Prolonged and continuous heat stress in cattle: Physiology, welfare, and electrolyte and nutritional interventions.

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This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

2005
Declaration

I declare that this is my own account of my research and contains as its main content work which has not been submitted for a degree at any tertiary institution.

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David Thomas Beatty
Abstract

The live export of cattle is an important industry for Australia. Concerns have arisen about animal welfare and in particular heat stress which may cause production losses and death. Cattle shipped live to the Middle East from a southern Australian winter can face continuous and prolonged periods of high heat and humidity as they cross the equator and arrive into a northern hemisphere summer, leading to heat stress and excessive heat load. Some live animal exporters treat heat stressed cattle with electrolyte supplements, but no scientific data exists as to whether this is beneficial to cattle in these unique environmental conditions.

In response to industry’s concerns, the experiments described here monitored the physiological responses of *Bos taurus* and *Bos indicus* to conditions similar to those experienced by cattle being shipped from southern Australian to the Middle East. Initial experiments were conducted in climate controlled rooms at Murdoch University where intensive monitoring was possible. In the *Bos taurus*, increases in core body temperature, reductions in feed intake, and increased water intake were measured. There were also changes in blood gas variables consistent with the observed panting causing a compensated respiratory alkalosis. Following the heating period, there were decreases in blood and urinary pH. *Bos indicus* showed similar responses to the heat, but the changes were less pronounced at the temperatures tested. A pair feeding experiment was conducted to separate the effects of heat from the reductions in feed intake, and this indicated that the major measured effects were due to the responses to heat. On the basis of the measured responses, an electrolyte supplement was formulated and tested on *Bos taurus*, in the climate controlled rooms, and then on a commercial live export vessel. Results from these experiments indicated improved buffering capacity and a weight advantage for supplemented cattle, even in the absence of extreme heat stress.
A final experiment investigated the effects of amount and quality of roughage in a pelleted feed on core and rumen temperature and feed intakes in *Bos taurus* subjected to hot environmental conditions in climate controlled rooms at Murdoch University. Both pelleted feeds had approximately the same metabolisable energy and crude protein but differed in content and type of roughage. There were no differences in feed intake, core temperature or rumen temperature between diets.

This work has led to a greater understanding of the physiological responses of cattle to prolonged and continuous high heat and humidity, the requirements and effects of supplemental electrolytes in these conditions, and the effect of manipulating export diets. The demonstration of advantages in weight and buffering capacity with the electrolyte supplement highlights future areas of research to investigate electrolyte doses, route and types of supplementation, and dietary manipulation.
Acknowledgements

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<tbody>
<tr>
<td>AUD</td>
<td>Australian dollar</td>
</tr>
<tr>
<td>ABE</td>
<td>Acid base excess</td>
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>ADH</td>
<td>Antidiuretic hormone</td>
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<tr>
<td>AG</td>
<td>Anion gap</td>
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<tr>
<td>bpm</td>
<td>Breaths/beats per minute</td>
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<td>BW</td>
<td>Body weight</td>
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<td>Calcium</td>
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<tr>
<td>Cr</td>
<td>Creatinine</td>
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<tr>
<td>DCAD</td>
<td>Dietary Cation Anion Difference</td>
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<tr>
<td>DMI</td>
<td>Dry matter intake</td>
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<tr>
<td>EHL</td>
<td>Excessive heat load</td>
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<tr>
<td>FER</td>
<td>Fractional Excretion Ratio</td>
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<tr>
<td>GH</td>
<td>Growth hormone</td>
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<td>Bicarbonate ion</td>
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<tr>
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<td>Hydrogen ion</td>
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<td>H₂CO₃</td>
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</tr>
<tr>
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<td>Water</td>
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<tr>
<td>K</td>
<td>Potassium</td>
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<tr>
<td>Mg</td>
<td>Magnesium</td>
</tr>
<tr>
<td>MLA</td>
<td>Meat and Livestock Australia</td>
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<tr>
<td>MEI</td>
<td>Metabolisable energy intake</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
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<tr>
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<td>pCO₂</td>
<td>Partial pressure carbon dioxide</td>
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<tr>
<td>ppm</td>
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<tr>
<td>P</td>
<td>Phosphorous</td>
</tr>
<tr>
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<td>RR</td>
<td>Respiratory rate</td>
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<td>RH</td>
<td>Relative humidity</td>
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<tr>
<td>SID</td>
<td>Strong ion difference</td>
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<tr>
<td>Tₐ</td>
<td>Ambient temperature</td>
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<tr>
<td>Tₖ</td>
<td>Core body temperature</td>
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<td>T₄</td>
<td>Thyroxine</td>
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<tr>
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<td>Triiodothyronine</td>
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<td>WB</td>
<td>Wet bulb</td>
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<td>WBT</td>
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Published and Submitted Papers and Conference Proceedings

Published papers

Submitted papers

Published conference proceedings
Chapter 1: Literature Review

1.1 Introduction

The scope of this thesis began at a welfare level in response to “heat stress” events on board live stock vessels carrying cattle to the Middle East. Very little is understood of the physiological response of animals to prolonged, continuous periods of heat and humidity as can occur in cattle on board live stock vessels. The purpose of the literature review is threefold. Firstly, it provides a brief background of the live export industry and how this industry differs to others where environmental conditions, in particular heat stress, represent a major problem. Secondly, it reviews the heat exchange mechanisms available to animals and how heat stress affects the animals’ body and how animals respond to heat at both a physiological and behavioural level. Finally, the review highlights the literature where attempts have been made to alleviate heat stress in other industries, with particular emphasis given to electrolyte and nutritional interventions.

1.2 Live export

1.2.1 Review of industry

Keniry et al. (2003) conducted an in depth review of the live export industry in response to concerns about animal welfare. Apart from reviewing the live export industry in general, the review’s main focus was to examine the adequacy of welfare model codes of practice, the adequacy of regulatory arrangements, the types of livestock suitable for export, supervision of voyages to ensure accurate reporting, and the factors that contributed to excess mortalities on the MV Cormo Express.
This literature review will briefly highlight the history of the live export industry as well as its regulation and economic and welfare characteristics.

The number of cattle exported from Australian ports has risen almost linearly since the trade began in the late 1980’s. In 1990 just over 100,000 cattle were exported live from Australia. This had risen to almost one million in 2002 (LiveCorp, 2004). The country of destination of live animal export has also changed. In the early 1990’s nearly all cattle were exported to Asian countries. In 1995 the “long haul voyage” to Middle Eastern and North African countries was established. In 2002, 20% of all cattle exported were destined for this market (LiveCorp, 2004). Both *Bos taurus* and *Bos indicus* are exported on long haul voyages with the majority of *Bos indicus* exported from northern ports and *Bos taurus* from southern ports. Voyage duration to the Middle East and North Africa ranges from 13 to 18 days (Clarke, 2000). This may be increased to 24 days or more if vessels discharge at more than one port. Large vessels on long haul voyages to the Middle East are capable of carrying up to 14,000 head of cattle and 40,000 sheep or 7,000 head of cattle and 100,000 sheep.

### 1.2.2 Regulation of industry

There are numerous government and industry organisations involved in the regulation and control of live animal exports from Australia. On a Federal Government level, the trade is regulated primarily through the *Australian Meat and Livestock Industry Act 1997* and the *Export Control Act (1982)* (Timms & Gehling, 2000). The Australian Quarantine and Inspection Service (AQIS) has a specific legal role in the export of goods in accordance with the *Export Control Act (1982)* and the accompanying Orders and Regulations (McDonald, 2000). The export of live animals is specifically covered in the Animal Orders attached to the *Export Control Act (1982)* and requires that all exported animals undergo a veterinary inspection within 48 hours of
export (McDonald, 2000). The primary industry organisations involved in the regulation of the live export industry are the Australian Livestock Exporters Council (ALEC) and Meat and Livestock Australia (MLA) through the Australian Livestock Export Corporation (LiveCorp). The Australian Livestock Export Corporation was established in 1998 and developed and implemented the Livestock Export and Accreditation Program (LEAP) which sets industry standards and quality assurance (Meerwald, 2000).

1.2.3 Economics and welfare

Australia is the largest exporter of live cattle and sheep in the world (Clarke, 2000). In 2002, the value of Australian live cattle exports was $610 million (LiveCorp, 2004). Animal welfare is probably the single most important issue impacting upon the continuation of the trade (McDonald, 2000) and was the reason for the 2003 Keniry report (Keniry et al., 2003) which was a major review conducted by the Australian Government in response to concerns about animal welfare.

Animal welfare has been defined as, “a characteristic of an animal which varies from poor to very good and can be defined by discrete measures, such as changes in hormone concentrations, body temperature and normal behaviour” (Silanikove, 2000). There is a lot of overlap between productive and welfare measures, such as disease, mortality risk, growth, milk yield and reproduction (Silanikove, 2000). Animal welfare will be impaired when an animal has difficulty coping with its environment and changes in the above parameters are observed. Given the above definition of animal welfare, any stressor placed on an animal will impair animal welfare. Animals can be stressed by either psychological stress (restraint, handling or novelty) or physical stresses (hunger, thirst, fatigue, injury or thermal extremes) (Grandin, 1997). During the transport of animals, stress due to extremes in the thermal environment has the biggest impact on animal welfare (Schrama et al., 1996).
Apart from Norris et al. (2003), no scientific literature exists on the animal welfare of Australian exported cattle. In the live export industry, heat stress is a major cause of reduced animal welfare and increased mortality (Norris et al., 2003). Due to the large numbers of animals exported on a single ship and the relative lack of scientific literature on measurable welfare parameters, mortality rate is the easiest and accepted measure of animal welfare for the live export industry (Norris et al., 2003). Norris et al. (2003) undertook a retrospective study analysing cattle deaths on voyages from Australia to all destinations between 1995 and 2000. Results indicated a mortality rate of 0.24% out of the four million cattle exported. However, a significantly greater percentage of deaths occurred on voyages to the Middle East (0.54%). The risk of death on these voyages was three times greater for animals exported from southern ports in Australia compared to northern ports. This study relied on masters’ reports submitted to Federal authorities to get mortality figures. These reports are compiled by stockmen on board the vessel and through the chain of personnel involved can occasionally become misleading (M. McCarthy, unpublished data). Furthermore, using mortality rates as an indicator for animal welfare has limitations in that a much larger proportion of animals may have impositions placed on their well being without death being the end result. Norris et al. (2003) did not measure many other behavioural and physiological variables due to practical reasons and the retrospective approach. Had other welfare measurements been made, they may have indicated that a larger proportion of animals had impositions placed on their welfare.

Norris et al. (2003) also analysed four long haul livestock voyages to the Middle East which were accompanied by a veterinarian to observe the cattle and to conduct necropsies on any that died during the voyage in order to determine the cause of death of cattle during live export. The risk of death was significantly greater for Bos taurus cattle exported from southern ports. Heat stroke was the major cause of death and was
diagnosed from clinical signs prior to death and necropsy findings. All of the deaths from heat stroke were in *Bos taurus* breeds and occurred in the later half of the voyage. Prior to death animals were observed to be panting heavily and to have rectal temperatures greater than 41.5°C. At necropsy, the eyes were sunken and core body temperatures (Tc) were consistently greater than 43°C. An additional consistent lesion was severe acute diffuse pulmonary congestion and oedema. Trauma was the next most common cause of death due to both lameness and injury. Respiratory disease was the third most common cause of death and animals were grossly and histologically diagnosed with fibrinous pneumonia and pleuropneumonia (Norris *et al.*, 2003). Studies in the feedlot industry verify that heat stress or heat stroke contributes to cattle production losses and death (Stokka *et al.*, 1996).

### 1.2.4 Conditions on ship

The Australian Maritime Safety Authority (AMSA) sets and monitors safety standards for livestock ships through *Marine Orders Part 43 “Cargo and Cargo Handling – Livestock”* (Timms & Gehling, 2000). Cattle-carrying live export ships vary markedly in size, carrying capacity and environmental conditions. Livestock vessels have a series of decks, some of which are split in two for sheep and others remain as one for cattle. Some of the decks are “open”, meaning they are above sea level and open at the sides allowing air to flow through the deck depending on wind and ship direction. Other decks are “closed”, meaning that they are enclosed and rely on supply fans to force air from the outside into the deck and exhaust fans to remove air. Artificial ventilation on closed decks varies between vessels. The following is based on personal experience, communication with live exporters and the Australian Livestock Export Standards (LiveCorp, 2001). The Australian Livestock Export Standards is the national code of practice for the livestock export industry.
1.2.4.1 Stocking densities

Depending on the ship, cattle can be transported in either open or closed decks. If a ship is transporting cattle and sheep, it is usual for the cattle to be housed below in the closed decks. Pen size varies considerably depending on the area of the ship. However, stocking rates are carried out as per Australian Livestock Export Standards (LiveCorp, 2001). Stocking rates will vary markedly depending on the breed, age, and sex of animal and also the time of year, port of departure and port of destination. For example, the minimum pen area (m$^2$/head) for a 400 kg beast shipped from a port south of the 26$^{th}$ parallel from 1$^{st}$ May to 31$^{st}$ October is 1.668 m$^2$/head. The same animal shipped from a port south of the 26$^{th}$ parallel from 1$^{st}$ November to 30$^{th}$ April would need 1.450 m$^2$/head.

1.2.4.2 Environmental conditions

The fundamental differences in climatic conditions experienced on livestock vessels compared to other intensive cattle industries are the lack of both solar radiation and diurnal variations in environmental climatic conditions, the use of forced ventilation, and high temperature and humidity during the northern hemisphere summer months. Routine measurements on all livestock vessel decks and the bridge are made daily of ambient temperature ($T_a$) and wet bulb temperature (WBT). Air temperature is a measure of the temperature of the air-vapour mixture registered on a thermometer. Wet bulb temperature is the temperature of moist air measured by a thermometer evaporatively cooled by contact with a water wetted wick. Wet bulb temperature gives a lower reading than $T_a$ due to the cooling effect of water evaporating from the wetted wick. Prolonged periods of over 30°C WB without night time cooling are not uncommon on livestock ships during the northern hemisphere summer (M. McCarthy, unpublished data).
The deck surface varies between ships. Some of the newer vessels have a hard rubber surface to prevent slipping. The deck surface of older vessels is hard metal. Sawdust is routinely used as a floor cover on all cattle decks, regardless of surface, to prevent cattle from slipping and to reduce excessive moisture build up. Sawdust is spread evenly throughout cattle pens to a depth of approximately 2cm. Depending on the ship and time of year, sawdust is reapplied 2 to 3 times throughout the voyage after washing.

1.2.4.3 Ventilation and washing

All livestock vessels require ventilation through decks to provide fresh air to animals, remove gaseous pollutants and help with cooling. Open decks rely on prevailing winds and ship speed and direction. Closed decks rely on forced ventilation. Air from outside is circulated through pens from supply intakes and removed via exhaust outlets. The efficacy of this forced ventilation depends on the individual ship and decks within the ship. The air being forced through the pens is at ambient temperature and humidity, and intakes will depend on wind speed and direction. Ventilation problems arise when hot, humid ambient conditions and tail winds are encountered.

It is routine practice to wash cattle decks during a long haul voyage to remove the build up of urine and faecal matter. This is done by stockmen using large volumes of seawater via hoses. Depending on the ship, stockmen will usually start washing at either the bow or stern of the ship and work their way towards drainage points where water and waste drain into the ocean. Invariably, cattle get wet during this process and relative humidity increases (M. McCarthy, unpublished data). Washing intervals vary depending on ship, the time of year and length of voyage. It is not uncommon for a ship to wash
cattle decks 5 to 6 times during a voyage. Sawdust is not always replaced after washing down and leg injuries due to animals slipping are more common after washing.

1.2.4.4 Food and water

Most live exporters feed cattle 2 to 2.5% (of live weight) in two equally divided feeds, one in the morning between 0600 and 0700 h and the other in the early afternoon between 1300 and 1400 h. Feed is in a pelleted form consisting of straw, lupins, barley and lime to give a maintenance ration of approximately 9 to 10 MJ of metabolisable energy (ME) per kg DM and 11 to 12% protein (amounts and constituents vary depending on time of year). There are usually 2 feed troughs per pen varying in size depending on the ship. Some of the newer ships have automated feed systems and others feed out by hand. The water system is automated and desalinised water is available ad libitum. There are usually 2 water troughs per pen which are gravity fed and refill as cattle drink. Stockmen clean feed and water troughs as required.

1.2.5 Gaseous pollutants

1.2.5.1 Ammonia

Ammonia gas (NH₃) is generated by bacterial ureases in the faeces breaking down urea in the urine, manure and bedding (Accioly et al., 2003). The live export industry has been relying on diets that are high in rumen degradable nitrogen to achieve both palatability and nutritional performance. As a consequence many of the diets have high urinary nitrogen output and thus greater potential for ammonia production (Accioly et al., 2003). Ammonia is a concern either as an undesirable irritant pollutant to the local neighbourhood or to continental (European) soil acidification (MAMIC, 2000c). With regards to live export shipping, NH₃ is seen as an irritant pollutant within the ship affecting the comfort and health of both animals and stockmen. The allowable ammonia
concentrations vary between countries and industries but fall in the range of 10 to 50 ppm, with 25 ppm being a common figure (MAMIC, 2000c). The rate of NH₃ production and concentrations obtained on livestock ships are unclear. In a review undertaken by Costa et al. (2003), it was determined that the atmospheric ammonia concentration on board live stock vessels was typically 15 ppm. However, common readings below decks reach 20 to 30 ppm. Concentrations will vary depending on the ventilation capacity of individual ships. MAMIC (2000c) suggests that NH₃ should be controlled by typical ventilation rates; however, it may be a problem on decks with low air turnover and in ventilation dead spots. Costa et al. (2003) suggest that the critical value of atmospheric ammonia above which cattle welfare and production could be adversely affected should be set at 25 ppm.

1.2.5.2 Carbon dioxide

Carbon dioxide (CO₂), in export ships will be generated almost exclusively by the metabolic activity of the cattle. The heat derived from the various biological reactions which form CO₂ are constant and so there is a close relationship between metabolic heat and generation of CO₂ (MAMIC, 2000c). The European Community “Standards for the microclimate inside animal transport road vehicles” give the CO₂ limit as 0.3% and this, along with work done by Phillips et al. (1998) on CO₂ production, suggest that CO₂ is unlikely to be an issue as a pollutant on a live export vessel; however, it remains useful as a measure of ventilation rate (MAMIC, 2000c).

1.2.5.3 Methane

Methane is a greenhouse gas but not toxic to animals (MAMIC, 2000c). Harper et al. (1999) found that a highly digestible feedlot diet produced 0.07 kg/day/animal of methane while a pasture diet gave 0.23 kg/day/animal. Given these figures, normal
ventilation rates on vessels, and shipper diets, methane is very unlikely to be an issue on board live export vessels. (MAMIC, 2000c).

1.3 Thermoregulation

1.3.1 Review of thermoregulation

Cattle are considered to be homeothermic in that they maintain a relatively constant $T_c$ over a wide range of environmental extremes. Homeothermy requires that the amount of heat produced or gained from the environment must be equal the heat loss to the environment, as indicated by the equation:

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

where $M$ equals the metabolic heat production, $K$ is the heat exchanged by conduction, $C$ is the heat exchanged by convection, $R$ is the heat exchanged by radiation, and $E$ is the heat exchanged by evaporation (Robertshaw, 1985).

