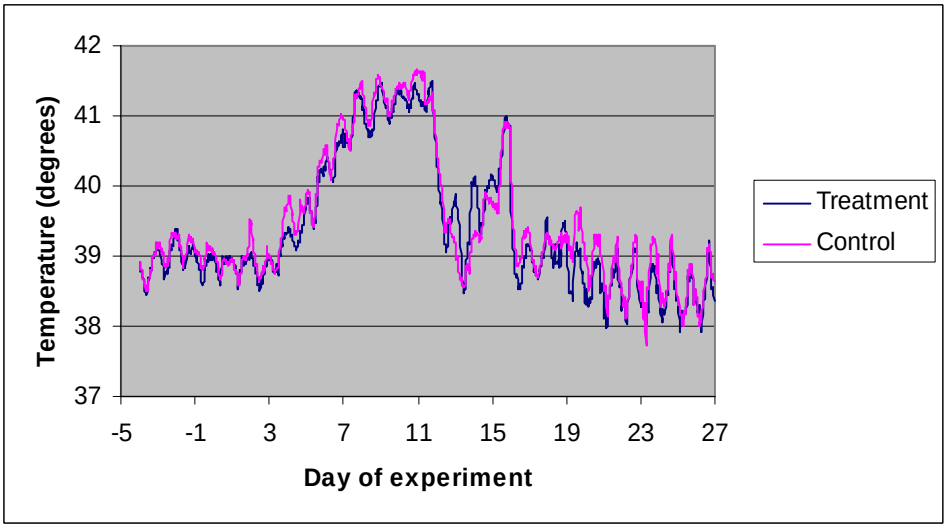
3.2.2.2 Clinical signs

All animals were compromised by the sustained period of high heat and humidity and exhibited clinical signs of heat stress, with high respiratory rates and elevated panting scores up to 4.5 indicating sustained open mouthed panting. In the first replicate, one control animal had to be removed from the rooms on welfare grounds early on day 11 when her core temperature rapidly rose over 43 °C, and she was unable to stand, and was severely depressed. Measurements from this animal are included only up to the time of removal. Intensive treatment of this animal with aggressive cooling, copious drenching with electrolyte supplemented water, and frequent assistance to stand eventually resulted in her complete recovery. Without intervention, it is highly probable that this animal would have died.

3.2.2.3 Core and rectal temperature

The rise in body temperatures was similar to that seen in previous experiments, with core temperatures rising above normal once conditions were over 30 °C wet bulb (Figure 5). There were no differences in core or rectal temperatures between control and treated animals.

Figure 5 Mean core body temperature of treatment and control animals in replicate experiments A and B.



3.2.2.4 Feed and water intake

The rise in core body temperature was associated with a decrease in feed intake, such that the animals were eating virtually nothing during the hottest period. The animals regained their appetite as the temperature of the rooms dropped, with a further small decrease in intake during the second heat insult. There was no significant difference between treated and control animals, and neither group was back to their full feed intake by the time they left the climate controlled rooms. However, treated animals did drink significantly more than control animals ($P<0.05$). There was no indication from the blood or urine samples that either group was dehydrated.

3.2.2.5 Body weight

Both groups of animals lost weight during the period within the climate controlled rooms. Although there is no significant difference between treated and control animals, there was a trend towards treatment animals losing less weight than control animals during the trial. On day 19, the last day in the climate-controlled rooms, the treatment animals had lost 5.1% body weight (mean) and control animals had lost 7.9% body weight (mean). At the next weighing, 7 days later, both groups had regained some weight to be the same percentage of their starting weight.

3.2.2.6 Respiratory rate, blood gas

Respiratory rates and panting scores of both groups rose during the hottest periods, and there were no differences between the groups. The blood gas measurements showed similar patterns to those recorded in the first experiments, with decreases in blood carbon dioxide and bicarbonate concentrations, maintenance of blood pH during the heat period, and a decrease in blood pH in the cooling period. There were no obvious serious detrimental effects of a short second heat insult on blood gas measurements, and no differences between the treatment and control animals.

The urine pH was significantly different between the groups. Urine from the control animals had a significantly lower pH that that from the treatment animals on days 13, 14, 15, 17 and 18 ($P<0.05$).

3.2.2.7 Electrolytes

Plasma concentrations of electrolytes were not different between the two groups. However, fractional excretion ratios (FER) of the sodium, potassium and chloride were significantly higher in the treatment than the control animals ($P<0.05$).

3.3 Shipboard trial of electrolyte supplement : Experiment 7

3.3.1 Background

The demonstration of a trend for a positive weight advantage in animals given electrolyte supplements in the drinking water prompted the consideration of an experiment with more animals. The best way to conduct such an experiment was to monitor animals on a commercial shipment to the Middle East, weighing them at the beginning and end of the voyage. There would be limited opportunity to monitor such animals closely, so no intensive physiological measurements could be taken.

3.3.2 Materials and Methods

3.3.2.1 Animals

Two groups of *Bos taurus* crossbred steers were sourced from southern Western Australia for the voyage. The prior origin of these cattle is unknown, but we were assured that these animals had spent the winter at these sites, and therefore were assumed to be winter-acclimatised, as they all had long hair coats.

3.3.2.2 Pen assignment

An area of the ship suitable for the transport of *Bos taurus* at that time of year was allocated for these experimental animals and good environmental symmetry was obtained in six pens, three on the starboard side (treatment) and three on the port side (control). The area was on an artificially ventilated closed deck, with doors that opened on to the main deck, which was an open deck. Thirty-nine steers were assigned into the treatment group and 41 into the control group. The allocation of animals to the pens was done randomly without mixing the cattle from different sources so there was one pen of Geraldton and two pens of Gingin sourced cattle for both treatment and control. The resulting stocking density was 1.57 and 1.55 m^2 per head for treatment and control respectively. The pen air turnover (PAT) of the experimental pens was 312 m/h, with a constant jetting of air from the forced ventilation. The air supply came from the vents in the walls of the ship, so animals in the outside pens were subject to increased air movement, compared to animals further from the vents, but conditions were similar for both sides of the ship, and therefore for both treatment and control pens.

The animals were loaded on the ship in Fremantle in late August 2003, and given three days acclimatisation to conditions before the experiment began on Day 1 and electrolytes were added to the water, over the course of the following 18 day voyage.

3.3.2.3 Feed and water

Feed was a standard shipper pellet, fed in approximately two equally divided feeds at 6-7am and 1-2pm daily. This was standard throughout the cattle decks on board. Each experimental pen had two feed troughs of equal size. It was not possible to keep an accurate record of feed intake by the groups because of the way feed was added.

Water was available *ad libitum* via two 1000L water tanks installed and located two decks above the experimental animals which gravity fed the two water troughs per pen. One tank supplied the treated animals and one the controls. The 1000L tanks were filled as necessary via the ships drinking water supply and electrolytes were added for the treatment group at a rate of 1.8g/L sodium bicarbonate and 3.5g/L potassium chloride, as per previous climate control room experiments. It was only possible to estimate the total water drunk by each group, and this could not be separated into pens.

3.3.2.4 Measurements

Environmental conditions were recorded at two symmetrically similar locations on the port and starboard side of the ship. Dry bulb temperature and relative humidity were recorded on Dataloggers (T-Tec) every 30 minutes, and two-hourly averages compared. Carbon dioxide (CO_2) and ammonia concentrations were recorded three times daily at 6am, 12pm and 6pm using the hand-held instruments as for room monitoring. Bridge dry bulb temperature, wet bulb temperature and wind speed and direction were

recorded by the ship's duty officer every four hours and seawater temperature was recorded daily at midday.

The cattle were weighed on Day 1 of the experiment (whilst in port in Port Headland) and on day 18 (the day of discharge). Morning feed was withheld on the day of weighing and treatment animals were weighed first on each occasion.

The respiratory rates and characters of three animals randomly chosen in each pen were recorded three times daily, and urine samples were collected on Day 8 (10 animals per group) and Day 16 (15 animals per group). Other daily measurements recorded were drinking water temperature and subjective bedding score of 1-5, where 1 is normal, a light cover of manure with or without sawdust, and 5 is deep wet manure above the level of the animals' dew claws.

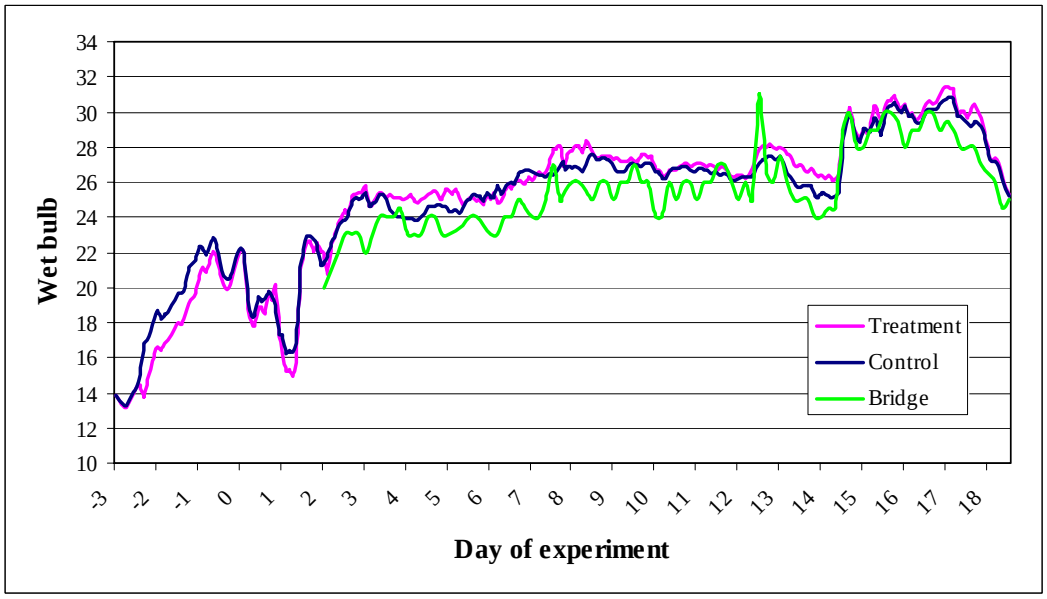
3.3.3 Results

3.3.3.1 Environmental conditions

The temperature and humidity gradually rose during the journey from Western Australia to the Middle East, as shown in Figure 6. The maximum wet bulb temperature recorded was 31.8 °C on the treatment side, on day 18, at which time the maximum wet bulb temperature for the control side was recorded at 31.1 °C. The conditions experienced by the treatment and control animals were similar. Conditions on other parts of the ship were more extreme. The peak in temperature on day 12 recorded on the bridge may have been an incorrect reading, or a transient spike in conditions that was not reflected on the deck when conditions were averaged for that two-hourly period.

Ammonia and CO_2 concentrations remained relatively low throughout the experiment. There were no differences between sites where measurements were taken. The maximum ammonia level recorded was 10ppm and CO_2 level 0.089%.

Figure 6: Recorded wet bulb temperature (°C) from Dataloggers and ship's bridge over the length of the voyage.



3.3.3.2 Clinical signs and observations

Respiratory rates did increase up to a mean of around 100 breaths per minute towards the end of the hottest period, but no open mouthed panting was observed in any animals during the experiment, and the animals did not suffer clinical heat stress in the way the animals held in the climate controlled rooms did.

3.3.3.3 Water and feed intake

The total amount of water consumed was markedly increased for the treatment group, but because our measurements were of total daily water consumption by the whole group, statistical analysis is not feasible. Water intakes were consistent with those calculated during climate controlled room experiments at Murdoch University where animals had individual water troughs. Animals seemed to have adequate access to water at all times as there was no observed evidence of dehydration in either group of animals. Behavioural observations did not show any dominant or aggressive behaviour around water troughs.

Feed intakes were very difficult to quantify because the animals were fed by hand, as the stockmen used buckets to add feed to the troughs, and so accurate measurements were not obtained. However, a very crude estimation was calculated by determining the total number of "buckets" fed to each group each day. This estimation revealed no difference between treatment and control groups. There was a slight increase in intakes towards the end of the voyage in both groups. This was attributable to the fact that animals were being offered more feed towards the end of the voyage.

3.3.3.4 Body weight

The cattle in this experiment gained weight over the 18 days of the voyage, consistent with their maintaining feed intake during that period. There was a 2.9 $\pm$ 1.7 % greater live weight gain in treated compared to control animals ($P< 0.001$). While heavier animals tended to have a lower percentage increase in weight for both treatment and control groups, the data was consistent with the treatment being equally effective at any weight.