The rate of the heat transfer from an animal to the surrounding environment is dependant on the temperature or vapour pressure gradient. Conduction, convection and radiation are referred to as the sensible heat transfer processes because they involve differences in the temperatures of the materials involved (Hansen, 2004). Evaporation is referred to as insensible heat exchange because it involves latent heat and a change in the kinetic energy of molecular arrangement without a change in material temperature (Sparke et al., 2001).

1.3.2 Heat input

Heat gain results from three sources: chemical, mechanical and thermal (Smith, 1996). Chemical and mechanical sources relate to metabolic processes and represent the body’s main heat sources. The influence of the thermal environment on an animal is
primarily exerted through energy exchanges which involve convection, conduction, radiation and evaporation.

1.3.2.1 Metabolism

Metabolism is defined as the sum of the physical and chemical processes by which living substance is built up and maintained (anabolism), and by which large molecules are broken down into smaller molecules to make energy available to the organism (catabolism) (Blood & Studdert, 1993). Cells use carbohydrates, fats and proteins from food to generate adenosine triphosphate (ATP), which is the body’s main energy source (Ruckebusch et al., 1991). Of the energy in foods, 35% becomes heat during ATP formation (Guyton & Hall, 1996). Still more energy becomes heat as it is transferred from ATP to the functional systems of the cells, so not more than 27% of all the energy from food is finally used by the functional systems (Guyton & Hall, 1996). Metabolism, or heat production, ranges from a basal level (chemical sources of metabolism), to a level that may be twenty times higher (mechanical sources of metabolism) which relate to work, such as reproduction, muscular work, production and thermoregulation (Smith, 1996). An increase in body temperature also increases the metabolic rate (Cunningham, 2002).

1.3.2.2 Conduction

Conductive heat transfer is due to the physical contact of the animal with a surface, air or liquid. The rate of heat flow is dependant on the area of contact, the thermal conductivities of the material involved, the distance the heat needs to flow, and the temperature gradient (Sparke et al., 2001).

For heat gain to occur via conduction, the animal’s skin or mucosal linings must be in contact with a hotter object. Animals when standing are mainly in contact with the
air (except hooves which represent 2% of body surface area and are in contact with the ground), therefore, most heat transfer takes place with air, and since air has a poor thermal conductivity, conductive heat transfer plays a small role in the total heat transfer in the environment. If either air temperature or the temperature of the ground on which the animal is lying is greater than the skin temperature, then the animal will gain heat by conduction, adding to the metabolic heat load (Robertshaw, 1985).

1.3.2.3 Radiation

All solid objects emit electromagnetic radiation in the infrared range. Warm objects emit on a shorter wavelength and more emissions per unit time than do cool objects. When these emissions strike another object, some are absorbed and thus transfer heat (Cunningham, 2002). Net heat transfer is from warm to cool objects, so if the temperature of the surroundings is greater than the temperature of the body, a greater quantity of heat is radiated to the body than is radiated from the body (Guyton & Hall, 1996).

1.3.3 Heat loss

1.3.3.1 Convection

Heat loss by convection occurs when air or water is warmed by the body (Cunningham, 2002). For animals to lose heat by convection, heat must first be conducted to the air from the skin then carried away by convection currents (Guyton & Hall, 1996). As the temperature of the air rises, the density decreases and the air moves upwards and away from the animal. This is known as free convection (Robertshaw, 1985). Forced convection involves a cooler fluid (air or water), moving over the skin surface. This is more effective than natural convection because the thermal gradient is
maintained by the constant renewal of the cooler air or water that blankets the surface of the skin (Cunningham, 2002).

1.3.3.2 Conduction

For heat loss to occur via conduction, the animal’s skin or mucosal linings must be in contact with a colder object. If either the air temperature or the temperature of the ground on which the animal is lying is less than the skin temperature, then the animal will lose heat to the cooler surface.

1.3.3.3 Radiation

Net heat transfer is from warm to cool objects so if the temperature of the body is greater than the temperature of its surroundings, a greater quantity of heat is radiated from the body than is radiated to the body (Guyton & Hall, 1996).

1.3.3.4 Evaporation

When water evaporates from the body surface, 2.43 joules of heat is lost for each gram of water that evaporates (Guyton & Hall, 1996). Evaporative heat loss occurs through the diffusion of water through the skin and by loss of water vapour from the respiratory tract. Rates of evaporative heat loss from surfaces are dependant, so far as environmental factors are concerned, on air temperature and humidity (thus capacity of the ambient air to take on water vapour), and the movement of the air which exchanges the air laden with moisture with adjacent air containing less moisture (Sparke et al., 2001). Evaporative heat loss is important when the ambient temperature approaches body temperature; it is the only form of heat loss available once ambient temperature exceeds body temperature (Cunningham, 2002) as long as there remains a vapour pressure gradient across the air boundary layer from the respiratory tract or moist skin to the atmosphere (Sparke et al., 2001). Its effectiveness is reduced if relative humidity
increases and the air becomes more saturated with water vapour. In warm humid conditions, as the air approaches temperatures near to that of the skin, skin evaporative cooling tends to be limited. However, respiratory cooling may still be effective because the temperature of the inhaled air is warmed further to body core temperature and can still take on more water vapour. The temperature of the inspired air can only be raised to core body temperature and saturated with water vapour at that temperature. If the air being inhaled is already near to core temperature and near saturation the scope for respiratory cooling is limited (Esmay, 1969 as cited by Sparke et al., 2001).

1.3.4 Thermoneutral Zone

The Thermoneutral Zone (TNZ) is defined as, “the range of ambient temperature within which metabolic rate is at a minimum, and within which temperature regulation is achieved by non evaporative physical processes alone” (Bligh & Johnson, 1973). The lower critical ambient temperature range point and the upper critical ambient temperature range point define the limits of the TNZ. The ambient temperature below which the rate of heat production of a resting homeotherm increases to maintain thermal balance is the lower critical temperature (LCT). The upper critical temperature (UCT) may be defined as the ambient temperature when the: (a) metabolic rate increases; (b) evaporative heat loss increases; or (c) tissue thermal insulation is minimal (Silanikove, 2000). Below and above these temperatures heat production is increased. The width of the thermoneutral zone will vary depending on age, species and breed, level of nutrition, previous state of temperature acclimation or acclimatization, level of productivity, housing conditions, insulation, and behaviour etc (Yousef, 1985c). In order to more clearly define the TNZ, Silanikove (2000), suggested that the subdivision of the TNZ into a zone of thermal well being is most suitable to describe the relation between an
animal and its environment. The schematic presentation and definition of the zone of optimal well being is described in a review article by Silanikove (2000).

Schrama et al. (1996) also reviewed thermoregulation and thermal thresholds (both upper and lower critical temperatures) during transport of animals. Generally, both sheep and cattle have lower LCT than pig and poultry. With age both the UCT and LCT decrease; however, the decline in the LCT is greater than the decline in the UCT. During transport, the elevated heat production due to high stocking densities results in an overall lowered UCT thus enhancing the susceptibility of animals to heat stress. Under hot conditions an increase in air velocity will increase both the UCT and LCT, thus reducing heat stress. Animals can be exposed to a high relative humidity during transport due to inadequate ventilation. Relative humidity does not affect LCT, but is of importance for UCT, because it determines the amount of heat an animal can lose to its environment by evaporation. In air saturated with moisture, UCT is lower than in dry air (Schrama et al., 1996).

1.3.5 Heat stress

The normal core body temperature range given for adult cattle living in temperate climates is between 36.7 and 39.1°C (Cunningham, 2002). At this temperature, cellular and biochemical activities operate most effectively. If tissues get too cold, metabolism is reduced. If body cells and tissues become too hot, metabolism speeds up. As metabolism increases there is greater metabolic heat production and thus the tissues get even hotter, causing metabolism to accelerate even more. The consequence is uncontrolled metabolism and a situation called “run-away hyperthermia” which will result in death (Young & Hall, 1993, as cited by Sparke et al., 2001). With body core temperatures above 42°C there is a risk of this phenomenon developing (Sparke et al., 2001). In addition, high tissue temperatures lead to denaturing
of proteins, the disruption of cell membrane integrity and possible permanent tissue damage resulting in long term morbidity and poor production performance (Guyton & Hall, 1996).

Animals can be stressed by either psychological stress (restraint, handling or novelty) or physical stresses (hunger, thirst, fatigue, injury or thermal stresses) (Grandin, 1997). There is some debate as to the best definitions or terms used to describe heat stress. Stott (1981) reviewed animal stress and found a working definition of stress by Lee (1965) which is often used by physiologists:

(1) Stress denotes the magnitude of forces external to the bodily system which tend to displace that system from its resting or ground state; (2) ‘strain’ is the internal displacement from the resting or ground state brought about by the application of the stress.

Given this working definition, heat stress would be the external stressor due to the thermal environment. This heat stress would produce a strain on the animal causing a displacement of various internal parameters from their resting or ground state i.e. an increase in core body temperature from its resting or ground state. The magnitude of the environmental stress can only be measured indirectly through the response of the animal. The acute responses may be a measurement of the strain due to stress. After consistently longer periods of stress, the measurable response is probably an adaptation (Stott, 1981).

Excessive heat load (EHL) has also been used to describe heat stress (Young, 1993). Excessive heat load occurs where a combination of local environmental conditions and animal factors leads to an increase in body heat content beyond the animal’s normal physiological range, and its ability to cope (Sparke et al., 2001) as evidenced by an increase in core body temperature (Young, 1993). The components of the thermal environment which influence heat load in cattle include ambient
temperature, relative humidity, wind, solar radiation (Yousef, 1985b), ground temperature, (Higgins & Dodd, 1989) and rainfall (Kabuga, 1992). The influence of the thermal environment on cattle is primarily exerted through energy exchanges which involve convection, conduction, radiation and evaporation (Blackshaw & Blackshaw, 1994) which have been previously discussed. Many animal factors (eg breed, health status, body condition, coat colour) determine metabolic intensity, rate of thermal exchange and thermal insulation which contribute significantly to the heat balance of the animal.

Sparke et al. (2001) and Young (1993), believe the term “heat stress” lacks clear quantitative definition, and is difficult to assess objectively, whereas, EHL is a thermodynamic description allowing affected animals to be assessed quantitatively, and to identify and describe differences between animals and situations (Sparke et al., 2001). For this literature review the term “heat stress” is as defined by Lee (1965) and assumes heat from the thermal environment is the external stressor and that heat stress places a strain on the animal. Excessive Heat Load is used as described and defined by Young (1993) and Sparke et al. (2001).

1.3.6 Heat stress indices

In hot climates, high ambient temperatures, humidity, wind speed, and high direct and indirect solar radiation are the main environmental stressing factors that impose strain on animals (Finch, 1984, as cited by Silanikove, 2000). Heat stress indices range from simple measurements of ambient air temperature to indices that try to provide a weighted estimation of all environmental factors involved. Given the fact that heat stress is caused by multiple environmental or thermal factors, it would seem unacceptable to use ambient temperature alone as the indicator of heat stress.
The Temperature Humidity Index (THI) has been widely used as a heat stress index in beef and dairy industries (Stokka et al., 1996; West, 1999; Mader et al., 2002). The THI takes into account only dry bulb temperature and relative humidity and is expressed in a formula as follows:

\[
\text{THI} = 0.8T_a + \text{RH} \times [(T_a - 14.3) + 46.3]
\]

where \( T_a \) = ambient dry bulb temperature (°C) and RH = relative humidity expressed in decimal form (Thom, 1959, as cited by Gaughan et al., 1999). Temperature humidity index values of 70 or less are considered comfortable, 75 to 78 stressful, and values greater than 78 cause extreme distress and animals are unable to maintain thermoregulatory mechanisms or normal body temperature (Silanikove, 2000). The THI fails to reflect the contributions of other environmental factors, such as airflow and thermal radiation.

A heat load index for dairy heifers has been developed by Yamamoto et al. (1994), who calculated effective temperature (ET) from ambient temperature and radiation (black globe temperature (BGT)). They found that:

\[
\text{ET} = 0.24 T_a + 0.76 \text{BGT}
\]

Black globe temperature integrates the influence of air temperature, radiation and air movement and is measured by a black globe thermometer. The ET equation indicates that solar radiation, as measured by BGT, contributes more to the heat load on cattle than does \( T_a \), though given the lack of solar radiation below decks on livestock vessels, this index would also appear to be of little value.

Baeta et al. (1987) developed an Equivalent Temperature Index (ETI) based on combined effects of temperature, humidity and air movement for housed lactating dairy cows at temperatures above thermoneutral conditions. For a combination of temperature, humidity and air movement, the ETI is defined as the equivalent dry bulb temperature at 40% relative humidity and 0.5 m/s air movement. Equivalency is based
on milk production and heat loss rates (Baeta *et al.*, 1987). The exclusion of solar radiation and inclusion of wind velocity would make ETI a realistic index for assessment of ship board conditions.

Wet bulb temperature (WBT) is the temperature of moist air measured by a thermometer evaporatively cooled by contact with a water wetted wick. The difference between wet and dry bulb temperatures provides for the estimation of relative humidity. Due to the large contribution humidity has on heat load on board live stock vessels, and the fact that wind velocity is relatively constant due to forced air ventilation, it would appear that wet bulb temperature may be the simplest and most beneficial indicator of heat load (MAMIC, 2000c).

### 1.4 Animal responses to heat load

Metabolism and the inevitable energy transformation which occurs within animal tissues produce heat. Under high heat load, about 15% of this heat is lost directly from the body core via the respiratory tract. The remainder must be transferred to the skin where it is then dissipated evaporatively (Finch, 1986). Heat stress and EHL in most cattle industries occurs during daylight hours, when there are extremes of environmental temperature and humidity, and at night, when there is a lack of night time cooling following daytime extremes. For EHL to occur, heat gain during the day from solar radiation and metabolism exceeds heat loss from radiation, convection, and evaporation so that heat is stored and body temperature rises. At night, when environment temperatures are generally lower, the heat flow reverses and stored heat is dissipated back into the environment and body temperature falls. This night time cooling effect is lost on board livestock vessels given the extreme conditions which can be encountered during the northern hemisphere summer where there is little or no
diurnal variation in environmental conditions. No scientific literature exists as to the physiological response of cattle to these extreme conditions.

1.4.1 Core temperature

For well hydrated beef cattle living in temperate climates, the range of normal \( T_c \) is 36.7 to 39.1°C (Cunningham, 2002). Heat inputs cannot be equal to heat outputs at all times, therefore, the maintenance of body temperature within this range is achieved through a system of temperature regulation.

To regulate body temperature the animal has a variety of temperature sensors located within the body. There are specific temperature sensitive neuronal structures located in the skin and in the mucous surfaces of the buccal cavity, in regions of the spinal cord, and in the hypothalamus of the midbrain (Bligh, 1985). These sensors relay information to the preoptic area of the hypothalamus, which is thought to be the main centre for temperature regulation. The pre-optic area of the hypothalamus then initiates mechanisms to either increase or decrease heat loss or production (Cunningham, 2002). In the regulation of body temperature the hypothalamus behaves as if it has a normal “set-point”. When core temperature rises above or falls below the set-point, heat losing or heat gaining mechanisms are initiated (Cunningham, 2002).

1.4.1.1 Measuring core body temperature

Many studies have used core body temperature as an indicator of cattle comfort and performance (Berman, 1968; Finch et al., 1982; Gaughan et al., 1999; Mader et al., 2002), therefore, it is important to use suitable methods to measure \( T_c \). Tympanic temperature probes (Mader et al., 2002), rectal temperature probes (Gaughan et al., 1999) and carotid artery thermocouples (McLean et al., 1982), all attached to data loggers, allow for continuous collection of data. Similarly, data loggers surgically
implanted directly into the abdomen have been used in Impalas (Kamerman et al., 2001). This method has the added advantage in that once implanted, there is no external hindrance to the animal.

Although data loggers are being widely used to monitor Tc, their main disadvantage is that data can only be retrieved when the logger is downloaded onto a computer. Gaughan et al. (1999) overcame this problem by having a digital readout of rectal temperatures in an adjacent room to allow continual monitoring of rectal temperatures of animals in climate controlled rooms. Lefcourt & Adams (1996) also overcame this problem by using radiotelemetry to measure body temperature. In this study temperature transmitters were surgically implanted into the peritoneal cavity and broadcast in a pulsatile manner with the inter pulse interval proportional to body temperature. A computer data acquisition system was used to monitor body temperatures every 3 minutes.

1.4.1.2 Diurnal rhythm of core body temperature

There are small circadian or nychthemeral (24 h) fluctuations in core body temperatures of cattle in both the natural environment and in steady temperature environments (Zhang et al., 1994; Mader et al., 1999). The diurnal rhythm for internal body temperature is often a reflection of the pattern of changes in the environmental temperature (Sparke et al., 2001). Animals exposed to a natural and fluctuating environment will show diurnal variations of less than 1°C (Bligh & Harthoon, 1965, as cited by Robertshaw, 1985). Knowledge of the relationship between the circadian change in body temperature and environmental thermal conditions is needed to increase our understanding of maintenance requirements and limitations to productivity performance (Araki et al., 1984). Likewise, such information is essential for the evaluation of the benefits of any environmental modification (Igono & Johnson, 1990,
as cited by Sparke et al., 2001). Zhang et al. (1994) demonstrated a circadian rhythm in beef calves exposed to hot and cold stress conditions. Core body temperature was measured continuously for 10 days in animals exposed to cold (1°C), thermoneutral (21°C) and hot (32°C) conditions in controlled environmental chambers (relative humidity 50%). Lights remained on from 0800 to 2100 h and animals were fed at 0800 and 2000 h. A daily fluctuation in $T_c$ of approximately 1°C was noted for each environmental condition. Furthermore, the time at which the highest and lowest core temperatures occurred differed between environmental conditions. Animals under cold conditions reached a maximum core temperature between 1600 and 2100 h compared to thermoneutral 0300 to 0500 h and hot 1800 to 1900 h. Similar results were obtained by McLean et al. (1982) who showed that animals kept at constant environmental temperatures had diurnal fluctuation in $T_c$ of less than 1°C. These results differ slightly to the work done by Berman & Morag (1971) who looked at seasonal thermoregulatory responses of Holstein cows during 4 days at 3 h intervals in summer and winter in their normal environment. This study found that the amplitude of the nychthemeral cycle (24 h period) in rectal temperature was 3.3 times larger in the summer as in the winter (approximately 1.2°C compared to 0.4°C). However, the large difference in amplitude of the nychthemeral cycle in rectal temperature was characterised in both seasons by a maximum attained at 1800 h and a minimum reached at 0600 h. Mader et al. (1999) reported similar results when they found the range in rectal temperatures of Hereford steers fed high energy feedlot diets to be approximately 0.7 and 1.3 °C under thermoneutral and hot environmental conditions (mean THI 75.4 and 79.7 respectively in climate chambers). Peak rectal temperatures for animals in the hot conditions peaked at 1800 h, which was similar to the results of Berman & Morag (1971) who were working in a natural environment.
There is evidence to suggest that diurnal body temperature rhythms lag behind environmental temperature rhythms. Berman & Morag (1971) suggested that the time lag between the peak in black globe temperature and that in the core temperatures was about 3 h in the summer and 6 h in the winter. This time frame is supported by both Mader et al. (1999) and Hahn et al. (1997).