3.3.3.5 Urine and bedding

Treatment animals had more alkaline urine than control animals at day 8 ($P=0.06$) and day 16 ($P<0.05$). The urine pH decreased from day 8 to 16 for both groups but this decrease was statistically significant only for the treatment animals ($P<0.05$).

Subjective bedding scores tended to rise higher for the treatment groups than for the control groups, but all pens were washed four times throughout the voyage and this alleviated any bedding problems.

3.3.4 Discussion

This experiment was the culmination of the electrolyte work undertaken at Murdoch University to assess the physiological effects of prolonged heat and humidity and to determine the efficacy of electrolyte replacement therapy. The difference in percent body weight change between the treatment and control animals was of a similar amount to that seen in the climate control room work testing electrolyte supplementation, and can largely be explained by the difference in amount drunk, as there were no apparent differences in feed intake. The *Bos taurus* tested on the voyage were not exposed to conditions as extreme as those in the climate controlled rooms, being transported on decks with good ventilation and at stocking densities consistent with recommendations of the heat stress risk management model (HS), and they did not develop clinical heat stress. There was no observed open mouthed panting and no apparent decrease in feed intake. The presence of a significant weight advantage in the shipped cattle, in the absence of clinical heat stress, indicates that electrolyte supplementation may also provide a weight advantage in other situations, such as long haul voyages at other times of the year, with different classes of animals, and in feed lotting of animals.

This electrolyte formulation cost approximately $4 per head, not including infrastructure for delivery. The treated animals tested here had a weight advantage of at least 11kg by the end of the experiment over the control animals. Figures quoted for the value of these extra kilograms vary, but even at $1/kg, there is an economic advantage.

While only *Bos taurus* were tested in this experiment, it is possible that *Bos indicus* supplemented with electrolytes may similarly have a weight advantage at the end of the voyage, even though they may not suffer heat stress. The *Bos indicus* on the particular voyage described were on decks experiencing more extreme conditions up to 34 °C wet bulb, and they did show clinical heat stress with open-mouthed panting. While the heat stress threshold of *Bos indicus* is higher than that of *Bos taurus*, their responses to heat stress appeared from our original work to be essentially the same once that threshold had been passed, and they may also benefit from supplementation of electrolytes if they do suffer heat stress.

The difference in urine pH implies that there was also an effect of the supplement on acid-base balance, but further interpretation of this result is difficult, given the limited measurements possible. The effect of increased drinking on increased urine output and deteriorating bedding was alleviated by the regular washing of the decks, but could present a problem if washing was not as frequent.

3.4 The physiology of heat stress in sheep : Experiment 5

3.4.1 Materials and Methods

3.4.1.1 Animals

Sheep typical of the live export trade were sourced from southern Western Australia. Twelve three-year old Merino wethers and six two-year old Awassi rams were randomly allocated to be exposed to the heat and humidity within the climate controlled rooms, and a further six Merino wethers were allocated to a control group, to be individually penned in another room and not exposed to heat. All groups averaged just over 53 kg body weight.

3.4.1.2 Preparation

The animals were handled and introduced to pelletted feed in the two weeks before the experiment. They were surgically implanted with temperature loggers and telemeters into the right side of the abdominal cavity ten days before the experiment, for continuous monitoring of core body temperature, using similar techniques and technology as for the cattle.

They were held off feed and water for 15 hours, weighed, and then randomly allocated to individual pens within the climate controlled rooms.

3.4.1.3 Feed and water

The sheep were fed standard sheep export pellets at 2.25% body weight (as fed), divided into two equal feeds offered at 7 am and 12 noon. The pellet composition is presented in appendix 3.

Water was available *ad libitum* in 2.5 L buckets, which were topped up manually as required. Feed residues were measured and weighed before the morning feed, and the daily water intake calculated at that same time.

3.4.1.4 Room conditions

After the animals entered the pens, the heat and humidity were gradually increased over several days, and the animals were exposed to three days set at over 30 °C wet bulb. An electrical storm caused equipment failure, which meant that this period was not as long as intended, and after 24 hours at ambient conditions, the heat was again increased, with the animals subjected to four more hot days around 30 °C wet bulb. The animals were then monitored for several more days as the rooms were cooled down.

Temperature, humidity, carbon dioxide and ammonia were monitored daily within the rooms, using hand held instruments. Dataloggers were also used in the two climate controlled rooms.

3.4.1.5 Measurements

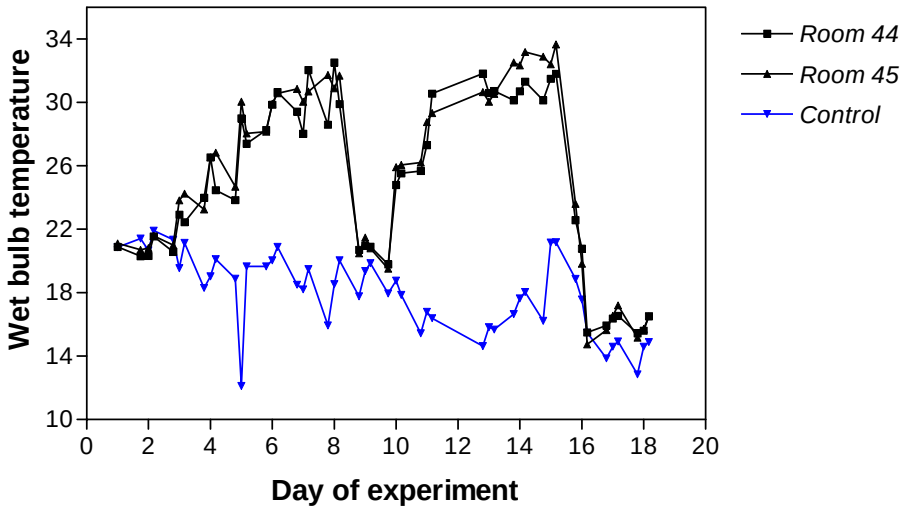
Heart rate and rectal temperature were measured once daily, and respiratory rates recorded three times daily throughout, with blood and urine samples taken on strategic days. Blood was collected from the jugular vein by venipuncture, and urine was collected by the use of harnesses. Blood gas analysis, plasma and urine electrolyte concentrations, packed cell volume and plasma protein, and complete blood count, were assessed on each sample.