1.4.1.3 Changes under heat load

In a simple study in climate controlled chambers Zhang et al. (1994) showed that there was a significant difference in the $T_c$ of beef calves kept at constant thermoneutral (21°C) and hot (32°C) conditions for 10 days. The maximum $T_c$ of cattle was 39.1 and 39.6°C respectively ($P < 0.05$). Lefcourt & Adams (1996) studied body temperatures of *Bos taurus* crossbred feedlot steers during the summer months and found that daily maximum body temperatures increased linearly with maximum ambient temperatures once daily maximum ambient temperature reached 25.6°C (0.42°C per 5°C; $P < 0.01$). During the summer months in which the study was undertaken, relative humidity remained relatively high and THI correlated closely with maximum, mean and minimum ambient temperatures (0.98, 1.00 and 1.00 respectively), therefore, no data were presented concerning THI. In addition, sharp peaks in body temperature were seen in the late evening (approximately 2200 h) well after ambient temperatures had decreased to below maximum values. Yamamoto et al. (1994) showed that the mean daily rectal temperatures of dairy heifers during the summer (dry bulb temperature varied from 26.5 to 32.8°C) and autumn (16.2 to 19.9°C) was 40.3 and 39.3°C respectively. Gaughan et al. (1999) demonstrated the effect of hot conditions (THI > 90) on Hereford, Brahman and crossbred steers (Hereford x Brahman, Hereford x Tuli and Hereford x Boran) when exposed to a test period of 10 h in climate controlled rooms. The Hereford had the highest rectal temperature at 40.3°C. The Brahman steers
had significantly lower rectal temperatures of 39.0°C (P < 0.05). The crossbred steers had rectal temperatures that were intermediate to the Brahman and Hereford. This agreed with Carvalho (1995) who walked cattle 7 km in 37°C at 60 to 65% relative humidity and found that rectal temperatures before the walk were significantly greater for imported *Bos taurus* (40.52°C), than in native *Bos taurus* (38.92°C) or native *Bos indicus* (38.90°C; P < 0.001). Rectal temperatures after the walk were greater in native *Bos taurus* (39.87°C) than in *Bos indicus* (39.46°C; P < 0.001). Imported *Bos taurus* were unable to finish the walk.

All of these studies were assessing the affects of short term excessive heat load over one to several days with a period of diurnal respite or night time cooling. Shipboard conditions for cattle can differ markedly as long periods of high heat and humidity can be encountered without any night time cooling. The effect this has on core body temperature and diurnal rhythm is unknown. What is understood is that changes in core body temperature over time are a useful indicator of an animals’ ability to cope with hot conditions.

### 1.4.2 Vasodilation and skin temperature

When cattle are exposed to hot environmental conditions, the initial response is vasodilation, which increases skin and limb blood flow. The resulting increase in skin temperature and the extension of core temperature down the limbs, increases the temperature gradient between the skin and the environment, resulting in more heat loss by radiation and convection (Cunningham, 2002). If skin temperature approaches core temperature, resistance to heat removal must decrease or heat will accumulate and body temperature increase (Finch, 1986). Temperature sensitive neurons are located in the pre-optic area of the hypothalamus, skin and viscera. Information from these central and peripheral heat sensitive neurons is integrated in the hypothalamus to regulate...
vasodilation and increased skin blood flow. Information from central temperature receptors predominates over skin and visceral receptors, so a rise in core temperature of only 0.5°C causes a sevenfold increase in skin blood flow (Cunningham, 2002).

Skin temperature, although dependant on skin colour, may be a good indicator of heat stress (Ingram & Whittow, 1962). Allen (1961) showed that *Bos taurus* and *Bos indicus* had similar responses in skin temperature to increases in ambient temperature and the increase in skin temperature resulted in increased sweating rates. The increase in skin temperature was approximately linear with the rise in air temperature. Differences arose when skin temperature was correlated to sweating rate. *Bos taurus* showed an early and linear rise in sweating rate as skin temperature increased from 32°C to 38°C, whereas *Bos indicus* sweating rate did not increase until skin temperature reached 35°C.

Direct skin thermal conductance has also been measured, with Brahman cattle exhibiting the highest rate increase in trunk skin thermal conductance as ambient temperature increased from 25°C to 41°C (Finch, 1985). The rate of increase of skin conductance (Watt m⁻² °C⁻¹ per degree rise in ambient temperature) was 3.95 for Brahman, 2.33 for Brahman cross and 2.09 for Shorthorn (Finch, 1985). The same study showed the mean tissue conductance (Watt m⁻² °C⁻¹) of Brahman was 63.5, Brahman cross 56.1 and Shorthorn 47.8. The author concluded that *Bos indicus* demonstrated an ability to lower resistance to internal heat transfer to a far greater extent than *Bos taurus* breeds and sustained this low resistance at high levels of heat stress. This would result in higher levels of free convection in the *Bos indicus* breeds. Why tissue resistance varies is not known (Finch, 1986).

If vasodilation and the resulting increase in skin blood flow is ineffective in maintaining temperature within the thermoneutral zone, evaporative cooling is increased by sweating or panting.
1.4.3 Sweating

At high temperatures, evaporative cooling is the principle mechanism for heat dissipation in cattle (Blackshaw & Blackshaw, 1994) and is the only form of heat loss once the ambient temperature exceeds the skin temperature (Cunningham, 2002). There is some variation in sweating rate and sweating response reported in the literature. This appears to be due to a number of factors including: the site of measurement (Dowling, 1955), method of collection (Johnson, 1970), breed (Finch et al., 1982), shape of sweat gland (Carvalho et al., 1995), plane of nutrition (Dowling, 1955), whether the animals are acclimatised to hot conditions, climatic conditions prior to and during measurement (Johnson, 1970; Gaughan et al., 1999), whether cattle are inside or outside (Gaughan et al., 1999), closeness to other cattle, and availability of drinking water (Sparke et al., 2001).

Early studies by McLean & Calvert (1972) examined the balance between heat production and heat loss, and the partition of heat exchanges of cattle in relation to air humidity, at two different air temperatures using a direct (gradient-layer) calorimeter. Results indicated that heat loss by evaporation amounted to 18% of the total heat loss at 15°C and 84% of total heat loss at 35°C. In cattle, sweating is the major form of evaporative heat loss (Cunningham, 2002). The Mclean and Calvert (1972) study found that 62% of the total evaporative heat loss at 35°C was due to sweating whilst the remaining 38% was due to respiratory evaporation.

Sweating occurs from apocrine sweat glands located in the dermis and found in association with hair follicles (Cunningham, 2002). Each hair follicle is accompanied by a sweat gland which has a duct opening onto the skin surface at the mouth of the follicle. There is a close anatomical association of capillary beds with sweat glands and the amount of blood directed to these capillary beds has an effect on the rate of sweat
production in cattle under heat loads (Schleger & Bean, 1971). Thus, increasing blood flow to the sweat gland in the skin facilitates both thermal heat transfer and sweat production (Finch, 1986).

Efforts have been made to establish the populations of hair follicles and apocrine glands in *Bos indicus* and *Bos taurus* breeds and findings would suggest that *Bos indicus* cattle have greater capacity to sweat due to greater densities of sweat glands (Dowling, 1955). However, there is considerable variation in the literature in regard to breed and sweating rate. Allen (1961) found that the difference between *Bos taurus* and *Bos indicus* in observed maximum and minimum sweating rates was not great (maximum 208 and 216 g m\(^{-2}\)h\(^{-1}\) respectively, and minimum 56 and 56 g m\(^{-2}\)h\(^{-1}\) respectively), although there were noticeable differences between individuals in maximum sweating rates. However, *Bos taurus* did show an early and almost linear increase in sweating rate with rise in air and skin temperature (from 18°C and 32°C respectively), whereas the sweating rate of *Bos indicus* did not increase until air temperature had risen to at least 29°C and skin temperature to 35°C. Similarly, Johnson (1970) found that sweating rates increased significantly for both *Bos taurus* and *Bos indicus* as ambient temperature rose to 45°C. For both *Bos taurus* and *Bos indicus*, sweating rates were highest on the shoulder and lowest on the lumbar region. The species difference was not significant, although the mean sweating rates at air temperatures of 40°C and 45°C were consistently higher at all sampling sites for *Bos indicus* compared to *Bos taurus*. More support for similar sweating rates for *Bos taurus* and *Bos indicus* comes from Gaughan (1999) who reported little difference in sweating rate, 171 and 175 g m\(^{-2}\)h\(^{-1}\) for Brahman and Hereford steers respectively, that were exposed to THI > 90 for 10 h. However, the sweating rate of Brahman x Hereford steers was significantly greater (P < 0.05) at 221 g m\(^{-2}\)h\(^{-1}\). This was suggestive of a heterosis effect, which supports the findings of Schleger & Turner (1965) who also found that the
sweating rate of *Bos indicus* cross cattle was greater than for *Bos taurus* when exposed to excessive heat load.

Nay and Hayman (1956) reported differences in the location of sweat glands and density of sweat glands in *Bos taurus* and *Bos indicus* species. They found that *Bos indicus* had larger and more numerous sweat glands than *Bos taurus*. The sweat glands of *Bos indicus* were more numerous on the midside than on the dewlap, and closer to the surface than for *Bos taurus* (Nay & Hayman, 1956). These findings were supported by Carvalho et al. (1995) who found sweat gland perimeter to be greater (*P < 0.001*) in *Bos indicus* (540 μm) than in native *Bos taurus* (382 μm) or imported *Bos taurus* (497 μm). Carvalho et al. (1995) and others (eg Yeates et al. 1975 as cited by Carvalho et al., 1995) suggest that the difference in sweat gland morphology rather than sweat gland perimeters leads to greater cooling capacity of *Bos indicus*.

Finch et al. (1982) analysed sweating rates in relation to rectal temperature to yield a measure of sweating response. Measurements were done in a natural radiant environment and they found that the sweating response was greater for *Bos indicus* (294 g m\(^{-2}\)h\(^{-1}\)°C\(^{-1}\)) compared to *Bos indicus* cross (146 g m\(^{-2}\)h\(^{-1}\)°C\(^{-1}\)) and *Bos taurus* (194 g m\(^{-2}\)h\(^{-1}\)°C\(^{-1}\)), which did not differ. Furthermore, between animals, the relationship of sweating response to mean rectal temperature was negative (*P < 0.01*), thus the measure of sweating response may be a good indicator of the thermoregulatory ability of the animal. Finch et al. (1982) also reported that between animals within breeds, the sweating response was negatively correlated with metabolic rate. This suggests that cattle with high sweating rate may have lower metabolic potential.

Little is known of the composition of secretions from cattle skin. In one of only a few studies published, Johnson (1970) determined that the amounts of sodium and potassium in sweat were very small and variable at low air temperatures (20°C) but increased significantly as air temperature rose to 45°C. At the higher ambient
temperature the sweat collected contained at least four to five times more potassium than sodium. However, the total sodium and potassium loss through the skin was estimated to be no more than 1 to 3% of the sodium and potassium intake in the feed and did not result in changes in plasma electrolyte concentrations of either sodium or potassium. No significant differences in the amounts of electrolyte recovered from sweat were recorded between Bos taurus or Bos indicus or between different sites of collection.

1.4.4 Respiration

The other method of evaporative heat loss available to cattle is via respiration. Respiration rate (RR) has long served as a gross indicator of heat load in animals during hot weather, increasing when animals need to maintain homeothermy by dissipating excess heat as other avenues become inadequate (Hahn, 1999). The normal RR of cattle under thermoneutral conditions is 20 to 60 breaths per minute (bpm) (Smith, 1996). A RR of 80 to 120 bpm is indicative of cattle under moderate thermal stress, while over 120 bpm cattle are considered to be under EHL (Mount, 1979, as cited by Gaughan et al., 1999). Cattle with RR greater than 140 bpm are under considerable strain and cooling is required (Sparke et al., 2001).

Respiratory rate is primarily influenced by ambient temperature, solar radiation, relative humidity, and wind velocity (Sparke et al., 2001). Of these, ambient temperature has been identified as the most important variable (Hahn, 1999). In work undertaken in environmental laboratories, Hahn et al. (1997) found that RR was strongly associated with ambient air temperature, once air temperature was above 21°C, and increased 4.3 bpm per °C above a baseline RR of 60 bpm, with a lag of about 2 h behind ambient temperature.
Further work by Gaughan et al. (2000) has shown that RR can be used as an indicator of EHL in cattle provided animal condition, prior exposure, ambient conditions (increasing or decreasing ambient temperature) and previous cooling strategies are considered. In one experiment, three repeated cycles of thermoneutral conditions (12 days) followed by hot conditions (9 days), revealed that the RR response of *Bos taurus* to ambient temperature during the chronic hot period tended to change over time, with RR at a given ambient temperature increasing over the 3 repeated cycles. It was hypothesised that the animals’ increased susceptibility to heat stress over time was due to an increase in body condition as animals grew and that the increased fatness was more of a factor than acclimation to the hot conditions. These results are in direct contrast to Spiers et al. (1994, as cited by Sparke et al., 2001) who found that Angus cattle exposed to 32.2°C for 7 days followed by a 20 day recovery period, exhibited a lower RR to the same range of dry bulb temperatures when tested a second time. It was suggested that this represented acclimation of cattle to heat stress. If respiratory rate does decrease with acclimation as suggested, then there must be a much more efficient evaporative water loss per breath. In a second experiment by Gaughan et al. (2000), *Bos taurus* were either day cooled or night cooled during conditions which mimicked typical hot summer conditions. Day cooled cattle showed an increased RR at night when ambient temperature was decreasing even though their peak RR were lower (133 bpm) than for night cooled cattle (200 bpm). In night cooled cattle there was a tendency for RR to decrease prior to decreasing ambient temperature and cooling being imposed. This is consistent with other studies which have shown RR to decrease while animals were still exposed to hot environments (Gaughan et al., 1999). A decreasing RR is therefore not always indicative of an animal coping with hot conditions and is likely due to a shift in RR dynamics from rapid shallow breathing to slower open mouth panting (Gaughan et al., 2000).
It is well recognised that RR differences exist between *Bos taurus* and *Bos indicus* with increasing heat load. At THI > 90, Gaughan *et al.* (1999) reported mean RR of Brahman steers to be 104 bpm, which was significantly lower than Hereford (168 bpm), Hereford x Boran (171 bpm) and Hereford x Tuli (166 bpm). Hereford x Brahman was intermediate with RR of 139 bpm. This compares with RR of 33, 68, 50, 55 and 48 bpm respectively when THI < 77. Differences between *Bos taurus* and *Bos indicus* RR were also reported by Allen (1961) who found that *Bos taurus* had higher RR than *Bos indicus* at all ambient temperatures from 18°C to 41°C (relative humidity ranged from 45 to 55%). However, the increase in RR was approximately the same until the temperatures reached above 35°C, after which the *Bos taurus* increased more rapidly. Cross breeds respired at rates similar to *Bos taurus* until an air temperature of 32°C was exceeded, when their RR became intermediate to the two breeds. Colditz & Kellaway (1972) examined RR of Friesians, Brahmans and Brahman x Friesian subjected to 28 days heat stress (38°C) or cool ambient conditions (17°C). The RR of Friesians and Brahman x Friesians at 38°C was twice that of their respective genotypes at 17°C (82 and 70 bpm compared to 34 and 35 bpm respectively). At both temperatures the RR of the Brahman was lower than the other two genotypes (26 and 37 bpm).

In one of the few published studies undertaken on board live export vessels, Norris *et al.* (2003) analysed the RR of cattle during 4 research voyages. Breed and temperature were each significantly associated with RR (P < 0.001). The highest respiratory rates were in Hereford and Shorthorn cattle (mean 78.2 and 73.8 bpm respectively), which were higher than Droughtmaster (55.1 bpm; P < 0.05) which were higher than Brahman (40.1 bpm; P < 0.05). The RR for all cattle increased by 5.7 bpm for each increase of 1°C for ambient temperatures above 25°C. The ambient temperature was 31.4 ± 0.3°C (mean ± SEM) and the mean relative humidity was 80.6 ± 0.8% (Norris *et al.*, 2003).
1.4.4.1 Changes in respiratory character

Panting or an increase in respiratory rate, is a process by which the aqueous mucous secretions of the buccal area are helped to evaporate by the constant movement of inhaled and exhaled air over the buccal surfaces (Robertshaw, 1985). In the panting animal, vascular engorgement of the respiratory and oral mucosa and increased salivation increase heat loss through evaporation. Panting in cattle is not as effective as sweating in providing evaporative cooling (Mount, 1979). In the first phase of rapid shallow panting, under mild heat stress, the dead space ventilation increases more than the alveolar ventilation, so severe hyperventilation and respiratory alkalosis are avoided (Mount, 1979; Cunningham, 2002). Under more severe heat stress, when core body temperature approaches 41°C, second phase panting occurs, in which respiratory frequency falls and tidal volume increases. Second phase panting increases alveolar ventilation up to five-fold and leads to increased loss of carbon dioxide and respiratory alkalosis (Mount, 1979).

It has already been suggested that a RR ceiling may exist that is associated with a shift from rapid shallow breathing to slower open mouth panting (Sparke et al., 2001). Gaughan (2002) concluded that a decreasing RR while ambient temperatures were rising was not always indicative of an animal coping with hot conditions and may indicate failure to cope. Therefore, it is important that RR observations are made in conjunction with panting observations. A simple panting score has been used to assist with this (Gaughan, 2004).

1.4.5 Acid base balance

Activities of almost all enzyme systems in the body are influenced by hydrogen ion concentration or pH (Guyton & Hall, 1996). An increase in blood hydrogen ion concentration leads to a decrease in pH and a decrease in hydrogen ion concentration
leads to an increase in blood pH. Serious deviations of blood pH outside the normal range can drastically disrupt cell metabolism and body function (Cunningham, 2002). The normal blood pH for ruminants is 7.4 with a range of 7.31 to 7.54 (Smith, 1996). Hydrogen ions are produced via the carbonic anhydrase reaction when carbon dioxide (CO₂) is transported from the tissues to the lungs. If the lungs eliminate CO₂ as fast as it is produced in the tissues, there is no net hydrogen ion gain by the body (Cunningham, 2002). Hydrogen ions are also a product of protein metabolism (which produces sulfuric and phosphoric acids), fat metabolism, and the incomplete oxidation of glucose to lactic acid (Cunningham, 2002). Maintaining acid base homeostasis (regulation and maintenance of pH) requires the action of various buffers as well as respiratory and renal mechanisms.

1.4.5.1 Buffering mechanisms

Buffers are combinations of salts and weak acids that prevent major changes in pH by reversibly binding hydrogen ions (Guyton & Hall, 1996). Any body buffering system can be represented by the Henderson-Hasselbalch equation, which shows that the pH of a solution is determined by the ratio of the concentration of base (the hydrogen ion acceptor) and that of the undissociated acid (the hydrogen ion donor), and by the pK of the buffering system (Cunningham, 2002). Haemoglobin and bicarbonate buffering systems are the most important blood buffers and provide the most immediately available source of buffers to prevent changes in blood pH. In ruminants, bicarbonate is also present in large concentrations in saliva (approximately 80 mmol/L). The production of bicarbonate in saliva acts as a buffer in the rumen to neutralise fatty acid production (Smith, 1996). Proteins and inorganic phosphates are intracellular buffers within the body tissues and provide a reserve buffering capacity (Cunningham, 2002). When body pH is threatened by a change in production or elimination of
hydrogen ions, the first line of defence is provided by buffers in the blood and tissues which can prevent drastic changes in pH. However, they cannot correct the problem by increasing or decreasing the elimination of hydrogen ions or by replacing lost buffering capacity (Cunningham, 2002). Base excess and base deficit refer to an increase or decrease in total buffer base respectively where total buffer base is the sum of the concentrations of available blood buffers (Cunningham, 2002).

1.4.5.2 Bicarbonate-carbonic acid buffering system

The bicarbonate buffering system (HCO₃⁻/H₂CO₃) is important for two reasons. Firstly, there is a large amount of bicarbonate in the blood (24 mmol/L), and secondly, the concentration of bicarbonate ions (HCO₃⁻) can be regulated by the kidneys, and the concentration of carbonic acid (H₂CO₃) can be regulated by the lungs (Cunningham, 2002). The concentration of H₂CO₃ in solution is directly proportional to the carbon dioxide tension (pCO₂) and can be calculated as 0.03 x pCO₂. Therefore, the bicarbonate buffering system can be represented by the Henderson-Hasselbalch equation as follows;

\[
pH = pK + \log \frac{[HCO_3^-]}{[0.03 \times pCO_2]}
\]

Under normal conditions blood pH requires a \([HCO_3^-] / [0.03 \times pCO_2]\) ratio of 20:1. An increase or decrease in this ratio increases or decreases pH respectively (Cunningham, 2002).

1.4.5.3 Respiratory regulation of acid base balance

Carbon dioxide is formed continually in the body by intracellular metabolic processes (Guyton & Hall, 1996). As blood flows through the tissues, CO₂ diffuses into the plasma and erythrocytes (95% diffuses into the erythrocyte), where carbonic acid forms (in the red cell the presence of carbonic anhydrase accelerates the hydration of
CO₂ several hundred fold) and then dissociates into hydrogen ions (H⁺) and bicarbonate ions as indicated by the following reaction;

\[
H₂O + CO₂ \rightarrow H₂CO₃ \rightarrow H^+ + HCO₃^{-}
\]

As the initial concentration of HCO₃⁻ in the blood is greater than that of H₂CO₃, the relative increase in the concentration of H₂CO₃ is greater than the increase in the concentration of HCO₃⁻, so the \([HCO₃^-] / [H₂CO₃] \) ratio (i.e. \([HCO₃^-] / [0.03 \times pCO₂]\) is decreased and pH decreases. In the lungs CO₂ leaves the blood and pH increases again. Normally the lungs remove CO₂ as fast as it is produced by the tissues and so the pCO₂ and pH remain constant (Cunningham, 2002).

The lungs can cause rapid changes in blood pH by increasing or decreasing the elimination of CO₂. When ventilation increases in relation to CO₂ production, pCO₂ decreases, the \([HCO₃^-] / [0.03 \times pCO₂] \) ratio increases, and pH increases resulting in respiratory alkalosis. Conversely, when ventilation decreases in relation to CO₂ production, pCO₂ increases, the \([HCO₃^-] / [0.03 \times pCO₂] \) ratio decreases and pH decreases resulting in respiratory acidosis (Cunningham, 2002).

1.4.5.4 Renal regulation

The lungs alter pH by removal of CO₂ whereas the kidneys are capable of secretion or excretion of hydrogen ions, reabsorption of bicarbonate ions or production of new bicarbonate ions. For every hydrogen ion secreted into the renal tubules, a bicarbonate ion is reabsorbed. When there is an excess of bicarbonate ions (a rise in pH), the excess can not be reabsorbed so are excreted in the urine. When there is an excess of hydrogen ions or decrease in pH, all the bicarbonate is reabsorbed and there is an excess of hydrogen ions in the urine. These excess hydrogen ions are excreted by binding to buffers such as phosphate or ammonia, which in turn generates new
bicarbonate ions in the tubular cells. These new bicarbonate ions can return to the blood helping to replenish extracellular stores of bicarbonate (Guyton & Hall, 1996).

1.4.6 Acid base response to high heat load

As discussed previously an important thermal regulatory reaction to heat stress is increased RR, which aids in heat dissipation via evaporative cooling (West et al., 1991; West et al., 1992; Blackshaw & Blackshaw, 1994). With increased respiration or second phase panting, the expiration of CO₂ exceeds the rate of its formation in the body (Sanchez et al., 1994b). Therefore, pCO₂ of blood declines, creating a deficit of H₂CO₃ and thus an increase in pH or respiratory alkalosis (Sanchez et al., 1994b). Dale & Brody (1952) were the first to characterise thermal hyperpnea and acid-base balance in cattle. They set out to clarify the reasons for the reduction in the CO₂-combining capacity of the blood previously noted in heat stressed dairy cows that had reduced feed intakes. Cows were either subjected to increasing temperatures of 18 to 40.5°C (with constant relative humidity) or fasted for 120 h. It was concluded that the observed decrease in the CO₂-combining capacity of the blood was due to respiratory alkalosis (or blowing off CO₂) rather than metabolic acidosis or the excessive production of ketone bodies and other metabolic acids as a result of the progressive reduction in feed intake. West et al. (1991) confirmed a decrease in pCO₂ during the hot phase of an experiment with dairy cows due to elevated respiratory rates. During the hot phase, mean THI ranged from 72.5 to 84.2 for 14 days and mean pCO₂ was 35.85 mmHg. During the cool phase, mean THI ranged from 59.0 to 74.8 and pCO₂ was 39.02 mmHg. Alkalemia depresses the rate of renal secretion of hydrogen ions, but increases the excretion of filtered HCO₃⁻, which subsequently leads to a reduction in blood HCO₃⁻ concentration, a more normal ratio of [HCO₃⁻] / [0.03 x PCO₂] leading to a decrease in pH towards normal (Sanchez et al., 1994b). Although the ratios are adjusted towards
normal, the final corrections for low pCO₂ and HCO₃⁻ occur only after heat stress and accelerated RR subside (Sanchez et al., 1994b).

Respiratory alkalosis is considered to be the major physiological consequence of heat stress and primary change in acid-base status; however, it may not be the only abnormal state of acid-base physiology (Sanchez et al., 1994b). In order to compensate for the high blood pH, renal excretion of HCO₃⁻ increases to adjust the ratio of [HCO₃⁻] / [0.03 x pCO₂] towards normal. The lower concentration and pool size of HCO₃⁻ can represent compensatory metabolic acidosis and this may be particularly evident during cooler parts of the natural nycthemeral period (Sanchez et al., 1994b). Few studies have characterised the diurnal pattern of acid-base status with sufficiently frequent measurements. Research with heat stressed dairy cows has involved taking blood samples during the hottest part of the day, when cows were near maximal heat stress (Schneider et al., 1984; West et al., 1991; West et al., 1992), resulting in responses indicative of respiratory alkalosis.

Schneider et al. (1988) characterised the nycthemeral patterns of acid-base status of lactating dairy cows both in an environmental chamber and under natural conditions where animals experienced heat stress during the day followed by night time cooling. Measurements from blood and urine were made hourly for 26 h to characterise the patterns of physiological measurements. In both studies measurements of rectal temperature, RR and blood gas composition indicated heat stress. During the hot part of the day, blood and urine pH were higher and blood pCO₂ and HCO₃⁻ lower for heat stressed cows compared to cows in the cooler environment. During the cooler part of the day, blood and urine pH of cows in heat stressed treatments fell below those of controls, and blood HCO₃⁻ was lower, which suggested compensatory metabolic acidosis. In both experiments, cows exhibited respiratory alkalosis only during hours of heat stress (Schneider et al., 1988). It has also been suggested by Sanchez et al. (1994b),
that if heat stressed cows consume most of their food during the cooler part of the day (Schneider et al., 1984), this would increase ruminal acid production post feeding in the cooler part of the day, when cows may also be experiencing compensatory metabolic acidosis.

During heat stress the demand for cations by the kidney is increased (Sanchez et al., 1994b). Respiratory alkalosis results in the renal secretion of H⁺ being depressed and the urine excretion of HCO₃⁻ being increased. However, the excretion of HCO₃⁻ must be accompanied by a cation. Sanchez et al. (1994b) suggests that sodium (Na⁺) or potassium (K⁺) are possibilities with Na⁺ being more likely. El-Nouty et al. (1980) noted that during prolonged heat stress, Na and K concentrations were reduced in blood serum and urinary excretion of Na increased 80% in a hot environment compared with excretion in a cooler environment. Urinary excretion of K increased only 18%.

In humans, elevation of body temperature (heat stroke) is primarily associated with respiratory alkalosis. Often, but not invariably, development of hyperventilation and respiratory alkalosis is rapidly followed by tissues releasing lactic and pyruvic acids. The reason for this occurring is unknown (Davenport, 1974). The effect on plasma pH and HCO₃⁻ concentration is the same as that produced by renal compensation; return of pH towards normal and reduction of HCO₃⁻ concentration (Davenport, 1974). Shapiro and Cristal (1987) suggest that the respiratory alkalosis is replaced later on by metabolic acidosis due to the development of hyperlactaemia. Turkeys exposed to 35°C and 80 to 85% relative humidity had significant decreases in body weight, food intake and pCO₂, this coinciding with an increase in blood pH (Yahav et al., 1998). Similarly chickens exposed to heat stress had lowered pCO₂, HCO₃⁻, but increased blood pH and plasma lactate (Koelebeck & Odom, 1994).

Nothing is known of the acid-base response in cattle during continuous and prolonged periods of heat and humidity as can occur on livestock vessels.
1.4.7 Hormonal changes with heat stress

Both acute and chronic thermal stress require metabolic adaptations to accommodate altered nutrient utilization caused by the stress. Due to the considerable involvement of the endocrine system in the coordination of metabolism, it is not surprising that thermal stress results in alteration of hormone concentrations in the blood (Beede & Collier, 1986). Hormones involved in adaptation to thermal stress include thyroxine, glucocorticoids, antidiuretic hormone, aldosterone, prolactin and growth hormone (Beede & Collier, 1986).

1.4.7.1 Thyroid hormone

The anterior lobe of the pituitary gland produces the hormone thyrotropin (TSH), which acts primarily on the thyroid gland to produce thyroxin (T₄) and triiodothyronine (T₃) (Cunningham, 2002). It is likely that thyroid hormones are the primary determinants of basal metabolism. It has been recognised that thyroid hormones increase oxygen consumption of tissues and, as a result, heat production (Cunningham, 2002). Thyroid activity and concentrations of T₃ and T₄ are reduced under heat stress conditions (Johnson, 1985; Beede & Collier, 1986; Silanikove, 2000). The response of T₃ and T₄ to heat stress is slow and it takes several days for levels to reach a new steady state (Silanikove, 2000). It is not an immediate response to acute heat stress, and instead can be involved in the acclimatisation of animals to a sustained heat load. A decrease in thyroid hormones will act to decrease metabolic rate and reduce the amount of heat produced by the cells (Beede & Collier, 1986). It has also been suggested that the effect of heat stress on thyroid gland activity reduces gut motility and rate of passage (Beede & Collier, 1986). Whether T₃ and T₄ decline due to thermal inhibition of the hypothalamus or indirectly because of lowered feed intake and metabolism is not clear (Johnson, 1985).
1.4.7.2 Glucocorticosteroids

Activation of the pre-optic area stimulates the hypothalamus to release a corticotrophin-releasing factor that acts on the anterior pituitary to release adrenocorticotropic hormone (ACTH). This stimulates the adrenal cortex to produce glucocorticosteroids, in particular cortisol (Silanikove, 2000). Activation of the hypothalamic-pituitary-adrenal axis and the consequent increase in plasma cortisol concentration are the most prominent responses of an animal to stressful conditions (Silanikove, 2000). Cortisol concentrations are elevated during acute but not chronic heat stress (Alvarez & Johnson, 1973; Johnson, 1985; Beede & Collier, 1986; Silanikove, 2000). However, El-Nouty et al. (1978) found that the effect of heat (2 days at 33°C and 50% humidity) on cattle plasma glucocorticoids was not significantly different from no heat (2 days at 20°C and 50% humidity). Reasons for differing responses are unknown (Beede & Collier, 1986). Alvarez & Johnson (1973) suggest that the depression of plasma hydrocortisone they found with chronic heat stress may be related to the fact that glucocorticoids exert a stimulatory effect on heat production in cattle and depressed adrenocortical function during chronic heat stress might contribute to depression in heat production. Roman Ponce et al. (1981) (as cited by Beede and Collier, 1986) suggested lower glucocorticoid and higher progesterone concentrations in heat stressed animals may be related to reduced conversion of progesterone to cortisol.

1.4.7.3 Antidiuretic hormone

Vasopressin or Antidiuretic hormone (ADH) is the most important hormone for the control of water balance. The pituitary gland alters ADH secretion in response to changes in plasma osmolality. An increase in plasma osmolality, or decrease in blood volume, leads to ADH secretion which acts to retain water in the kidneys (Cunningham, 2002). ADH concentrations have been shown to increase during heat stress in cattle (El-
Nouty et al., 1980; Johnson, 1985); however, this was not associated with a rise in serum osmolality (El-Nouty et al., 1980). El-Nouty et al. (1980) found that cattle exposed to heat (35°C and 50% relative humidity) and with access to water ad libitum, had significantly increased ADH concentrations and significantly lower serum osmolality compared to animals under thermoneutral conditions. The decrease in serum osmolality under heat also coincided with a significant reduction in total plasma protein, a significant increase in water intake and a slight but significant increase in urine output. El-Nouty et al. (1980) suggested that the increase in plasma ADH concentration in cattle during heat exposure was due to a direct effect of heat on the neuroendocrine system at the level of the hypothalamus and/or pituitary gland. Beede & Collier (1986) suggested that increases in ADH were associated with the need to conserve water and increase water intake as water losses via the respiratory tract and sweating increased.

1.4.7.4 Aldosterone

Aldosterone is a mineralocorticoid produced by the adrenal gland and it is secreted in response to a reduction in blood volume. An increase in aldosterone secretion results in an increase in Na reabsorption and increase in K secretion. Therefore, aldosterone causes Na to be conserved in the extracellular fluid while increasing K excretion in the urine. With Na reabsorption in the kidneys there is simultaneous osmotic absorption of water, thus increasing blood volume. There is also a small increase in extracellular sodium concentration which is enough to stimulate thirst and increase water intake thus also increasing blood volume (Guyton & Hall, 1996). During heat stress in cattle aldosterone concentrations in the plasma have been shown to decline (El-Nouty et al., 1980; Collier et al., 1982; Johnson, 1985). El-Nouty et al. (1980) suggested that the decrease in serum K may be the main factor that inhibits aldosterone release during heat exposure. Furthermore, the decrease in aldosterone
levels during heat exposure of cattle may be the main factor contributing to the increase in urine output during heat exposure. The decline was associated with reduced concentrations of Na and K in blood serum and of K in urine. However, urinary Na excretion increased, perhaps to aid in the conservation of K as it was lost via cutaneous evaporation.

1.4.7.5 *Growth hormone*

Growth hormone (GH) does not function through a target gland but exerts its effects on almost all tissues of the body (Guyton & Hall, 1996). Growth hormone is produced by the anterior pituitary gland and is implicated in nutrient partitioning and homeorhesis and for the initiation and maintenance of lactation (Beede & Collier, 1986). Growth hormone concentrations decline with both short and long term exposure to heat (Johnson, 1985). It is unclear whether GH decline is due to thermal inhibition of the hypothalamus or comes about indirectly because of lowered feed intake and metabolism (Johnson, 1985).

1.4.7.6 *Prolactin*

Prolactin is similar to GH and is also produced by the anterior pituitary gland. Prolactin concentrations increase in response to heat stress in cattle, the reasons for which are unclear (Johnson, 1985).

1.5 *Behavioural responses to heat load*

1.5.1 *Seek shade*

Physical protection from solar radiation with artificial or natural shade offers one of the most immediate and cost effective approaches for enhancing performance of cattle in hot environments (Blackshaw & Blackshaw, 1994). Blackshaw & Blackshaw
(1994) reviewed the effects of shade on the behaviour of beef cattle. They concluded that during hot weather, cattle will use shade if available, although *Bos indicus* use shade less than *Bos taurus*. In many situations shade improves cattle production and prevents increased rectal temperature and respiration rate. Gaughan *et al.* (1998) examined the shade preferences of lactating Holstein-Friesian cows under Australian summer natural conditions. Four different shade types were offered as well as no shade. Cows selected the galvanised iron roof shade type most frequently when temperatures rose above 30°C, with no significant differences between other shade types (vines on trellis, 70% shade cloth and natural shade from trees). At temperatures below 30°C, animals did not seek shade. Overall, 43.2% of cows selected some form of shade at temperatures between 26 and 29°C. At temperatures between 30 and 34°C, 90.2% of cows selected some form of shade and at temperatures above 34°C, 94.6% of cows were under shade (Gaughan *et al.*, 1998). Vandenheede *et al.* (1995) reported that Belgian Blue bulls increased their use of shelter from 10% to 49% of the day when maximum ambient temperature exceeded 20°C.

Obviously cattle below decks on livestock vessels experience complete shade with no direct solar radiation to influence heat load. The benefits listed above may be largely offset by lack of and/or variable air movement, overcrowding, and indirect radiation from engine rooms and bulk heads. The influence of these factors on physiological parameters during live export shipping is unknown.

### 1.5.2 Feed intake

Food intake by the animal is directly related to all aspects of energy metabolism with the release of heat for maintenance, activities and production (Finch, 1986). Metabolism generates about one third of the heat load of an animal standing in a hot radiant environment (Finch, 1976). This is supported by Purwanto *et al.* (1990), who
concluded that total heat production within the animal is dependant, in part, on dry
matter intake (DMI). High feed intake increases metabolic rate and water intake; hence,
there is a greater need for thermoregulatory effort. A reduction in feed intake is an
immediate response to heat stress (Conrad, 1985; NRC, 2000). This is followed by a fall
in metabolic rate and, therefore, reduced maintenance, which helps to balance heat
production with heat loss (Turner & Taylor, 1983). The causes of reduced voluntary
feed intake of cattle due to rising environmental temperatures have been reviewed by
Bianca (1965). It was proposed that the hypothalamus acted as an integrator for
regulating food intake and other functions involving energy balance. This was supported
by Andersson and Larsson (1961, as cited by Bianca, 1965) who found that warming the
pre-optic area and rostral hypothalamus of goats with thermodes caused the hungry
animals, which had just begun to eat with good appetite, to stop eating within 1 minute.

The extent to which feed intake (DMI) is reduced due to heat stress varies in the
literature. At temperatures of 15 to 25°C, normal feed intake will occur (Conrad, 1985).
Temperatures between 25 and 35°C can be expected to cause a noticeable reduction in
feed intake (3 to 10%), but temperatures above 35°C can result in a 10 to 35% reduction
in feed intake (Conrad, 1985). Colditz & Kellaway (1972) also reported a 15%
reduction in feed intake when *Bos taurus* were fed at 38°C compared to 17°C; however,
there was no reduction in feed intake of either *Bos indicus* or *Bos indicus x Bos taurus*.