3.4.2 Results

3.4.2.1 Room Conditions

The wet bulb temperatures recorded by the Dataloggers in the rooms are shown in Figure 7. In both rooms, wet bulb temperatures of over 30 °C were achieved for two sustained periods. Concentrations of ammonia (below 14 ppm) and carbon dioxide (range 0.07 – 0.1 %) remained low, similar to reported values from ships. Animals in the control room were not exposed to excessive heat or humidity.

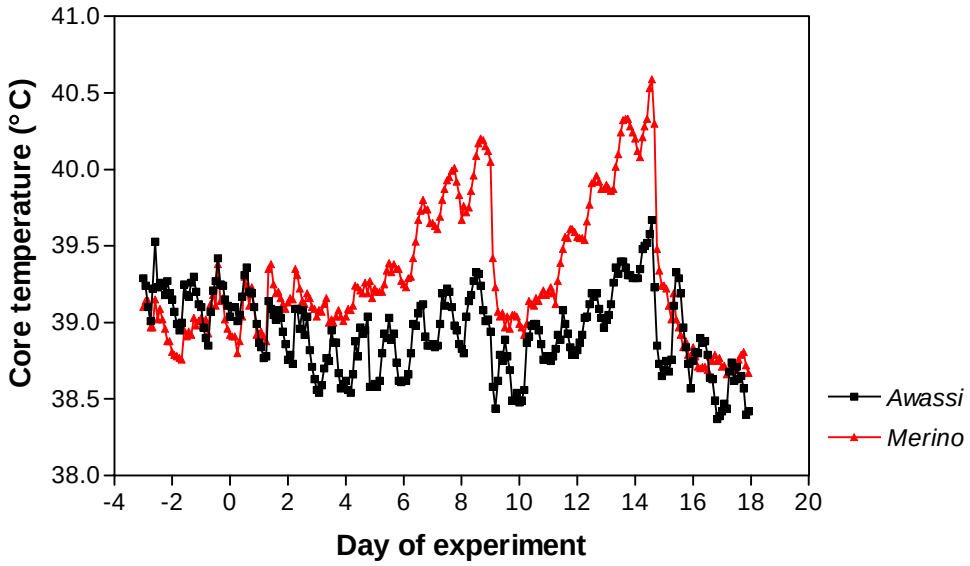
Figure 7. Wet bulb temperatures of the climate-controlled rooms 44 and 45, and the control room, for the duration of the sheep experiment, calculated from dry bulb temperature and relative humidity taken using a hand held Testo 445 instrument.



3.4.2.2 Core and rectal temperature

Core temperatures of the sheep rose with continued exposure to the hot conditions, but did not rise as drastically as the cattle (Figure 8). There was continued elevation of core temperature of the Merino wethers once the rooms were at 30 °C wet bulb, which would correspond to their heat stress threshold. The core temperatures of the Awassi rams did not rise above 39.5 °C until the end of the second hottest period, when the temperature was nearing 32 °C wet bulb, indicating a higher heat stress threshold for these animals.

Figure 8. Core body temperatures (two-hourly averages) of Awassi and Merino sheep for the duration of the experiment. The hottest periods were days 6-9 and days 11-15.



3.4.2.3 Clinical signs

As the exposure to the heat progressed, there were some Merino wethers with very high respiratory rates, and open mouthed panting, considered to be signs of heat stress. None of the Awassi rams progressed to open mouthed panting. The majority of the sheep appeared alert and appetent throughout the experiment.

3.4.2.4 Feed and water intake

Feed intake of most sheep remained good throughout. The Awassi rams ate everything offered very quickly, and their loss in body weight over the period within the rooms indicated that they should have been fed at a greater rate than the Merino wethers. They were in leaner body condition at the start of the trial, and as rams, may have a higher metabolic rate. Most Merino wethers ate well, whether it was hot or not, and finished the experiment slightly heavier than they started. Several Merino wethers in both the hot and control groups ate poorly at the start, but towards the end of the experiment, all the control Merino wethers were eating everything they were offered, while a few of the hot Merinos continued to be poor feeders. Water intake of both groups of Merino wethers did not change, and was not different, while the Awassi rams increased their water intake during the hotter periods.

3.4.2.5 Respiratory rate, blood gas

Respiratory rates were the most useful indication that animals were responding to the increased heat and humidity, with some Hot Merino wethers breathing more than 200 breaths per minute. The increased respiratory rate of the sheep during the periods of high heat and humidity did result in a drop in blood carbon dioxide with an associated drop in blood bicarbonate, and these changes were more marked in the Merino wethers. Blood bicarbonate concentrations rapidly returned to initial values once the heat was turned off, unlike the situation seen in the cattle. Blood pH of the Awassi and Merino animals was different at the start of the experiment, but after that all groups (including the control Merino wethers) followed a similar pattern, without the dramatic drop in blood pH seen in the cattle.

Urine pH of the hot Merino wethers followed the expected pattern of increasing during the heat periods, and decreasing when the heat was turned off, indicating renal compensation for the respiratory alkalosis. The Awassi rams did not follow this pattern and their urine pH was more variable.

3.4.2.6 Electrolytes

The plasma concentrations of electrolytes remained within normal ranges throughout, with no changes attributable to the exposure to heat, and there were no marked changes in urine electrolytes.

3.4.3 Discussion

The sheep did not appear as affected by the hot and humid conditions as the cattle did. This may have been because they did not experience as sustained a period of high heat and humidity over their heat stress threshold as the *Bos taurus* did. However, even those animals that were panting very fast with open mouths did not have as rapid or high a rise in core temperature as the *Bos taurus*, nor did they experience perturbations in blood gas measurements to the same extent as the cattle. This would seem to indicate a different degree of dead space ventilation in the sheep, where very rapid shallow panting does not result in blowing off of carbon dioxide to the same extent as happened in the cattle. It was not until the end of the second hot period that there were marked deceases in blood carbon dioxide and bicarbonate. Of interest was the diurnal pattern in respiratory rate shown by all sheep, which followed diurnal changes in core temperature, whether the animals were exposed to heat or not. The animals were cooler and panted less early in the morning, compared to the afternoon.

The other difference to the cattle experiments was the retention of appetite by most sheep, which would help in maintaining a supply of electrolytes and bicarbonate, helping with homeostasis. Several Merino wethers ate poorly, regardless of the temperature, while the Awassi rams all ate everything and would appear to have higher maintenance requirements than the wethers. Water intake of the Merino wethers was not different between animals exposed to the heat and the control animals, while the Awassi rams increased their water intake in the hotter periods, and their urine specific gravity decreased indicating that much of the increased intake was excreted via the kidney and not utilised for evaporative cooling. It was unexpected that the Awassi animals, considered the more desert adapted breed, should drink and therefore urinate so much.