### 1.5.3 Water intake

The water needs of cattle are sourced from free drinking water, water contained
in foods, and metabolic water produced by oxidation of organic nutrients (Beede &
Collier, 1986). Water losses are principally from urine, faeces and evaporation from the
body surface and respiratory tract. As already stated, heat loss via evaporation requires
water. As evaporation (both via sweating and panting) becomes the more important
mechanism for heat loss during excessive heat load, water intake also becomes important. Water intake is influenced by many factors in addition to ambient temperature, among which are feed intake, liveweight, type of diet and physiological state. Water needs during heat stress may rise 1.2- to 2-fold compared with requirements in thermoneutrality (Beede & Collier, 1986). Normally, beef cattle will consume from three to five times more water than dry matter (Conrad, 1985). At environmental temperatures above 35°C, water intake may be 8 to 15 L/kg of DM (Conrad, 1985). In dairy cattle, increasing the environmental temperature from 4.4 to 26.7°C caused an increase in water intake from 3.1 to 5.2 L/kg DM. Increasing the environmental temperature from 26.7 to 37.8°C caused water intake to increase from 5.2 to 15.6 L/kg DM (Conrad, 1985).

*Bos indicus* have been shown to have consistently lower water intakes per unit of body weight and per unit of dry matter intake than *Bos taurus* (Winchester & Morris, 1956; Phillips, 1960; Colditz & Kellaway, 1972). Water intake (free water plus feed water) was similar for Brahman, Brahman x Friesian and Friesian at 17°C whilst at 38°C the water intake was 109, 61 and 46 % higher for Friesian, Brahman x Friesian and Brahman respectively (Colditz & Kellaway, 1972). Macfarlane (1959) proposed that the reasons for the differences between *Bos taurus* and *Bos indicus* were that *Bos taurus* had a higher water turnover than *Bos indicus*. Colditz & Kellaway (1972) suggest that any differences may be due to large variations between individuals, whilst (Phillips, 1960) suggest that lower water requirements for *Bos indicus* may be explained by the lower moisture content in the faeces compared to *Bos taurus*. However, Yousef & Johnson (1985) were uncertain whether there was any real difference in water intakes between *Bos taurus* and *Bos indicus* types.

The mechanism responsible for heat-induced increases in water consumption seems to involve the hypothalamus (Bianca, 1965). Warming the pre-optic area and
rostral hypothalamus of goats with thermodes evoked a large increase in water consumption (Andersson et al., 1960).

1.6 Electrolytes

1.6.1 Electrolyte overview

Conservation of the body’s supply of water and electrolytes, primarily sodium (Na), potassium (K), chloride (Cl) and bicarbonate (HCO₃⁻), is a high priority for sustaining life (Cunningham, 2002). The gastrointestinal (GI) tract plays a major role in this conservation, as it is the portal of entry for replenishment and because water and electrolytes in GI secretions must be efficiently reclaimed to maintain body composition (Cunningham, 2002). Once electrolytes enter the body via the GI tract, homeostasis is maintained via the kidney. The kidneys filter the blood and secrete or reabsorb electrolytes depending on whether or not they are in excess or deficit.

1.6.1.1 Sodium

Sodium is the major cation in extracellular fluid and it is involved in maintaining osmotic pressure, controlling water balance, and regulating acid-base balance (NRC, 2000). There are at least three distinct mechanisms of Na absorption from the gut. The first is the secondary active transport of Na via sodium co-transport proteins. The second is via the Na⁺/H⁺ ion exchanger in which intracellular H⁺ is exchanged for luminal Na⁺. This transport mechanism is often called “coupled sodium chloride transport” as intracellular HCO₃⁻ is exchanged for luminal Cl⁻. The third mechanism of Na absorption is via simple diffusion through ion channels in the enterocyte apical membrane (Cunningham, 2002). Aldosterone release in response to hypotension enhances Na reabsorption in the kidneys, which in turn enhances water reabsorption in order to correct volume depletion (Cunningham, 2002).
Requirements for Na in non lactating beef cattle do not exceed 0.06 to 0.08%, while lactating beef cows require approximately 0.1% (NRC, 2000). Signs of Na deficiency are non specific and include pica, and reduced feed intake, growth and milk production. When Na intake is low the body conserves Na by increasing reabsorption from the kidney in response to aldosterone (NRC, 2000). Serum or plasma Na concentration is not a reliable indicator of Na deficiency. Dietary Na concentration is a good measure of Na adequacy (NRC, 2000). The most reliable guide to Na status is the Na:K ratio in saliva which is generally greater than 20:1 in Na replete animals. When the ratio decreases below 10:1 there is an increased likelihood of a production response to a sodium supplement (Corbett, 1990). The maximum dietary concentration of salt tolerable to cattle has been estimated at 9%. Salt is much more toxic when present in the drinking water of cattle. Growing cattle were able to tolerate 1% added salt in drinking water without adverse effects; however, the addition of 1.25 to 2% salt resulted in anorexia, weight loss and reduced water intake and collapse (NRC, 2000).

1.6.1.2 Chloride

Chloride is the major anion in extracellular fluid and like Na is involved with maintaining osmotic pressure, controlling water balance, and regulating acid-base balance. Chloride requirements are not well defined but Cl deficiencies do not seem likely in practical conditions (NRC, 2000). There are three major mechanisms of Cl absorption from the gut. The first is coupled Na/Cl absorption. The second is paracellular chloride absorption that occurs in association with Na co-transport because of the electrical gradient created and the third is by direct exchange for HCO$_3^-$ (Cunningham, 2002).
1.6.1.3  Bicarbonate

Bicarbonate has an important role in blood buffering against changes in blood pH and acid-base balance (Cunningham, 2002). Bicarbonate ion is secreted by several digestive glands and must be recovered from the gut if body acid-base balance is to be maintained. Much HCO$_3^-$ is absorbed by the neutralisation of HCl from the stomach. The remaining HCO$_3^-$ is reabsorbed by an ion exchange mechanism in which HCO$_3^-$ is reabsorbed as NaHCO$_3$ (Cunningham, 2002). Bicarbonate is secreted or absorbed in the kidneys depending on the animals acid-base balance. For every hydrogen ion secreted into the renal tubules, a HCO$_3^-$ ion is reabsorbed. When there is an excess of HCO$_3^-$ ions in the blood (a rise in pH), it is excreted in the urine. When there is an excess of hydrogen ions in the blood or decrease in pH, all the HCO$_3^-$ is reabsorbed by the kidneys and there is an excess of hydrogen ions in the urine.

1.6.1.4  Potassium

Potassium is the third most abundant mineral in the body and the major cation in intracellular fluid. Potassium is important in acid-base balance, regulation of osmotic pressure, water balance, muscle contractions, nerve impulse transmission, and certain enzymatic reactions (NRC, 2000). The primary mechanism of K absorption is via paracellular passive diffusion, which occurs rapidly in response to a large concentration gradient which exists between the intestinal lumen and blood. Potassium absorption is directly coupled to water absorption. The major route of K excretion is the urine and excretion is enhanced by aldosterone release (Cunningham, 2002). Estimates of requirements for cattle indicate a general value of 5 g K/kg DM (Corbett, 1990). A deficiency of K results in reduced feed intake and weight gain, pica, rough hair coat, and muscular weakness. Dietary K concentration is the best indicator of K status because serum or plasma K are not reliable indicators (NRC, 2000).
1.6.2 Methods of evaluating electrolyte balance

1.6.2.1 Blood and urine concentrations

Homeostatic mechanisms ensure that extracellular fluid concentrations of electrolytes and minerals are maintained within a narrow range during all but severe disease states. This means that the evaluation of serum electrolyte and mineral concentration may be of limited use in subclinical or mild clinical conditions or early in the disease process (King, 1994). The kidney is a major route of excretion of electrolytes (Na, K, Cl, calcium (Ca) and phosphorous (P)) and electrolyte homeostasis is affected mainly through changes in renal excretion. Measuring the urine concentration of electrolytes in the urine can be misleading because the concentration is dependant in part on the volume of urine produced, which is determined by the glomerular filtration rate (GFR). Creatinine can be used to determine the GFR because it is produced at a fairly constant rate and is its excretion by the kidney is virtually constant (King, 1994). Expressing the urinary excretion of a substance as a percentage of creatinine excretion (the fractional excretion), gives an indication of the body’s efforts to conserve or excrete that substance (King, 1994). The fractional excretion ratio (FER) of a substance is calculated by the following equation (King, 1994):

\[
FE_x = \left( \frac{[X]_{\text{urine}} \times [\text{Cr}]_{\text{serum}}}{[X]_{\text{serum}} \times [\text{Cr}]_{\text{urine}}} \right) \times 100
\]

Where \( X \) = electrolyte under investigation

\([\ ]_{\text{urine}} = \text{urinary concentration of electrolyte}\)

\([\ ]_{\text{serum}} = \text{plasma concentration of electrolyte}\)

An animal’s response to excessive heat load results in a decrease in PCV and a subsequent increase in plasma volume (Van Beaumont et al., 1981). Increases in plasma volume will subsequently influence plasma constituent concentrations. It is therefore
important to measure not only plasma electrolyte concentration but also the total circulating mass of plasma electrolytes. The change in total circulating mass of plasma electrolyte can be calculated from the following equations (Van Beaumont et al., 1981):

\[
\frac{T_2}{T_1} = \frac{P_2X_2}{P_1X_1}
\]

and

\[
\frac{P_2}{P_1} = \frac{H_1(1 - H_2)}{H_2(1 - H_1)}
\]

where

- \( H \) = PCV or hematocrit
- \( X \) = concentration of electrolyte (mmol/L)
- \( T \) = total circulating mass of electrolyte (mmol)
- \( P \) = plasma volume

1.6.2.2 Blood gas

Blood gas analysis provides information concerning pulmonary gas exchange and ventilation and measures the partial pressures of oxygen (pO2) and carbon dioxide (pCO2) as well as hydrogen ion (H⁺) concentration (George, 1994). Bicarbonate ion (HCO₃⁻) concentration is measured indirectly using either a nomogram or the total CO₂ measure (Smith, 1996). Actual base excess (ABE) is the concentration of titrable base when the blood is titrated with a strong base or acid. Positive values (base excess) indicate a relative deficit of non-carbonic acids; negative values (base deficit) indicate a relative excess of non-carbonic acids.

Heparinized whole blood is used for blood gas analysis. Samples must be measured immediately if kept at room temperature or within a few hours if kept on ice. Only arterial samples are suitable for pO2 determinations. Either arterial or venous samples are adequate for pH, pCO₂ and HCO₃⁻ measurements but reference ranges differ (George, 1994). Ideally, blood gas analyses should be performed on arterial blood, since the values of the variables in jugular venous blood are determined not only by their
values in the arterial blood, but are also influenced by cranial metabolism and cranial blood flow. In sheep it has been demonstrated that under hot conditions cranial blood flow and blood flow through arterio-venous anastomoses increases markedly to facilitate convective heat loss (Hales, 1973). Given that cranial metabolism remains much the same during heat stress and blood flow increases, reductions in jugular pCO$_2$ will result as animals move from thermoneutral to a hot environment. Similarly, even if the arterial pH of an animal does not change, venous pH will drop when an animal moves from a hot to a thermoneutral environment, and peripheral blood flow drops. There is no data available to confirm that this is the case with cattle or how much variation there is between arterial and venous blood gas constituents for cattle in thermoneutral and hot environments. Other authors have reported venous acid base changes in cattle under hot and thermoneutral conditions (Dale & Brody, 1952; Bianca & Findlay, 1962; Schneider et al., 1984) and for practical and financial reasons, jugular venous blood has been used for acid base variables throughout this thesis.

1.6.2.3 Dietary cation anion difference

The dietary cation anion difference (DCAD) is an approximation of the unmeasured anions in excess of the main monovalent cations in the diet dry matter (Ramberg et al., 1996). The equation assumes equi-potency of the components of the equation on a chemical equivalent basis. The DCAD equation concerns only monovalent dietary electrolytes. Organic ions and inorganic ions with higher valences are ignored. The supposed reason for this is that binding of ionized substances with valences higher than one, and variable but incomplete intestinal absorption, would complicate the interpretation of the effects on acid base balance. This is in contrast to inorganic monovalent ions which have high intestinal absorption and homeostasis is maintained primarily by urinary excretion (Ramberg et al., 1996)
The relationship of DCAD to acid-base physiology has been investigated in non-ruminant species and several equations have been developed to describe the balance of inorganic ions in the diet. Of these, the equation receiving most attention from poultry nutritionists relies upon the almost complete dietary availability of Na, K, and Cl and is described as follows: \( \text{DCAD} = (\text{Na} + \text{K}) - \text{Cl} \) (mmol/kg DM) (Tucker et al., 1988). To convert % dry matter of the ration to mmol/kg the following formula is used:

\[
\text{DCAD} = (438.98 \times \% \text{Na} + 255.74 \times \% \text{K}) - (282.06 \times \% \text{Cl})
\]

1.6.2.4 Anion gap

The Anion gap (AG) is similar to the DCAD in that in the blood, the total cation concentration (Na + K) should approximately equal the total anion concentration (Cl + HCO₃). Usually, the total cations exceed the total anions, so there is an anion gap. This gap is due to unaccounted-for anions from fixed acids, such as lactate. In metabolic acidosis, the AG increases because of increased production of fixed acids (Cunningham, 2002).

1.6.2.5 Strong ion difference

Another approach to acid-base chemistry proposes that \( \text{H}^+ \) concentration in body fluids is determined by pCO₂, strong ion difference (SID = sum of strong cation concentrations minus the sum of the strong anion concentrations) and the total concentration of non-volatile weak acid under normal circumstances (Stewart, 1983). Given the law of electroneutrality it was proposed that a decrease in SID below normal results in acidosis, or increase in \( \text{H}^+ \), and an increase in SID above normal results in alkalosis or a decrease in \( \text{H}^+ \) (Whitehair et al., 1995).
1.6.3 Evaluating electrolyte balance during heat stress

It has been established that heat stress will cause an increase in respiratory rate and sweating rate in cattle as they endeavour to reduce their heat load (Allen, 1961). Furthermore, the increase in panting associated with heat stress has been shown to cause respiratory alkalosis and possible compensatory metabolic acidosis during night time cooling (Dale & Brody, 1952; Schneider et al., 1988; Sanchez et al., 1994b). 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. In order to correct the acid-base imbalance, excretion of HCO$_3^-$ by the kidney increases and the rate of renal secretion of H$^+$ is depressed (Sanchez et al., 1994b). In alkalosis, K$^+$ will exchange with H$^+$ and enter cells to maintain electroneutrality. Potassium will also exchange with hydrogen in the renal tubules, and along with sweat losses, this urinary loss can lead to a total body deficit of K$^+$. Reductions in plasma K concentration during heat stress in cattle have been confirmed (Johnson, 1970; El-Nouty et al., 1980; Mallonee et al., 1985). Sodium is conserved as much as possible in the animal, being the major cation involved in water balance, but when there is low body K, there is less aldosterone release, and the main drive for reabsorption of Na from the urine is reduced. This can lead to further Na losses in the urine. El-Nouty et al. (1980) found that during prolonged heat stress, aldosterone concentrations in Holstein cows decreased, and associated with this were reduced concentrations of Na and K in the blood serum and of K in the urine. Na excretion in the urine was increased perhaps to aid in the conservation of K. West et al. (1991) found that both Na and K urinary excretion increased in a hot environment.
Supplementing animals with electrolyte formulations is not new. Recent research with electrolyte supplementation in cattle has been based on balancing DCAD in an attempt to reduce transport stress (Schaefer et al., 1992; Schaefer et al., 1997), increase DMI and milk yield in dairy cows (Tucker et al., 1988; Sanchez et al., 1994a), improve performance in growing steers (Ross et al., 1994) and reduce the effects of heat stress in dairy cows (Schneider et al., 1984; Schneider et al., 1986; West et al., 1991; West et al., 1992; Sanchez et al., 1994b).

Schaefer et al. (1997) reviewed and collated data from various experiments and demonstrated that a positive effect of antemortem electrolyte therapy exists on live and carcass weight loss in transported and handled cattle. Pre- and post- transport treatment significantly improved hot carcass yield of cattle and decreased liveweight loss from 6.7% to 4.9% in pre transport treated cattle (P < 0.001). It was concluded that at least partial attenuation of live weight and carcass loss as well as meat quality improvements were attributable to electrolyte supplementation (Schaefer et al., 1997). No indication was given as the composition or dose rates of electrolytes involved in these reviewed experiments.

Similar reductions in liveweight loss were reported by Schaefer et al. (1992), who studied the effect of electrolytes in drinking water pre-slaughter with respect to lairage time. In this experiment cattle were given 0, 12, 24 or 36 h in lairage before slaughter. Half the animals in each group had access to electrolyte drinking water, the other half were without food and water. The electrolyte solution contained (weight vol\(^{-1}\)) 0.02% NaCl, 0.02% KHCO\(_3\), 0.01% magnesium sulphate, and 0.005% each of alanine, lysine, phenylalanine, glutamate, tryptophan, methionine, isoleucine, leucine and valine mixed in a 5% glucose solution. Electrolyte treated animals retained 1.1, 1.7 and 1.9%
more live weight relative to the respective 12-, 24-, and 36-h lairage treatment without electrolyte (P = 0.001). There was no difference in any measured blood gas variable or serum electrolyte. There were significant decreases in urine Na and K concentrations and increases in Cl urine concentrations in those animals given electrolyte supplements. These differences may be attributable to hydration alone, and control animals given access to water may have given similar results as electrolyte supplemented animals. Parker et al. (2003), in a study of Bos indicus steers transported for 48 h without feed and water, concluded that the primary challenge to the animals was the elevation of total weak acids via an increase in plasma albumin concentration as a result of dehydration, and offering electrolyte solutions to dehydrated, transported, nutrient deprived and stressed Bos indicus was unlikely to resolve the physiological stressors any more than water alone.

Research into the effects of altering DCAD without any physical stressors in an attempt to improve productivity has been undertaken with dairy cows and growing steers (Tucker et al., 1988; Ross et al., 1994; Sanchez et al., 1994a). The concept of manipulating DCAD in an attempt to improve performance in cattle was first evaluated by Tucker et al. (1988), who fed lactating dairy cows diets with DCAD values of -100, 0, +100, +200 mmol/kg of dietary DM by manipulating Na, K, and Cl concentrations in the diets. The study found that the diet with the +200 DCAD improved DMI by 11% and milk yield by 9% compared with -100 DCAD diet, independent of the individual mineral elements used to vary DCAD. Blood pH and HCO₃⁻ increased linearly with increasing DCAD, presumably in response to either increased systemic bicarbonate generation accompanying the absorption of Na and K from the GI tract or a reduction in systemic free proton generation as Cl absorption decreased. The experiment concluded that it was the changes in DCAD rather than the manipulation of individual ions which resulted in the observed responses and the improvement in acid-base status.
Following on from this work, Sanchez et al. (1994a) evaluated the interactions of Na, K and Cl on lactation, acid-base status and mineral concentration in dairy cows. The objectives were to determine responses to graded dietary concentrations of Na, K and Cl and to cation anion difference. The study concluded that interrelationships between ions were abundant and were related to acid-base status and mineral element concentrations in plasma, whole blood and milk. Overall lactational performance was highest when DCAD was between +300 and +500 mmol/kg of dietary DM, somewhat higher than the +200 mmol/kg DM reported by Tucker et al. (1988). Intermediary to these two results, a DCAD in the range of +150 to +300 mmol/kg seemed to provide normal homeostasis for growing steers (Ross et al., 1994). Again, this study showed that increases in blood pH and HCO$_3^-$ were associated with increases in DCAD from 0 to +450 mmol/kg DM after 28 days on supplement. After 84 days on supplements, the increase in blood pH and HCO$_3^-$ was quadratic in nature with +150 mmol/kg DM diet showing the maximum response. By day 84, feed intake had increased linearly with increasing DCAD. There is no indication given as to the mechanisms which may lead to an increase in feed intake.

Electrolyte supplementation and DCAD manipulation has also been undertaken in cattle during periods of heat stress (Schneider et al., 1984; West et al., 1991). Increasing the dietary cation anion balance or SID, results in an alkalosis. A concern may arise in providing a potentially alkalotic animal with more alkalising ions, as would be the case if supplementing with increased DCAD during the heat stress (Schneider et al., 1984). Schneider et al. (1984) suggests that lactating dairy cows are extremely effective at withstanding dietary challenges to acid-base homeostasis. However, increasing SID (in the form of NaHCO$_3$) may be beneficial because the plasma pool of HCO$_3^-$ is continuing to be reduced via urinary excretion (Schneider et al., 1984). Furthermore, the reduced HCO$_3^-$ pool may lead to a shortage in HCO$_3^-$ available for
buffering in the rumen via salivary secretion. Niles et al. (1980) showed lower ruminal pH in heat stressed dairy cows. Therefore, dietary supplementation with bicarbonate salts may be warranted to reduced ruminal acidosis (Schneider et al., 1984). Furthermore, increasing SID may be beneficial during heat stress and respiratory alkalosis because excretion of bicarbonate must be accompanied by the excretion of a cation. Sodium or potassium are possibilities; however, sodium is more likely to be required (Sanchez et al., 1994b). The increased demand of sodium for renal excretion and of potassium for sweating are consistent with the idea that dietary requirements of each increase with heat stress (Sanchez et al., 1994b).

In a study with lactating Holstein cows suffering heat stress, West et al. (1992) concluded that cows responded to increased dietary cation anion balance with greater dry matter intake and that this increase was independent of the cation source (Na or K) used to increase the DCAD. The same study also hypothesised that greater blood buffering capacity, indicated by blood base excess, a higher blood pH and bicarbonate content, may be responsible for the improved feed intake. Contrary to this work, Schneider et al. (1984) found that supplementing with 0.85% NaHCO₃ did improve feed intake of dairy cows under heat stress but had no effect on blood pH, HCO₃⁻, pCO₂, total CO₂, or ratio of HCO₃⁻ / pCO₂. This study also reported that supplementing with 1% KHCO₃ had negative effects on total feed intake and milk yield. Schneider et al. (1986) went on to evaluate the effects of source and quantity of dietary Na (NaHCO₃ and NaCl) and total diet K quantity on acid-base status, production, and mineral metabolism in lactating dairy cows during naturally occurring heat stress. Blood HCO₃⁻ and total CO₂ concentrations were higher in cows supplemented with NaCl (0.73% of DM) and NaHCO₃ (1% of DM) but there was no change in HCO₃⁻ / pCO₂ ratio. Higher dietary K (1.8% of DM) increased blood pH and HCO₃⁻ / pCO₂ ratio. Daily DMI was not
affected with additions of NaHCO$_3$ or NaCl, whereas cows receiving 1.8% total dietary K consumed 4.5% more dry matter than cows receiving 1.3% K (P < 0.05).

Similar studies have been undertaken with swine fed in high ambient temperatures (Haydon et al., 1990). In this study pigs were fed DCAD diets of either 25, 100, 175, 250, 325 or 400 mmol/kg DM. Live weight gain and daily feed intake measured over the entire growing-finishing period (21-105kg) improved linearly (P < 0.03) with increasing DCAD. Similarly blood pH, HCO$_3^-$, total CO$_2$, Na concentration, and base excess increased linearly as DCAD increased.

There is no indication that electrolyte supplementation has any effect on body temperature during heat stress in cattle. However, supplementing with 0.6% KCl in chickens’ drinking water was shown to reduce body temperature during heat stress in 5 to 7 wk old male chicks by as much as 1.2°C (Ait-Boulahsen et al., 1995).

In conclusion, responses when supplementing with electrolytes or increasing DCAD have been variable. In dairy cows it has been shown that increasing DCAD can improve blood buffering capacity by increasing blood pH and HCO$_3^-$ concentrations (Tucker et al., 1988; West et al., 1992; Ross et al., 1994) as well as increase feed intake (Schneider et al., 1984; West et al., 1991). However, other studies have shown no response in blood buffering capacity (Schneider et al., 1984) and variable effects on feed intake depending on the amount and source of electrolyte (Schneider et al., 1986). Lactating dairy cows are subjected to a continual loss of cations through milk. Responses to electrolyte supplementation during heat stress in dry cows and steers may be very different and little research has been undertaken in this area. No scientific literature exists as to the physiological effects supplementing electrolytes may have on cattle that have been exposed to prolonged periods of heat stress without night time cooling as can occur on live export vessels. Concerns exist with supplementing a bicarbonate buffer to an already potentially alkalotic animal (Schneider et al., 1984) and
this may be of even greater concern if animals remain under respiratory distress for longer periods.

1.7 Nutrition

1.7.1 Metabolism and heat production

Heat is a byproduct of all metabolic processes (Cunningham, 2002). The metabolic heat of maintenance is the heat generated from chemical processes for;

- basal metabolism for sustaining basic life processes
- voluntary activity and obtaining nutrients including the muscular activity of seeking and obtaining food, the processes of digestion, absorption, conversion of food into metabolisable forms, and the formation and excretion of waste products, and
- combating of external stressors related to an immediate and direct of stress on the animal.

Once the energy requirements of maintenance are met, energy is available for production i.e. growth and milk production. The metabolic heat of production is the heat generated from these productivity processes (Sparke et al., 2001).

1.7.2 Feed ingredients and metabolic heat

Digestion and metabolism of nutrients creates heat. Heat increment is defined as the increase in heat production following consumption of food by an animal in a thermoneutral environment. Included in heat increment is the heat of fermentation and energy expenditure in the digestive process as well as heat produced as a result of nutrient metabolism (Conrad, 1985). Feed ingredients influence metabolic heat by way of their individual characteristic heat increment and their influence on DMI (Sparke et al., 2001). There is greater heat production associated with metabolism of acetate
compared with propionate; therefore, fibrous ingredients, which ferment in the rumen to produce acetate, have a higher heat increment than concentrates which produce a greater proportion of propionate. Ingredients with the lowest heat increments are fats and oils. This is followed by oilseeds, grains or concentrates, then the roughages and forages with increasing heat increments associated with decreasing digestibility (Sparke et al., 2001). During periods of hot weather where cattle are able to satisfactorily thermoregulate their body temperature by dissipating excess body heat, theoretical heat production assessments favour the use of feed ingredients with a lower heat increment or diets with a lower roughage content and higher fat or concentrate content (West, 1999). Nutrient manipulation to reduce heat load in cattle will be discussed in a later section.

1.7.3 Effects of heat stress on nutrient acquisition and metabolism

As already discussed in a previous section, a reduction in feed intake is an immediate response to heat stress (Conrad, 1985). Under hot conditions, cattle will reduce DMI and consequently heat generated from rumen fermentation and metabolism, in an attempt to maintain homeostasis (Sanchez et al., 1994b). Reduced feed intake results in less metabolisable energy (ME) being consumed (Beede & Collier, 1986). Additionally, heat stress and lower feed intake reduce gut motility, rumination and rate of passage of ingesta (Attebery & Johnson, 1969; Warren et al., 1974). This results in increased gut fill and mean retention time (Schneider et al., 1988). It has been surmised that increased retention time may lead to improved digestibility (West, 1999). Mean total-tract retention time for cattle was 36.6 and 43.2 h respectively when the ambient temperatures were 18 and 32°C (Warren et al., 1974). However, Beede & Collier (1986) suggest that the advantage in ruminal digestion is largely offset by lower feed intake, resulting in less net total nutrients being available to the thermal stressed animal. In
general, the less digestible the diet fed to a heat stressed animal, the greater will be the rate and extent of reduction in consumption (Beede & Collier, 1986).

Although digestibility of dietary energy is enhanced in hotter environments, the efficiency of utilisation is reduced. This is due to higher maintenance requirements for heat stressed animals resulting from elevated body metabolism and activity, such as increased respiration, to alleviate excess heat load (Beede & Collier, 1986). For example, accelerated panting may increase maintenance requirements by 7 to 25%, depending on intensity (NRC, 2000). The greater the expenditure of nutrient energy on maintenance and production, the greater the body heat produced.

### 1.7.4 Nutritional manipulation to reduce heat load

Some controversy exists in the literature and practical situations, as to the feeding of concentrates versus roughages to heat stressed cattle. Many experimental studies have been undertaken to assess dietary roughage and grain content and their effects on heat stress in cattle (e.g. Gaughan et al., 1997; Mader et al., 1999). Theoretical heat production assessments favour the use of diets with low heat increments. The lower fibre and higher concentrate diets may reduce metabolic heat production and contribute to a lower heat load in the animal. Furthermore, the low fibre, high grain diets provide more efficiently used end products, which contribute to lower dietary heat increment (Sparke et al., 2001). This principle is supported for lactating dairy cows by both Beede & Collier (1986) and West (1999). However, Mader et al. (1999) suggested that it is the reduced dry matter intake (DMI) leading to reduced metabolisable energy intake (MEI) which leads to less metabolic heat production and lower body temperature. These conclusions were made after the effects of feedlot diet roughage level on MEI and DMI level were demonstrated under simulated feedlot conditions. Steers were assigned to 3 diet treatments under hot or thermoneutral conditions. Diet treatments were a 6%
roughage diet fed ad libitum (HE), or 90% of ad libitum (RE), or a 28% roughage diet fed ad libitum (HR) such that metabolisable energy intake (MEI) approximated the MEI of the RE group. DMI declined significantly for all diets under hot conditions, and significantly more so for HR diet, with corresponding MEI decline. Under hot conditions steers fed the HR diet had significantly lower body temperature and RR than those fed the HE or RE diets. Steers fed the RE diet had significantly lower body temperatures than the steers fed HE diets. The data suggests that under hot environmental conditions cattle with reduced DMI fed a higher roughage diet maintain a lower core body temperature. In essence, it is the reduced DMI leading to reduced MEI which leads to less metabolic heat production and hence lower body temperature (Mader et al., 1999). This was enhanced by feeding high roughage diet, contrary to suggestions by West (1999) and others who proposed feeding high roughage diets would increase body temperature due to the higher heat increments of those foods.

In another feedlot study by Gaughan et al. (1997), Bos taurus steers were exposed to thermoneutral or hot climatic conditions while being fed 40, 25 and 10% roughage diets for 5, 5 and 7 days respectively. Differences in DMI and MEI for hot and thermoneutral groups were only observed when cattle were fed the 10% roughage diet. Cattle fed the 10% roughage diet in hot conditions had an 18.3% reduction in feed intake (P < 0.05). It was concluded that under hot conditions, higher roughage diets (≥ 25% of diet DM), which are lower in ME density, appear to contribute less to metabolic heat load, and hot conditions compromise the ability of feedlot cattle to adapt to high energy (10% roughage) diets.

Following on from this work, it has been suggested that limit feeding of cattle, or reducing DMI and subsequently MEI, may be a successful tool in reducing heat load (Holt et al., 2000; Mader et al., 2002). Mader (2002) used Bos taurus crossbred steers in a study to investigate the effects of level and duration of limit feeding feedlot cattle in a
natural hot environment. Cattle were fed 75% of ad libitum DMI for either 21 or 42 days. Restricting feed intake for both 21- and 42-days reduced tympanic temperature when compared to ad libitum treatment groups under hot environmental conditions. Temperature reductions exceeded 0.5°C (P < 0.05) depending on the time of day. It was hypothesised that the reduction in tympanic temperature was most likely due to a reduction in metabolic heat load and/or a concurrent reduction in metabolic rate.

It has also been suggested that the time of feeding may be manipulated so as to reduce heat load (Gaughan et al., 1996). Purwanto et al. (1990) found that heat production increased during feeding and reached a peak 3 h after feeding in lactating dairy cows irrespective of stage of lactation. Minimum heat production was observed in the early morning before feeding. Reinhardt and Brandt (1994, as cited by Gaughan et al., 1996) reported that ruminal fermentation of high quality grain diets peaked within 12 h of consumption. This is supported by Mader et al. (1997), who suggests that ruminal fermentation of most high grain diets peaks within a few hours after consumption.

The effect of high temperature and feeding regime on DMI, MEI, RR, PR and rectal temperature of feedlot steers was examined by Gaughan et al. (1996). It was hypothesised that because ruminal fermentation of high quality grain diets peaks within 12 hrs after consumption (Reinhardt & Brandt, 1994, as cited by Gaughan et al., 1996), morning feeding would result in maximum heat from fermentation during the hottest part of the day. Therefore, cattle consuming the highest energy components of their diets during the late evening or at night during summer may be better able to cope with heat load and utilise metabolisable energy more efficiently than those fed in the morning immediately prior to maximum daily heat load. To evaluate this hypothesis three feeding times were imposed on 6 Hereford steers under either hot or thermoneutral conditions in climate controlled rooms. Cattle were either fed at 0800 (AM), 1600 (PM)
or a split fed regime at 0900 and 1600 (SP). The feeding treatments were a 14% roughage diet for the AM and PM fed animals and for the SP regime approximately one third of the dietary intake was provided from a 30% roughage diet fed at 0800 and the remaining dietary intake provided from a 6% roughage diet fed at 1600. Total diet consumed in the SP group approximated the composition of the 14% roughage of the other two treatments. Results indicated that SP fed steers under hot conditions had lower minimum RT than AM and PM fed steers. Steers fed PM under hot conditions had significantly lower MEI than other treatments, while steers fed AM or SP under hot conditions were able to maintain DMI at a level equal to or greater than those under thermoneutral conditions. The implications being, that under hot conditions, minimum RT may have a greater influence on subsequent feed intake rather than previous maximum RT. Cattle consuming large quantities of feed in the afternoon (PM) may not experience the degree of RT reduction normally associated with night time cooling. SP feeding under hot conditions resulted in DMI equal to or greater than any thermoneutral diet regime. Intakes appear to be maintained as a result of lower mean and minimum RT (Gaughan et al., 1996).

1.7.5 Pelleted feeds

Straw based pelleted feedstuffs are routinely used on long haul live export voyages. The effect pelleting has on roughages and grains was reviewed by NRC (2000). Pelleting of roughages improves daily feed intake in cattle by 11%. Grinding and then pelleting straw increased intake by 37% in bulls. Digestibility of pelleted roughage is decreased in proportion to the intake increase. Digestibility decreases are usually attributed to a faster rate of passage of food, with more digestion occurring in the hindgut. In contrast, pelleting roughages results in lowering of the heat increment so
that the net dietary energy from these roughages is often higher than for the parent product.

1.8 Other methods of alleviating heat stress

1.8.1 Cooling drinking water

It is well documented that high environmental temperatures decrease feed intake and increase water intake of cattle. In a warm environment, non-lactating cattle require up to 65L of water per day and preferably the drinking water should be no warmer than ground water temperature (Hahn, 1985). Research has been undertaken to assess the effect of chilling drinking water on heat load in cattle, with particular attention given to rectal temperature and respiratory rate. Purwanto et al. (1996) undertook a study to determine the effect of heat dissipation from drinking water (8L at 10, 20 and 30°C) on the heat balance and thermoregulatory response of 4 dairy heifers housed at 24, 29 and 34°C. This study found that the RR, skin temperature, and rectal temperature all decreased with decreasing drinking water temperature but returned to previous values 120 to 180 minutes after watering. Rectal temperature reached a minimum 20 minutes after watering. Environmental temperature and drinking water temperature did not influence heat production and heart rate. Similar transient reductions in body temperature of dairy cows offered chilled drinking water have been found by other researchers (Lanham et al., 1986; Milam et al., 1986; Stermer et al., 1986). Milam et al. (1986) offered drinking water ad libitum at 10 or 28°C at 1400 h (after the animals had been denied water from 0900 h) to investigate the effects on RR, body temperature, DMI and milk production during natural hot conditions. There was found to be no significant difference in water intake between treatments, although cows drinking the 10°C water tended to drink less. No differences were found between treatments in RR
and rectal temperature. However, decreases in tympanic temperatures of 0.4 and 0.2°C (after 10 and 28°C water intake respectively) and the time taken for maximum decreases to occur (22.2 and 11.7 minutes) were significantly different (P < 0.05). Cows that drank the 10°C drinking water also had significantly increased DMI and milk yield. Both Lanham et al. (1986) and Stermer et al. (1986) reported decreases in RR and rectal temperatures after dairy cows were offered chilled drinking water; however, decreases were again only transient.

Under severe conditions (mean maximum 38°C, mean minimum 21°C, range 12-48°C), cooling the drinking water of Hereford cattle from about 32 to 18°C increased growth rate and decreased water intake. Brahman cattle supplied with warm water grew at the same rate as Herefords with cooled water (Ittner et al., 1951). Using similar water temperatures, Lofgreen et al. (1975), showed that cooled water (18.3°C) improved weight gain, DMI and energy utilisation of British cattle but had no beneficial effects on Brahman-British crossbreeds, which performed as well with warm water (32.2°C) as British cattle receiving cooled water. Warm water did not depress feed intake or energy utilisation in crossbred cattle as it did with the British cattle.

1.8.2 Wetting cattle

Research into wetting cattle as a means of cooling has been undertaken in the dairy and beef industries. Flamenbuam et al. (1986) conducted a detailed study into the effects of cooling dairy cattle by sprinkling and forced ventilation. This study used short periods (10, 20 and 30 sec) of large droplet spraying followed by forced ventilation at 3 m/s for 4.5 minutes thus attaining high evaporative cooling close to the skin and reducing the increase in ambient relative humidity. Results indicated that over 45 minutes the 20 second wetting regime reduced core body temperature by 1°C. This decrease was halved if stocking density was increased from 3.5 m² to 1.9 m², which
would be more consistent with shipboard conditions. Cooling for 30 minutes 5 times per day resulted in significantly reduced rectal temperatures ($P < 0.01$). Ambient conditions averaged 28.6°C and 65% (23.5°C WB) during experiments. Significant reductions in rectal temperature and respiratory rate have also been experimentally achieved by wetting cattle in the feedlot industry (Gaughan *et al.*, 2001). This is more pertinent to the live export industry in that it was carried out in environmental chambers where THI was 80. Results indicated that cessation of cooling may result in significant heat stress which may be of concern to cattle on board livestock ships as wetting regimes are irregular.
1.9 Aims and hypotheses

The broad aims of this PhD thesis were firstly to define and characterise the physiological response of both *Bos taurus* and *Bos indicus* to prolonged periods of continuous heat and humidity such as might be experienced during live export. Secondly, to develop interventions and/or strategies to help ameliorate any detrimental effects that these physiological responses may have on body homeostasis. Interventions primarily focused on electrolyte supplementation and nutritional manipulation.