There were no large alterations in blood electrolyte concentrations attributable to the effects of the heat. This may be because the animals ate so well throughout. High renal losses seen in cattle associated with excessive drinking, or with loss of bicarbonate, did not seem to be an issue in these sheep.

The decrease in blood bicarbonate during the period of exposure to high wet bulb temperatures indicates that inclusion of bicarbonate in sheep feeds may have a place, in ensuring sheep exposed to high heat and humidity maintain their body bicarbonate for buffering. The possible effects on palatability of the ration will be an important consideration in choosing an inclusion rate for sodium bicarbonate.

From this experiment, there seems limited place for the supplementation of electrolytes in water to shipped sheep exposed to similar conditions, as long as they are eating well. The issue of shy feeders seems very important, regardless of temperature, and we recommend continued work on detection and management of such animals, before they are shipped, and during transport. Supplementation of drinking water with products containing glucose and electrolytes may be of some use for animals that are not eating.

3.5 Feeding experiment, two different pelleted feeds for cattle : Experiment 8

3.5.1 Background

Type of feed may influence behaviour and physiology of animals exposed to heat. There has been debate about whether the heat of digestion as animals ferment diets high in poorly digestible roughage has a greater contribution to body heat than metabolic heat from high energy diets (West, 1999; Mader et al., 2002 a and b). The following experiment was conducted to compare two diets similar in metabolisable energy, but containing different amounts of different quality roughage. The experiment also provided the first opportunity to extend the temperature sensing technology employed in monitoring core body temperature to monitoring rumen temperature.

3.5.2 Materials and Methods

3.5.2.1 Animals and preparation

Six *Bos taurus* heifers (Angus cross) ranging in weight from 324-430 kg were selected from the Murdoch University herd. Nine days before the commencement of the experiment, temperature loggers were surgically implanted into the right abdominal. The day before the experiment started, a rumen capsule (Elanco AH0315 Rumensin Capsule), fitted with temperature telemeters and loggers, was passed orally into the rumen of each animal. The heifers were randomly allocated to one of two groups.

3.5.2.2 Feed

The animals were given seven days gradual introduction onto pelleted feed. Once the experiment started, the two diets used were a standard straw-based shipper pellet and a hay-based pellet, offered at 2.5% body weight (as fed). The content and analysis of the pellets in shown in appendix 4.

On Day 1, each group of three heifers was penned together for three days introduction to the diet, and the animals fed one or other of the diets. The total feed for the group for the day was given in two equal feeds at 7am and 1 pm. On Day 4, the animals were moved into individual pens in the climate controlled rooms, where they continued to receive the same diet and feeding regime. Allocation to pens in the rooms was organized so that each room had one or two animals on each diet. The animals spent nine days in the climate controlled rooms, five days at ambient conditions and four days exposed to higher heat, between 22 and 26 °C wet bulb.

On day 13 the animals were removed from the rooms, and the groups swapped diets, and the protocol was repeated. Water was available *ad libitum*. Feed residues were collected daily before the morning feed, at which times daily water intake was also calculated.

3.5.2.3 Measurements

Core and rumen temperature were recorded via the telemeters and loggers throughout. Animals were weighed on Days 1, 4, 8 and 13 after 15 hours feed curfew. Blood and urine samples were collected on Days 1, 8 and 13. During the exposure to heat, the heart rate, respiratory rate, and rectal temperature were recorded daily at 7am, 1pm, and 5pm.

3.5.3 Results

3.5.3.1 Room conditions

During both replicates there was no difference between rooms. On day 10 during the first replicate the humidifier in the first climate control room malfunctioned and as a result both humidifiers were shut down and rooms relied on their heater to reach a maximum temperature of 22 °C WB. Excessive ambient temperatures with high humidity on days 10 and 11 of the second replicate meant that there were differences in environmental conditions between the replicates.

3.5.3.2 Feed and water intake, body weight

While there were some differences in feed intake between the replicates due to the different conditions, there was little difference in feed intake between the two diets, except for a significantly lower intake of the straw diet at the end of the hottest period in replicate 2. For both diets, there was a significant reduction in feed intake once core temperature was above 39 °C ($P=0.003$). Animals on the hay diet during the second replicate drank significantly more water compared to straw diet and both diets in experiment 1 ($P<0.05$).

Animals on the straw diet during the first replicate had a significantly greater end weight ($P<0.05$) compared to animals on the hay diet, which cannot be explained by either feed or water intake. In replicate 2, both groups performed the same and lost a small amount of weight.

3.5.3.3 Temperature – rectal, core, rumen

In replicate 1, the mean core temperature of animals receiving the hay diet was significantly higher for the last two hot days than the animals on the straw diet, but in replicate 2 there were no differences between the diets. Mean core temperatures in replicate 2 were higher than for replicate 1 once the heat was turned

on, unexpected because the wet bulb temperatures in replicate 2 were the same as those for replicate 1 for the first two days.

Rumen temperatures fluctuated, dropping markedly when the animals drank. Maximum rumen temperatures were generally 1.5 to 2 °C hotter than the maximum core temperatures, regardless of diet or replicate. The maximum rumen temperature for animals eating the straw diet in replicate 2 was higher than that for replicate 1 on days 9 and 10, even though the environmental conditions were similar. There was no apparent effect of diet type on maximum rumen temperature.

3.5.3.4 Physical parameters

The animals were not considered heat stressed during this experiment, although the respiratory rates did increase during the hot periods, with associated changes in blood bicarbonate concentrations and pCO_2. There were no differences between diets in any of the measured parameters.

3.5.4 Discussion

While the technology worked well to record rumen temperature, there were no consistent differences in temperatures between diets. Core temperatures were similarly difficult to interpret due to the differences in the ambient conditions in replicates 1 and 2. In the first replicate the animals on the hay diet had a significantly higher mean core body temperature than those on the straw diet for the last two days of that replicate, perhaps indicating that animals on the hay diet were producing more heat from rumen digestion compared to the straw diet, although this was not reflected in maximum rumen temperatures. The lower core temperature of the animals on the straw based diet corresponded to the same animals having a significant improvement in weight gain compared to those animals on the hay diet in the first replicate. At this stage, the reason for the differences in core temperature and body weight cannot be determined, and with the small group size may simply indicate between animal variability in response, rather than an effect of diet.