The primary hypothesis is that prolonged and continuous heat and humidity will cause cattle to become heat stressed and that this heat stress will produce strain on the animals’ physiological responses causing a displacement of various internal parameters from their resting or ground state. It is hypothesised that these will include:

- A rise in core body temperature
- Reductions in feed intake and increases in water intake
- Acid-base imbalances, in particular a primary respiratory alkalosis
- Plasma electrolyte imbalances
- Haematological imbalances

Given the primary hypothesis to be proven correct, the secondary hypothesis is that the above displacements can be ameliorated to some extent by electrolyte supplementation and nutritional manipulation.
Chapter 2: Materials and Methods

2.1 Environmental rooms

All experiments conducted at Murdoch University took place in two climate controlled rooms. Each room had an electric duct heater (Email Grimwood PPN 400T240) and humidifier (Carel SD333P0415) capable of maintaining air temperature and moisture content as high as 60°C and 40 g of moisture per kg of dry air. The air flow rate was sufficient to study up to 1250 kg of beast per room, working on a fresh air inflow of 0.2 L/sec/kg body weight. The system was controlled by independent electronic temperature and absolute moisture controls, with one sensor in each room providing feed back control. The rooms were designed to provide a degree of control accuracy in the order of ± 1K temperature and ± 10% relative humidity. A control panel and Light Emitting Diodes (LED) display was mounted outside each room. Settings for dry bulb temperature and absolute moisture content could be manually entered on control panel and values inside rooms were shown on the LED display. Three animals were individually penned in each room. Each animal had a pen space of 2.3 m², with additional space in each pen occupied by a galvanised iron feeder trough (500 x 250 x 250 mm) and a water bucket (25 L). The animals could not take feed or water from their neighbours but had room to turn around and lie down (Figure 2.1 and Plate 2.1).

A mezzanine level was situated above the pens, which was occupied by 3 x 20 L plastic reservoir buckets which were used to top up respective drinking water buckets in the pens below. Researchers could also monitor animals from above and enter pen 3 without disturbing animals in the other pens.
Figure 2.1 Layout of pens within the climate controlled rooms.

Plate 2.1 Sampling inside climate controlled rooms.
In all climate control room studies, animals were exposed to 24 hours of continuous light for the duration of experiment. Natural light was negligible and artificial light was provided by fluorescent globes on the ceiling of rooms.

The walls of climate controlled rooms were double brick and a heavy duty insulation panel was fixed in front of the exit door in each room so heat loss to the outside environment was minimal. The mean radiant temperature was equal to air temperature, there were no windows and the average surrounding surface temperature was equal to the average air temperature. Airflow direction was from the hot air inlet to the exhaust fan which was situated in the ceiling above pen 3.

Sawdust was used as bedding in the climate controlled rooms and this was topped up or changed if it became particularly wet or if the room ammonia (NH₃) concentration exceeded 15 ppm.

### 2.2 Animals and management

All experimental work was approved by the Murdoch University Animal Ethics committee.

Experimental animals were selected based on weight and temperament. The maximum tolerable weight limit for each climate controlled room was 1250 kg of beast. For animals to be able to turn around and lie down comfortably, an individual weight range of between 300 and 400 kg was established for all *Bos taurus* experiments and 280 to 380 kg for *Bos indicus* experiments. Selection of experimental animals for good temperament was essential for both animal and researcher welfare given the close confines of the climate controlled rooms and the intensive sampling procedures.

All experimental animals were treated for internal and external parasites with 0.5 mg/kg Cydectin (Moxidectin 5 g/L) pour-on (Fort Dodge, New South Wales, Australia) and vaccinated against leptospirosis with a 2 ml subcutaneous injection of Leptoshield
vaccine (Commonwealth Serum Laboratories, Australia) prior to intensive handling. Animals were manually checked for pregnancy and oestrous cycles were synchronised with two 25 mg intramuscular injections of Lutalyse (dinoprost 5 mg/ml) solution (Pharmacia Animal Health, New South Wales, Australia) 14 days apart. For the 2 weeks before experiments began, cattle were handled daily in a crush to accustom them to the intensive sampling and monitoring they would receive in the climate rooms.

### 2.3 Core body temperature

When core body temperature ($T_c$) was required to be monitored during experiments animals were fitted with either temperature telemeters (Datamet, Potchefstroom, South Africa) or temperature loggers - Stowaway XTI (Onset Computer Corp, Massachusetts, USA). Radio signals from the temperature telemeters were received on an AR8000 receiver (AOC, Japan) interfaced with a personal computer running dedicated software. The software ran continuously and sequentially scanned each frequency, measured time taken to receive 30 pulses from the telemeter (to millisecond resolution), converted pulse period to temperature using individual calibration coefficients determined prior to implantation, and stored temperatures to disk in real time. Real time core body temperature could then be monitored on a computer running in an adjoining room. The temperature loggers were specially modified with a range of 32 to 46°C and resolution of 0.04°C. Accuracy after individual calibration was equal to 0.04°C. Scan interval was set to 10 min and data was downloaded after retrieval.

Each unit was covered with several layers of inert wax (Elvax). Incorporated in the wax covering was a 40 cm length of 0.4 mm diameter non absorbable suture material (Vetafil Bengen, Germany). When completed all units (telemeters and loggers) had external dimensions of about 50 x 45 x 20 mm and a mass of 40 g. Units were
sterilised for 24 h by immersing in 0.5% w/v Hibitane Disinfectant (Coopers Animal Health, New South Wales, Australia) in 70% v/v alcohol prior to implantation (Plate 2.2).

All units were surgically implanted into the peritoneal cavity in the region of the right paralumbar fossa two weeks prior to experiment (Plate 2.3). Surgery was performed with the animals standing. Epidural nerve blocks provided sedation plus some analgesia as well as local anaesthesia; 0.04 mg/kg xylazine (Ilium/Troy Laboratories, New South Wales, Australia) was made up to 2 ml with 2% plain lignocaine (Ilium/Troy Laboratories, New South Wales, Australia) and injected into the intervertebral space between the first and second coccygeal vertebrae using a 20 gauge 1½ inch needle. Lumbar paravertebral nerve blocks provided anaesthesia (Cakala, 1961). A 20 cm skin incision was made in the region of the right paralumbar fossa and muscle layers were blunt dissected down to the level of the peritoneum. An incision was made into the peritoneum big enough for the units to fit through. Each unit was suspended from the peritoneal wall from the suture material which was incorporated in the wax coating. The suture material was sutured in placed as part of the muscle layer. A continuous suture pattern using Ethicon Vicryl® (Johnson and Johnson medical, New South Wales, Australia) was used to close peritoneum and then muscle layers. A simple interrupted suture pattern using heavy Vetafil (0.4 mm diameter; Vetafil Bengen, Germany) was then used to close the skin incision. Animals were treated at the time of surgery with 20 mg/kg oxytetracycline 200mg LA intramuscularly (Ilium/Troy Laboratories, NSW, Australia) and 2.2 mg/kg Flunixin intramuscularly (Ilium/Troy Laboratories, NSW, Australia). Post surgery, heart rate (HR), respiratory rate (RR), core body temperature, rumen motility and the wound site were monitored daily for any signs of post operative complications. The same technique was used to surgically retrieve the units after the experiments.
Plate 2.2 Temperature telemeters

Plate 2.3 Surgical placement of temperature loggers and telemeters into the right peritoneal cavity.
For radiotelemetry $T_c$ data, the files that were recorded to disk were scanned manually for spurious data which were removed manually. Data were then smoothed (using a binomial smoothing procedure) and 30 min averages calculated for all analyses performed. For temperature loggers, core body temperature was logged temperature every 10 minutes. Data was downloaded using BoxCar Pro software (Onset Computer Corp, Massachusetts, USA) and 30 min averages were calculated for all analyses performed.

Both temperature telemeters and temperature loggers were calibrated pre and post surgery against a high accuracy certified quartz thermometer (Quat 100, Heraeus, Hanau, Germany) in an insulated water bath over the range of 37.5 to 42°C.

### 2.4 Indwelling jugular catheters

When experiments required intensive blood sampling, indwelling jugular catheters were sutured in place the day before the experiment began. Twenty four hours prior to procedure, 90 cm lengths of Teflon tubing; PTFE ID 1.2 mm, OD 1.8 mm (Jepson Bolton, England) were sterilized by immersing in 0.5% w/v Hibitane Disinfectant (Coopers Animal Health, New South Wales, Australia) in 70% v/v alcohol. An epidural was used for sedation (as described above) and 2% plain lignocaine (Ilium/Troy Laboratories, New South Wales, Australia) infused subcutaneously around the venipuncture and suture sites. A 12 gauge Dwellcaths catheter (Western Biomedical, Western Australia, Australia) was inserted into the left jugular vein and the Teflon tubing was passed through the catheter and 20 cm caudally into the vein. The catheter was then removed and a tab of Elastoplast (Smith and Nephew, Victoria, Australia) was attached to the Teflon and sutured to the skin. The 70 cm of Teflon remaining was covered with 70 cm Nylex clear vinyl tubing (Nylex plastics, Australia) and an 18
gauge needle glued (methacrylate) and capped onto the end of the Teflon. The 70cm of covered Teflon tubing was then sutured to the side of the neck every 20cm so the capped needle hub was at the top of the neck for easy sampling access (Plate 2.4).

### 2.5 Sample collection and measurement

Body weights (BW) were measured using an FX 1 electronic weighing system (Iconix, New Zealand). Heart rate (beats per minute) was assumed to be equal to the pulse rate and was measured by manual palpation of the coccygeal artery. The number of pulses over 20 sec were counted and the figure converted to pulses per min. Respiratory rate (breaths per minute) was calculated by counting the numbers of breaths each animal took over 30 sec and the figure converted to breaths per min. At the same time respiratory character was assessed and a subjective respiratory score assigned to each animal (Gaughan, 2004). A rectal thermometer (GEON Corp., Taiwan), inserted approximately 5 cm into the rectum, was used to measure rectal temperature.

A total of approximately 31 ml of jugular venous blood was collected from the indwelling jugular catheter at each collection. At the start of each collection, the first 5 ml of blood was discarded then blood was collected in 10 ml syringes and emptied into 2 x 9 ml tubes containing lithium heparin (Becton Dickinson Pty Ltd, New South Wales, Australia), 1 x 9 ml z serum clot activator (Becton Dickinson Pty Ltd, New South Wales, Australia), 1 x 2 ml EDTA (Sarstedt Australia, Technology Park, South Australia, Australia) and 2 ml into a heparin coated syringe. Blood samples were immediately placed in ice after collection. The catheter was then flushed and refilled with heparinised saline.

The samples containing the lithium heparin were used for analysis of plasma electrolytes. The z serum clot activator samples were used for hormonal analysis, the EDTA samples for complete blood counts and heparin coated syringe for blood gas
Plate 2.4 Indwelling jugular catheter.
analysis. The lithium heparin and z serum clot activator tubes were centrifuged at 300 rpm for 15 min and plasma and serum removed and stored at -20°C within 2 h of collection for analysis at a later date. All air was removed from the blood gas syringes and they were capped and analysis performed within 0.5 h of collection. EDTA samples were refrigerated and analysed within 24 h of collection.

Voided urine samples were collected into 50 ml plain urine pots (Sarstedt Australia, Technology Park, South Australia, Australia) and immediately placed on ice. Urine was removed from each sample and stored at -20°C for analysis of urine electrolytes at a later date.

Venous blood pH, partial pressure of carbon dioxide (pCO₂), partial pressure of oxygen (pO₂), actual base excess (ABE) and bicarbonate (HCO₃⁻) were measured using a blood gas analyser (Radiometer Pacific ABL 5 Blood gas system, Copenhagen, Denmark).

Plasma and urine concentrations of Na, K, and Cl were measured using diluted ion selective electrode method on an Olympus AU400 Automated Chemistry Analyser (Olympus Analysers, Tokyo, Japan). Urea, creatinine, Mg and Ca concentrations in plasma and urine were measured using respective liquid reagents (Integrated Sciences, Melbourne, Australia) on an Olympus AU400 Automated Chemistry Analyser (Olympus Analysers, Tokyo, Japan). Packed cell volume (PCV) and total plasma protein (PP) were measured by micro hematocrit.

Haematological parameters were measured using an ADVIA 120 version 2.2.06 (Bayer diagnostics, Dublin, Ireland). The following variables were measured; white blood cell count (WCC), red blood cell count (RBC), platelets (PLT), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).
Urine pH and specific gravity were measured within 1 h of collection. Urine pH was measured using a Shindengen ISFET pH meter (Japan) and specific gravity with a Leica VET 360 Veterinary Refractometer (USA).

The fractional excretion ratio (FER) for each urinary electrolyte was calculated from the following equation (King, 1994):

\[
\text{FE}_x = \frac{([X]_{\text{urine}} \times [\text{Cr}]_{\text{serum}})}{([X]_{\text{serum}} \times [\text{Cr}]_{\text{urine}})} \times 100
\]

Where \( X \) = electrolyte under investigation

\([ ]_{\text{urine}} = \text{urinary concentration of electrolyte}\)

\([ ]_{\text{serum}} = \text{plasma concentration of electrolyte}\)

2.6 Environmental monitoring

During experiments, measurements were taken inside the rooms either three or four times daily to monitor temperature, humidity and air quality. Three times daily at 0800, 1300, and 1700 h when climate rooms were turned off and four times daily at 0800, 1300, 1700 and 2200 h when climate rooms were on. Measurements of carbon dioxide, dry bulb temperature and relative humidity were made using a Testo 445 VAC measuring instrument (Testo Australia, Victoria, Australia) and NH\(_3\) levels were monitored using a Neotox MK5 ammonia meter (Nutech Australia, Western Australia, Australia). Measurements were taken at cattle head height at the back of the pen 1 and 3, as well as at the air outflow point above pen 3. Wet bulb temperature (WBT; °C) was calculated from dry bulb temperature and relative humidity (Hemp, 1989).

2.7 Statistical analysis

A 5% level of significance was used throughout. Specific statistical analyses varied depending on experiment and are described in detail in relevant chapters.
Chapter 3: Physiological responses of *Bos taurus* to prolonged and continuous high heat and humidity

### 3.1 Introduction

It is well recognised that *Bos taurus* are susceptible to hot environmental conditions (Finch, 1986; Blackshaw & Blackshaw, 1994; Norris et al., 2003). It is also clear from long haul voyage reports to the Middle East (MAMIC, 2000a; MAMIC, 2000b) and personal communication with the live export industry (M. McCarthy, personal communication) that *Bos taurus* are more susceptible to heat stress than *Bos indicus*. This was confirmed by Norris et al. (2003) who found that on 4 voyages to the Middle East between 1998 and 2001 *Bos taurus* were the only animals to die from heat stress.

Physiological responses of *Bos taurus* to acute periods of high temperature and humidity have been well described in both the dairy and beef industries. Among these are increased RR (Schneider et al., 1988), increased $T_c$ (Gaughan et al., 1999), increased sweating rates (Allen, 1961), increased water intake (Winchester & Morris, 1956), decreased feed intake (Colditz & Kellaway, 1972), respiratory alkalosis (Dale & Brody, 1952; Bianca & Findlay, 1962; Schneider et al., 1988), metabolic acidosis (Sanchez et al., 1994b; West, 1999), and plasma electrolyte imbalances (El-Nouty et al., 1980). Collectively, these changes present a considerable challenge to the animal.

Previous studies on *Bos taurus* under conditions of high heat load have not examined the effects of continuous high WBT, with high humidity and little or no diurnal variation as can be experienced on board livestock vessels. It is hypothesised that such conditions would lead to severe heat stress and an accumulated EHL. It would
be expected that the heat stress conditions experienced by these animals would produce a more pronounced physiological response than previously reported in other studies. The experiments described here were undertaken to characterise the physiological responses of *Bos taurus* animals during periods of continuous and prolonged high temperature and humidity such as might be experienced during live export.

### 3.2 Aims

The aim of the two experiments described here was to investigate and characterise the physiological responses of *Bos taurus* to prolonged and continuous periods of high heat and humidity.

### 3.3 Hypotheses

*Bos taurus* animals will develop physiological responses when subjected to prolonged and continuous high heat and humidity. Physiological responses will include; decreased feed intake, increased water intake, acid base imbalances in particular respiratory alkalosis due to increased respiratory rate, increased core body temperature, and plasma and urine electrolyte imbalances. Furthermore, *Bos taurus* animals subjected to the environmental conditions described above will develop clinical signs of heat stress.

### 3.4 Materials and methods

For detailed materials and methods refer to Chapter 2.

#### 3.4.1 Experimental design

Two experiments (*Bos taurus* 1 and *Bos taurus* 2) were conducted in the climate controlled rooms with 6 *Bos taurus* heifers used in each. The length of the experiments
and environmental conditions (Table 3.1) were based on voyage reports collated from voyages to the Middle East during the northern hemisphere summer (MAMIC, 2000a; MAMIC, 2000b). *Bos taurus* 2 was essentially a replicate of *Bos taurus* 1 except that animals spent an extra day (day 1) in the climate controlled rooms at the beginning of the experiment under ambient conditions (Table 3.1). Therefore, animals in *Bos taurus* 1 spent a total of 14 days in the climate controlled rooms with an additional 1 or 2 days of sampling after they exited, and animals in *Bos taurus* 2 spent a total of 15 days in the climate controlled rooms with an additional 1 to 3 days of sampling after they exited. Each day of the experiments was from midnight to midnight. The heaters and humidifiers for the rooms were turned on at 0800 h on day 3, and new room conditions set at 0800 h each day. The WBT was reduced at 1200 h on day 11 for *Bos taurus* 1 and 2200 h on day 11 for *Bos taurus* 2. Rooms were turned off at 0800 h on day 14 for both experiments.

### 3.4.2 Animals and management

For *Bos taurus* 1 and *Bos taurus* 2, six heifers of appropriate BW and reasonable temperament were chosen. *Bos taurus* 1 were Angus and Angus-cross heifers selected from the Murdoch University herd, and ranged in bodyweight from 336 to 408 kg (366 ± 14 kg; mean ± SEM), weighed after 18 h off feed. The total weight of animals in each room was 1120 and 1148 kg. *Bos taurus* 2 were Murray Grey-cross and Angus-cross heifers from sale yard stock sourced from southern Western Australia. These animals ranged in bodyweight from 312 to 368 kg (331 ± 10 kg; mean ± SEM), weighed after 18
Table 3.1 Set wet bulb temperature of climate control rooms for *Bos taurus* 1 and *Bos taurus* 2.

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Set wet bulb temperature (°C)</th>
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<tr>
<td></td>
<td><em>Bos taurus</em> 1</td>
</tr>
<tr>
<td>1</td>
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<td>2</td>
<td>Ambient</td>
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<td>3-4</td>
<td>26</td>
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<td>28</td>
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<td>13</td>
<td>26</td>
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<tr>
<td>14-17</td>
<td>Ambient</td>
</tr>
</tbody>
</table>
h off feed. The total weight of animal in each room was 992 and 986 kg. *Bos taurus 1* ran from 27th April to 12th May 2002, and *Bos taurus 2* from 13th June until 29th June 2002. This time of year (late spring/early winter) meant that all animals were developing long hair coats and were considered to be winter acclimatised.