There appears to be interaction between environmental temperature and both core and rumen temperatures, and also the possible carry-over effect of the first heat exposure into the second replicate, with rapid increases in core temperatures in replicate 2 even though the environmental conditions were similar between the replicates for the first few days.

The wet bulb temperatures to which these animals were exposed did cause some reduction in feed intake, but there were no differences between the diets at these temperatures.

4 SUCCESS IN ACHIEVING OBJECTIVES

All objectives or modified objectives have been met and milestone reports delivered in a timely manner.

The specific objectives for LIVE.209 were:

1. Establish an animal house facility suited to the study of heat stress;
2. To use the facility to better define the physiology of heat stress in cattle and sheep;
3. Develop heat stress therapies for use on live export ships.

The climate controlled rooms have been operational for two years, and used intensively for the experiments described here, as well as for experiments on ammonia production by cattle. Work is continuing to have the rooms again operating at their optimum.

Three cattle experiments and one larger sheep experiment have been conducted to define the physiological responses of animals to sustained periods of high heat and humidity.

Recommendations have been developed from the results of the physiological experiments.

Stage One specific objectives were:

1. Define the physiological response of both sheep and cattle to high heat load (continual temperature and humidity over time) as can be experienced in clinical heat stress on livestock vessels.
2. Compare the difference in physiological response between high grade B*os indicus* and high grade *Bos taurus* cattle.
3. Compare the difference in physiological response between selected sheep breeds (merino and British breed versus fat tailed sheep).
4. Based on the findings of objectives 1, 2 and 3, provide possible therapies and conduct experimental evaluation of these therapies following consultation with MLA.

Objectives 1 and 2 were completed in 2002, objective 3 was held over to LIVE.209B. Consultation with MLA in November 2002 led to specific objectives of LIVE.209B.

Stage 2 LIVE.209B Objectives were:

1. By 31 May 2003, define the physiological response of Merino and fat-tailed sheep to clinical heat stress as seen on long haul voyages to the Middle East.
2. By 31 July 2003, determine the animal performance and commercial effects of electrolyte replacement therapy in *Bos taurus* cattle subjected to clinical heat stress as seen on long haul voyages to the Middle East.
3. By 30 September 2003, determine the animal performance and commercial effects of electrolyte replacement therapy in Merino sheep subjected to clinical heat stress as seen on long haul voyages to the Middle East.
4. By 30 November 2003, determine the animal performance and commercial effects of replacing normal industry ship pellets with a high roughage pellet in *Bos taurus* cattle subjected to clinical heat stress as seen on long haul voyages to the Middle East.

The sheep experiment conducted in 2003 defined the responses of those animals to sustained periods of high heat and humidity, and resulted in a recommendation not to test electrolyte supplements for sheep subjected to heat stress. Objective 2 was conducted with two animal house experiments and a shipboard experiment. Objective 4 was conducted in late 2003.

5 IMPACT ON MEAT AND LIVESTOCK INDUSTRY

The experiments conducted in LIVE.209 have attempted to answer the question of whether electrolyte supplements should be recommended as part of normal practice for cattle and sheep shipped on the long haul voyages. A scientific approach to determining measured deficits in animals exposed to sustained periods of high heat and humidity allowed the development of an electrolyte supplement. Animal house and shipboard trials have shown positive responses in body weight, and postulated improvements in acid-base homeostasis with this supplement for *Bos taurus*. From this work, definite recommendations can be made for exporters, for both animal health and economic benefits.

Information has also been generated on the responses of sheep to high heat and humidity, with recommendations on the use of electrolyte supplements during shipping, and the suggestion of future work looking at shy feeding in sheep.

Additional useful information regarding the “heat stress threshold” has been gathered for *Bos taurus*, *Bos indicus* and Merino sheep, which can be used in risk management calculations. Techniques and technology for intensively monitoring animal temperature over long periods have been proven through use in a number of experiments.

6 CONCLUSIONS AND RECOMMENDATIONS

The experiments conducted and reported as LIVE.209 have answered some questions on the electrolyte changes measurable in cattle and sheep subjected to sustained periods of high heat and humidity. However, recommendations as a result of these experiments are limited by the confines of the testing performed. There were sufficient changes in cattle to warrant the testing of an electrolyte supplement in *Bos taurus*, with subsequent positive results on health and body weight. No conclusions can be made about the usefulness or otherwise of supplements not tested, or different concentrations of supplements, or the use of supplements in classes of animals not tested. The testing of other supplements, different concentrations, and other classes of animals would be of use, to determine whether the same positive results can be obtained. For instance, there may be weight advantages in *Bos indicus*, or in animals in feedlots or on short haul voyages. Similarly the lack of an indication for an electrolyte supplement for sheep under hot humid conditions only holds true for those animals which continue to eat, as the sheep examined here did. The usefulness of a supplement for sheep that do not have continual access to feed and water, such as when transported by truck, is unknown, as is the usefulness of water supplements containing an energy source for animals that do not eat. Finally, the lack of significant conclusions from the feeding of different pelleted feeds should not be interpreted to indicate there can be no advantages of different feed types.

Recommendations are:

1. Animals transported by live export vessel should have sufficient access at all times to drinking water.

Rationale: cattle particularly increase the amount they drink when exposed to high heat and humidity, in many cases drinking more than 10% of their body weight daily, e.g. over 40 litres per head per day for a 400 kilogram heifer.

2. *Bos taurus* shipped on long haul voyages subjected to periods of high heat and humidity should receive electrolytes in the drinking water. These electrolytes should be of an appropriate dose, which would appear to be more concentrated than many commercial supplements, but should not be over-concentrated, particularly in situations where there is any likelihood that animals will be unable to have free access for drinking.

Rationale: supplementation of sodium, potassium and bicarbonate will replenish those electrolytes measured to decrease during periods of high heat and humidity, and supplemented animals have improved body weight and buffering capacity compared to unsupplemented animals.

3. Further work should be conducted on *Bos taurus* to determine the repeatability of the weight advantage in supplemented animals, and investigate the nature of this weight advantage.

Rationale: The use of electrolyte supplements requires an outlay both in the cost of the supplements and in the infrastructure for the correct delivery of the supplements. Therefore there should be a demonstration that this result is repeatable. It appears from the increased fluid consumption by the supplemented animals that the weight advantage may be due to increased uptake of fluid, and further investigation is required to determine the physiology of the weight increase.

4. Current methods of electrolyte supplementation should be reviewed and recommendations developed for accurate supply.

Rationale: Currently electrolyte supplements are given in a variety of ways, some of which are not precise, and may mean the animals receive incorrect doses, which maybe wasteful or even harmful to the animals. For instance, hand dispensing of electrolytes into water troughs may mean that some animals receive a concentrated solution, and others miss out on any supplement. If electrolyte supplements are to be used, an optimal method, dose and formulation should be determined.

5. *Bos indicus* may not need electrolyte supplements if the conditions are not over their heat stress threshold, but the response of such animals to the supplement should be tested under real conditions experienced by such animals being transported to the Middle East during the northern summer.

Rationale: *Bos indicus* have a higher heat stress threshold than *Bos taurus*, and may not experience heat stress when transported to the Middle East on long haul voyages. However, given the significant weight advantage for the *Bos taurus* in the absence of clinical heat stress, it may still be of economic advantage to supplement the *Bos indicus*, hoping for a similar weight response. Additionally, there is evidence that *Bos indicus* are subject to more extreme conditions than were imposed in the climate controlled rooms, and sustained periods over 34 °C wet bulb will induce clinical heat stress in *Bos indicus*, and therefore the need to replace electrolyte deficits induced by the response to the heat.

6. There is no indication for electrolyte supplementation of sheep that are eating and drinking, even if they are subject to high heat and humidity. The usefulness of supplements for sheep subject to other stressors, and in situations where they are not eating should be investigated.

Rationale: There were no sustained deficits in electrolytes measured in the sheep studied here, most of which continued to eat well, whether it was hot or not. The blood gas response of the sheep to rapid panting under the test conditions was not as pronounced as that of the cattle, and quickly returned to normal after the heat insult. Those sheep that did not eat whatever the temperature may benefit from water based supplements that contain some energy, to maintain both body and rumen functions.

7. Further investigation of feed types, formulations and timing of feeding should be conducted to determine the optimum feed for animals subject to high heat and humidity.

Rationale: All animals tested showed diurnal fluctuations in core body temperatures, despite there being little variation in room conditions. Time of feeding may be altered to fit better with these fluctuations, which may improve feed intake, and/or limit detrimental effects of digestive heat on overall heat load of the animals. The one feed experiment conducted did not examine extremes of diet, such as the difference in heat generation by long roughage such as chaff or hay, compared to high concentrate grain-based diets. These feeding experiments may have more relevance to the feedlot industry, where there may be scope to alter the type of feed supplied depending on the environmental conditions.

7 BIBLIOGRAPHY

Adrogue, H.J. and Madias, N.E. (1998). Management of life-threatening acid-base disorders. Second of two parts. New England J. Med. 338: 107-111.

Alliance Consulting and Management (2001). Use of electrolytes to alleviate stress: Desk Top Study. LIVE.104. Meat and Livestock Australia Ltd.

Baker, C.C., Coppock, C.E., Lanham, J.K., Nave, D.H., Labore, J.M., Brasington, C.F., and Stermer, R.A. (1988). Chilled drinking water effects on lactating Holstein cows in summer. J Dairy Sci 71(10): 2699-708

Beede, D.K. and Collier, R.J. (1986). Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim. Sci. 62: 543-554.

Bligh, J.A. (1985). Temperature regulation. Chapter 8 in “Stress Physiology in Livestock Volume I Basic Principles”. Ed M.K. Yousef. CRC Press USA. Pp75-96.

Escabosa, A., Coppock, C.E., Rowe, L.D. Jnr, Jenkins, W.L. and Gates, C.E. (1984). Effects of sodium bicarbonate and calcium chloride on physiological responses of lactating dairy cows in hot weather. J. Dairy Sci. 67: 574-584.

Hales, J.R.S. (1976). Interactions between respiratory and thermoregulatory systems of domestic animals in hot environments. Progress in Biometerology. Ser.B. Animal Biometerology. 1(1): 123-131.

Haydon, K.D., West, J.W. and McCarter, M.N. (1990). Effect of Dietary Electrolyte Balance on Performance and Blood Parameters of Growing-Finishing Swine Fed in High Ambient Temperatures. J. Anim. Sci. 68: 2400-06.

Johnson, K.G. (1970). Sweating rate and electrolyte content of skin secretions of Bos taurus and Bos indicus cross-bred cows. J. Agric. Sci. Camb. 75: 397-402.

Johnson, H.D. (1985). Physiological responses and productivity of cattle. Chapter 1 in “Stress Physiology in Livestock Volume II Ungulates”. Ed M.K. Yousef. CRC Press USA. Pp 3-24.

Koelkebeck, K.W. and Odom, T.W. (1994). Laying hen responses to acute heat stress and carbon dioxide supplementation. 1. Blood gas changes and plasma lactate accumulation. Comparative Biochemistry and Physiology A 107: 603-606.

MAMIC Pty Ltd (2000). Investigation of ventilation efficiency on livestock vessels. Report on Voyage 2. Project SBMR.002. Report No. 3472.02/00. Registration No. 2054. Meat and Livestock Australia.

Mader, T.L., Gaughan, J.B. and Young, B.A. (2002a). Feedlot diet roughage level for Hereford cattle exposed to excessive heat load. The Professional Animal Scientist. 15: 53-62.

Mader T.L., Holt S.M., Hahn G.L., Davis M.S. and Spiers D.E. (2002b). Feeding strategies for managing heat load in feedlot cattle. J.Anim.Sc. 80: 2373-82.

Mitchell, J.H., Wildenthal, K. and Johnson, R.L. (Jnr) (1972). The effects of acid-base disturbances on cardiovascular and pulmonary function. Kidney International 1:375-389.

NRC (2000). Nutrient Requirements of Beef Cattle. 7th edition. Washington: National Academy Press.

Robertshaw, D. (1985). Heat loss of cattle. Chapter 6 in “Stress Physiology in Livestock Volume I Basic Principles”. Ed M.K. Yousef. CRC Press USA. Pp55-66.

Sanchez, W.K., Beede, D.K. and Cornell, J.A. (1994a). Interactions of sodium, potassium, and chloride on lactation, acid-base status, and mineral concentrations. Dairy Sci. 77: 1661-1675.

Sanchez, W.K., McGuire, M.A. and Beede, D.K. (1994b). Macromineral nutrition by heat stress interactions in dairy cattle: review and original research. J. Dairy Sci. 77: 2051-2079.

Schneider, P.L., Beede, D.K., Wilcox, C.J. and Collier, R.J. (1984). Influence of dietary sodium and potassium bicarbonate and total potassium on heat-stressed lactating dairy cows. J. Dairy Sci. 67: 2546-2553.

Sparke, E.J., Young, B.A., Gaughan, J.B., Holt, S.M., and Goodwin, P.J. (2001). Heat load in feedlot cattle. Project Number FLOT.307, 308, 309. Meat and Livestock Australia Ltd.

Stermer, R.A., Brasington, C.F., Coppock, C.E., Lanham, J.K., and Milam, K.Z. (1986). Effect of drinking water temperature on heat stress of dairy cows. J Dairy Sci 69(2): 546-51.

Stokka, G.L., Smith, J., Kuhl, G. and Nichols, D. (1996). Heat stress in feedlot cattle. Compend. Contin. Ed. Pract. Vet. 18: S296-302.

Thwaites, C.J. (1985). Physiological responses and productivity in sheep. Chapter 2 in “Stress Physiology in Livestock Volume II Ungulates”. Ed M.K. Yousef. CRC Press USA. Pp 25-38.

West, J.W., Coppock, C.E., Milam, K.Z., Nave, D.H., Labore, J.M. and Rowe, L.D. (1987). Potassium carbonate as a potassium source and dietary buffer for lactating Holstein cows during hot weather. J. Dairy Sci. 70: 309-320.

West, J.W., Mullinix, B.G. and Sandifer, T.G. (1991). Changing Dietary Electrolyte Balance for Dairy Cows in Cool and Hot Environments. J. Dairy Sci. 74: 1662-74.

West, J.W., Haydon, K.D., Mullinix, B.G. and Sandifer, T.G. (1992). Dietary Cation-Anion balance and Cation source effects on production and Acid-Base status of heat-stressed cows. J. Dairy Sci.75: 2776-86.

West, J.W. (1999). Nutritional strategies for managing the heat stressed dairy cow. J. Anim. Sci. 77 Suppl.2/J:21-35.

Wilks, D.L., Coppock, C.E., Lanham, J.K., Brooks, K.N., Baker, C.C., Bryson, W.L., Elmore, R.G., and Stermer, R.A. (1990). Responses of lactating Holstein cows to chilled drinking water in high ambient temperatures. J Dairy Sci 73(4): 1091-9.

Whitehair, K.J., Haskins, S.C., Whitehair, J.G. and Pascoe, P.J. (1995). Clinical applications of quantitative acid-base chemistry. J. Vet. Int. Med. 9:1-11.

8 APPENDICES

Appendix 1

Feed composition Shipper pellets Macco's 501

Composition per kg of dry matter:

Moisture	9.7%
Dry matter	90.3%
Ash	7.6%
Crude protein (N x 6.25)	11.9% of dry matter
Acid Detergent Fibre	19.7% of dry matter
Neutral Detergent Fibre	39.9% of dry matter
Metabolisable energy	8.6 MJ

Mineral content of the ration:

Phosphorus	0.184	%
Potassium	0.486	%
Sulphur	0.119	%
Sodium	0.065	%
Calcium	1.404	%
Magnesium	0.194	%
Chloride	0.221	%
Copper	3.05	mg/kg
Zinc	18.23	mg/kg
Manganese	43.2	mg/kg
Iron	323.9	mg/kg
Nitrate	46.4	mg/kg
Boron	7.13	mg/kg

Appendix 2

Composition of shipper pellets for electrolyte supplement experiments

Control pellets:

Moisture	9.8%
Dry matter	91.1 %
Ash	6.2%
Crude Protein (N x 6.25)	11.0% of dry matter
Acid detergent fibre	28.7% of dry matter
Digestibility	60.1% of digestible DM
Metabolisable energy	8.6 MJ/kg DM

Mineral content:

Phosphorus	0.207	%
Potassium	0.862	%
Sulphur	0.146	%
Sodium	0.259	%
Calcium	1.631	%
Magnesium	0.207	%
Chloride	0.613	%
Copper	5.51	mg/kg
Zinc	27.00	mg/kg
Manganese	83.3	mg/kg

Iron	332.0	mg/kg
Nitrate	38.9	mg/kg
Boron	8.51	mg/kg

Treatment pellets:

Moisture	8.4%
Dry matter	91.6 %
Ash	6.8%
Crude Protein (N x 6.25)	10.6% of dry matter
Acid detergent fibre	29.3% of dry matter
Digestibility	60.3% of digestible DM
Metabolisable energy	8.6 MJ/kg DM

Mineral content:

Phosphorus	0.193	%
Potassium	0.856	%
Sulphur	0.133	%
Sodium	0.602	%
Calcium	1.691	%
Magnesium	0.204	%
Chloride	0.652	%
Copper	5.50	mg/kg
Zinc	25.48	mg/kg
Manganese	81.2	mg/kg
Iron	301.4	mg/kg
Nitrate	43.2	mg/kg
Boron	8.46	mg/kg

Appendix 3

Composition of sheep export pellets

Moisture	9.5%
Dry matter	90.5 %
Ash	7.8%
Crude Protein (N x 6.25)	11.6% of dry matter
Acid detergent fibre	27.0% of dry matter
Digestibility	61.0% of digestible DM
Metabolisable energy	8.7 MJ/kg DM

Mineral content:

Phosphorus	0.150	%
Potassium	0.704	%
Sulphur	0.150	%
Sodium	0.090	%
Calcium	1.030	%
Magnesium	0.170	%
Chloride	0.367	%
Copper	4.24	mg/kg
Zinc	21.20	mg/kg
Manganese	51.0	mg/kg
Iron	844.8	mg/kg
Nitrate	57.2	mg/kg
Boron	10.59	mg/kg

Appendix 4

Composition of pelleted feeds for cattle feeding experiment

	Hay-based pellet	Straw-based pellet
Hay (%)	80 (60% IVD)	-
Straw (%)	-	40 (38% IVD)
Barley (%)	-	26
Lupin (%)	18	32
Lime (%)	2	2
Dry matter (%)	91.6	90.8
Crude protein (% of DM)	11	12.4
Dry matter digestibility	64.6	63.6
Metabolisable Energy (MJ/kg DM)	9.3	9.1