Animals were surgically implanted with temperature telemeters (Datamet, Potchefstroom, South Africa) 1 wk before the experiments started and indwelling jugular catheters were inserted the day before the animals entered the climate controlled rooms.

Animals had 7 days of adaptation to a commercial dietary cube; 8.6 MJ of ME, 11.9% CP, and 39.9% NDF per kg of DM (Macco Feeds, Western Australia, Australia). Full analysis and chemical composition of feed is shown in Appendix 1. Over the 7 days of adaptation feed on offer was 2.25% of BW (as fed). On day 1 of adaptation animals were offered 100% hay. On days 2 to 5 the percent of hay offered was reduced by 20% per day and the percent of pellet increased by 20% per day. On days 6 and 7 animals were offered 100% pelleted feed.

Upon entry into the climate controlled rooms, animals were randomly allocated to a specific pen for the whole of the experiment. Feed was divided into two equal amounts, given at 0700 and 1300 h daily. Residues were cleaned out and weighed before each morning feed. The previous day’s total feed intake was then calculated. Water was available *ad libitum*, in 25 L buckets. Water was topped up as necessary from reservoir buckets situated on the mezzanine level above the animals. Water buckets were weighed and cleaned out daily at 0830 h or more frequently if animals had defecated in a bucket. Total amount of water drunk was calculated once daily by subtracting the weighed residue of water each morning from the total amount of water given for the past 24 h.
3.4.3 Sample collection

Body weights were recorded on days 0, 16 and 22 for *Bos taurus 1* and days 0, 12, 16 and 22 for *Bos taurus 2* (after 18 h off feed but not water). Heart rate, RR and RT were measured and recorded 3 to 4 times daily at 0600, 1200, 1800 and 2200 h. At the same times environmental conditions (dry bulb temperature, relative humidity, and ammonia and CO₂ levels) were measured and recorded. Jugular venous blood and voided urine samples were collected at 0600, 1200, 2200 h on days 7 to 11 and once daily at 1200 h on all other days. Daily averages were calculated for all variables measured more than once daily.

3.4.4 Statistical analysis

For each separate experiment a two way ANOVA with animal and day as fixed factors was used to test for an overall change over days. When the overall effect of days was significant, Dunnett t-tests were used to compare each day with a control day or “pre-heat day”. Apart from Tc and BW, the pre-heat day was day 2 for *Bos taurus 1* and the average of days 1 and 2 for *Bos taurus 2*. Core body temperature changes were not statistically analysed for *Bos taurus 1* because measurements could not be recorded during ambient conditions or before climate control rooms were turned on (i.e. pre heat days). For *Bos taurus 2* the pre-heat day for Tc data was the average of days -3 to -1. The pre-heat day for BW was day 0 for both *Bos taurus 1* and *Bos taurus 2*. Unless otherwise stated, days on figures which were significantly different from the pre-heat day are represented by the symbol “a” for *Bos taurus 1* and “b” for *Bos taurus 2*.

Dunnett t-tests were performed to alleviate inflated probabilities of type 1 errors when examining which days were significantly different to normal ambient conditions.
A 5% level of significance was used throughout and all analyses were carried out using SPSS 11.0 for Windows.

3.5 Results

3.5.1 Environmental conditions

For all experiments conducted in the CCR, it became apparent that there was variation of WBT within a day greater than the CCR operating specifications. An average daily variation of approximately ± 2°C WB was due to the flushing of humidifiers and people entering and leaving the rooms. The within day variation did not affect the mean daily measured WBT and this remained within ± 1°C WB of the set daily WBT.

Figure 3.1 shows the measured WBT in the climate controlled rooms taken 3 to 4 times daily. Days 7 to 11 represent the hottest period of the experiments (set WBT 32°C). The maximum WBT measured was 32.6°C at 2200 h on day 8 for *Bos taurus 1* and 33.4°C at 1200 h on day 10 for *Bos taurus 2*. In general, environmental conditions were comparable with voyage conditions recorded on livestock voyages during the northern hemisphere summer (M. McCarthy, personal communication). Animals in *Bos taurus 2* were subjected to a slightly longer period of maximum WBT.

In general, environmental ammonia concentration increased as WBT increased and bedding deteriorated (data not presented). Ammonia concentration never exceeded 25 ppm for either *Bos taurus 1* or *Bos taurus 2*. When ammonia concentration neared 20 ppm, contaminated bedding at the rear of pens was removed and sawdust replaced. This happened on days 4, 8 and 12 for *Bos taurus 1* and on days 4, 10 and 14 for *Bos taurus 2*. 
Figure 3.1 Measured wet bulb temperature for *Bos taurus* 1 (○) and *Bos taurus* 2 (●).
3.5.2 Clinical responses

The clinical responses of the 12 Bos taurus heifers during the experiments were similar. At WBT at or above 30°C clinical signs of heat stress were seen. Animals exhibited open mouth panting, inappetence, drooling, reluctance or inability to rise, reduced rumen motility, increased licking of coat and general dullness including neurological signs with staring and glazed eyes.

3.5.3 Core body temperature

The mean $T_c$ calculated every half hour for both Bos taurus 1 and Bos taurus 2 is shown in Figure 3.2.i. Telemetry recordings for Bos taurus 1 started on day 3 when climate rooms were turned on and therefore, significant differences cannot be calculated as there was no data recorded during ambient or “normal” conditions. In order to gain an understanding of the normal circadian rhythm of $T_c$, telemetry recordings began 4 days prior to animals entering climate rooms in Bos taurus 2. There was a positive linear relationship between daily mean $T_c$ and daily mean WBT when the climate rooms were operating from days 4 to 14 ($R^2 = 0.85$ and $0.93$ for Bos taurus 1 and Bos taurus 2 respectively; Figure 3.2.ii).

The daily mean $T_c$ of animals in both experiments increased with increasing WBT (Figure 3.3.i). The maximum mean $T_c$ for Bos taurus 1 was 41.0°C (day 11 at 1100 h) and for Bos taurus 2, 41.2°C (day 10 at 0330 h). The maximum individual $T_c$ for Bos taurus 1 was 42.2°C (day 7 at 1430 h) and for Bos taurus 2, 41.9°C (day 10 at 2200 h). For Bos taurus 2 the daily mean $T_c$ was significantly increased on days 5 to 14 and day 17 when compared to the average of days -3 to -1 (ambient conditions). For Bos taurus 2, minimum $T_c$ was significantly increased on days 5 to 13 and maximum $T_c$ was significantly increased on days 5 to 15 and day 17 (Figure 3.3.ii). The daily circadian
rhythm (or range) in $T_c$ was maintained throughout the hot period at about 1°C for *Bos taurus* 2 (Figure 3.3.iii). After the hottest period the daily circadian rhythm increased and was significantly increased on days 12 and 17. For *Bos taurus* 1, the daily circadian rhythm was more variable. It remained at approximately 1.5°C until day 9 and then dropped to around 1°C.

### 3.5.4 Feed and water intake

Individual daily feed and water intakes (kg) were converted to daily intake as percent of starting weight by dividing daily intake by start weight and multiplying by 100.

Feed intakes decreased (Figure 3.4.i) and water intakes increased (Figure 3.4.ii) with during the hottest days of the experiment. Feed intakes for *Bos taurus* 1 were significantly reduced on days 5 to 13 compared to day 2. Feed intakes for *Bos taurus* 2 were significantly reduced on days 6 to 14 when compared to the average of days 1 and 2. Water intakes were more variable although significant increases were measured on days 3, 5, 6 and 8 to 12 in *Bos taurus* 1 and days 8 to 11 in *Bos taurus* 2. The maximum mean daily water intake (as a percent of starting body weight) for *Bos taurus* 1 and *Bos taurus* 2 was recorded on day 9 (13.6% and 9.8% of starting BW respectively). This corresponded to a greater than 2 fold increase in water intake for both *Bos taurus* 1 and *Bos taurus* 2. For *Bos taurus* 1, the maximum individual daily water intake was measured on day 5 and was 21% of starting BW (75 L). For *Bos taurus* 2, it was 15% of starting BW (48 L) and was measured on day 6.
Figure 3.3 (i) Daily core body temperature for *Bos taurus* 1 (o) and *Bos taurus* 2 (●); (ii) Daily maximum and minimum core body temperature for *Bos taurus* 2 (● maximum; ■ minimum); (iii) Daily range (maximum minus minimum) in core body temperature for *Bos taurus* 1 (o) and *Bos taurus* 2 (●). Points show mean ± SEM (°C).
Figure 3.4 Mean daily (i) feed intake and (ii) water intake expressed as a percent of starting body weight for *Bos taurus* 1 (○) and *Bos taurus* 2 (●). Points show mean ± SEM.
3.5.5 Live weight

Individual body weights (kg) were converted to live weight as a percent of starting weight (start weight = 100%) by dividing measured BW (kg) by start weight (kg) and multiplying by 100.

Associated with the decrease in feed intake was a decrease in live weight (Figure 3.5). Live weight losses remained evident until well after the experiment (day 22). Live weight was significantly reduced (P < 0.05) on days 16 and 22 (Bos taurus 1) and days 12, 16 and 22 (Bos taurus 2) when compared with the starting weight of 100%.

3.5.6 Respiratory rate and blood gas parameters

Respiratory rate increased with increasing WBT (Figure 3.6.i and 3.6.ii). For both Bos taurus 1 and Bos taurus 2 there was a positive linear relationship between measured WBT and RR (R² = 0.76 and 0.86 respectively). Mean daily RR was significantly elevated on days 3 to 13 for Bos taurus 1 and days 3 to 12 and day 14 for Bos taurus 2.

Changes in venous blood gas variables were similar in Bos taurus 1 and Bos taurus 2 (Figure 3.7). Increasing WBT resulted in a decrease in pCO₂ and HCO₃⁻ concentration. Partial pressure of carbon dioxide was significantly reduced on days 7 to 12 for Bos taurus 1 and days 6 to 13 for Bos taurus 2. Bicarbonate concentration remained significantly decreased after the hottest period. Venous blood HCO₃⁻ concentration was significantly reduced on days 3 to 15 for Bos taurus 1 and days 6 to 16 for Bos taurus 2. Blood pH remained unchanged until after day 11 or post heating period in Bos taurus 2 and was significantly decreased on days 12 to 16. For Bos taurus 1, blood pH was significantly decreased on days 4 to 6 and 12 to 15.
Figure 3.5 Live weight (mean ± SEM), shown as a percent of starting weight, for

*Bos taurus 1* (○) and *Bos taurus 2* (●).
Figure 3.6 (i) Mean daily respiratory rate (bpm) for *Bos taurus* 1 (○) and *Bos taurus* 2 (●). Points show mean ± SEM (ii) Relationship between respiratory and room wet bulb temperature for *Bos taurus* 1 (○) and *Bos taurus* 2 (●). $R^2 = 0.76$ and 0.86 for *Bos taurus* 1 and *Bos taurus* 2 respectively.
Figure 3.7 Daily venous blood gas parameters; (i) Partial pressure carbon dioxide; (ii) Bicarbonate concentration and (iii) Blood pH for *Bos taurus* 1 (○) and *Bos taurus* 2 (●). Points show mean ± SEM.
3.5.7 Urine

Urine pH for *Bos taurus* 2 followed a similar pattern to blood pH and was unchanged throughout the hottest period (Figure 3.8.i). After day 11 urine pH was significantly decreased (days 12 to 18). Similar changes were evident in *Bos taurus* 1, however, urine pH became significantly reduced earlier (day 8) and remained decreased for the duration of the experiment. The large amounts of water drunk resulted in dilute urine and a low specific gravity (Figure 3.8.ii). Animals in *Bos taurus* 1 had a lower SG than those in *Bos taurus* 2. Urine S.G. was significantly reduced on days 5 to 15 for *Bos taurus* 1 and days 9 to 13 for *Bos taurus* 2.

3.5.8 Electrolytes and urea and creatinine

Daily plasma and urine concentrations of sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), calcium (Ca), urea and creatinine (Cr) were measured for both experiments. Due to the dilute nature of the urine, urine concentrations of measured electrolytes were often below detectable levels. Fractional Excretion Ratios (FER) of the above electrolytes were calculated to give an indication of the animals’ efforts to conserve or excrete a particular electrolyte.

Plasma concentrations of Na, Cl, Mg, Ca and K are shown in Figure 3.9. In general, the measured changes were similar between experiments. Sodium concentration was significantly reduced on days 10 to 15 in *Bos taurus* 2 and day 8 in *Bos taurus* 1. By day 12 plasma Na concentrations were reduced by 2.3% and 3.7% for *Bos taurus* 1 and *Bos taurus* 2 respectively. Plasma Cl concentration generally increased over the length of the experiments. For *Bos taurus* 1 plasma Cl concentration was significantly increased on day 14 and for *Bos taurus* 2 was increased on days 10, 15, 17 and 18. The response of plasma Mg differed between experiments. Plasma Mg was
Figure 3.8 Mean daily (i) urine pH and (ii) urine SG for *Bos taurus* 1 (o) and *Bos taurus* 2 (•). Points show mean ± SEM.
Figure 3.9 Daily plasma electrolyte concentrations for *Bos taurus* 1 (○) and *Bos taurus* 2 (●); (i) Sodium; (ii) Chloride; (iii) Magnesium; (iv) Calcium; and (v) Potassium. Points show mean ± SEM.
significantly reduced on days 7 to 15 for *Bos taurus* 1, and remained unchanged except on day 16 for *Bos taurus* 2. Plasma Ca followed a similar pattern to Na. Plasma Ca was significantly reduced on days 11 to 14 (*Bos taurus* 1) and days 10 to 18 (*Bos taurus* 2).

The plasma concentration of K did not change in either experiment.

Plasma Urea and Cr concentrations follow similar trends in both experiments and increased with increasing WBT (Figure 3.10). In *Bos taurus* 1 plasma urea concentration was significantly increased on days 5 and 8 and plasma Cr concentration significantly increased on days 7 to 12. Urea concentration was significantly on day 14. In *Bos taurus* 2 plasma urea and Cr concentration were significantly increased on days 7 to 11 and 7 to 12 respectively. As with *Bos taurus* 1, plasma urea concentration for *Bos taurus* 2 was significantly reduced after the heating period on day 17.

Sodium FER in *Bos taurus* 1 was variable during the heating period but was consistently reduced after day 11 (Figure 3.11.i). *Bos taurus* 2 followed a similar pattern in that Na FER was reduced after day 12 for the remainder of the experiment; however, reductions were not significantly different from the average of day 1 and 2. The Na FER was significantly increased on days 6 to 8 and 10. Calcium FER was significantly increased on days 4 to 6 and day 8 for *Bos taurus* 1 whilst Ca FER remained unchanged for *Bos taurus* 2 (Figure 3.11.ii). Potassium FER for both experiments was more consistent, being reduced during and after the hottest period in both experiments. Potassium FER for *Bos taurus* 1 was significantly reduced on days 9 to 15 and for *Bos taurus* 2 on days 9, 11 to 16 and 18 (Figure 3.11.iii). No significant changes were measured for FER of Cl, Mg, or urea (data not presented).
Figure 3.10 Mean daily plasma (i) urea and (ii) creatinine for *Bos taurus* 1 (○) and *Bos taurus* 2 (●). Points show mean ± SEM.
Figure 3.11 Daily fractional excretions ratios of (i) sodium; (ii) calcium and (iii) potassium for *Bos taurus* 1 (○) and *Bos taurus* 2 (●). Points show mean ± SEM.
3.5.9 **Heart rate and haematological parameters**

Heart rates were variable for both *Bos taurus 1* and *Bos taurus 2* (Figure 3.12). In general, as WBT increased HR decreased. The mean daily HR was significantly decreased on days 6 to 15 for *Bos taurus 1* and days 7 to 16 for *Bos taurus 2*.

Measured haematological variables included packed cell volume (PCV), total protein (TP), haemoglobin (Hb), mean corpuscular volume (MCV), red blood cells (RBC), white blood cells (WBC), and platelets. Packed cell volume was significantly reduced on days 12 to 16 for *Bos taurus 2*. For *Bos taurus 1* there was no significant change measured in PCV, although a similar trend of reduced PCV during the heat heating period was evident (Figure 3.13.i). The reduction in PCV for *Bos taurus 2* resulted in a maximum increase in plasma volume of 31% on day 16 (data not presented).

Haemoglobin concentration followed a similar pattern to PCV and was significantly reduced on days 10 to 14 in *Bos taurus 1* and days 11 to 15 in *Bos taurus 2* (Figure 3.13.ii). Platelet concentration was significantly reduced on day 7, 10 and 11 in *Bos taurus 1* and day 4 and days 7 to 12 in *Bos taurus 2* (Figure 3.13.iii). Red and white blood cell counts decreased after the heating insult. Red cell count was significantly reduced on days 11 and 13 for *Bos taurus 1* and on days 11 to 15 for *Bos taurus 2* (Figure 3.13.iv). White cell count was significantly reduced on days 11, 12 and 14 for *Bos taurus 1* and days 11 to 13 for *Bos taurus 2* (Figure 3.13.v). Total protein and MCV did not change significantly in either experiment (data not presented).
Figure 3.12 Measured heart rate (bpm) for *Bos taurus* 1 (○) and *Bos taurus* 2 (●). Points show mean ± SEM.
Figure 3.13 Daily haematological parameters for *Bos taurus* 1 (o) and *Bos taurus* 2 (●); (i) Packed cell volume; (ii) Haemoglobin concentration; (iii) Platelet concentration; (iv) Red blood cells; (v) White blood cells. Points show mean ± SEM.
Platelets (mmol/L)

Red cell count (x10^9/L)

White cell count (x10^9/L)

Day of experiment
3.6 Discussion

3.6.1 Core body temperature

The prolonged and continuous high heat and humidity caused a significant rise in $T_c$ in both *Bos taurus* experiments indicating that the animals’ heat loss mechanisms could not compensate fully for the excessive heat load i.e. heat gain exceeded heat loss. Many other studies have confirmed a rise in $T_c$ when *Bos taurus* were exposed to hot conditions both in natural environmental conditions (Kabuga, 1992; Gaughan *et al.*, 1999) and climate controlled rooms (Zhang *et al.*, 1994; Gaughan *et al.*, 1999). No other scientific study has assessed the responses of *Bos taurus* to 11 days of continuous high temperature and humidity with no nocturnal respite and, therefore, a cumulative heat load. Normally in hot environmental conditions, heat gain during the day from the environment and metabolism exceeds heat loss from radiation, convection and evaporation so that some heat is stored and body temperature rises. At night, the heat flow reverses and stored heat is dissipated back to the environment and body temperature falls (Finch, 1986). Evidence of a cumulative effect was seen in *Bos taurus* 2. On days 3 and 4 the set WBT was 26°C and the mean $T_c$ was not significantly increased. On day 13, after 11 days of continuous and prolonged varying degrees of high heat and humidity, the set WBT was also 26°C and the mean $T_c$ was significantly increased. The cumulative effect of the previous 11 days heat and humidity suggested that not all stored body heat was able to be dissipated to the environment as it was on days 3 and 4 when there was very little cumulative heat load. It was not until rooms were turned off and nocturnal cooling present (day 14), that $T_c$ returned to pre heat levels.

The clinical signs of heat stress observed in *Bos taurus 1* and *Bos taurus 2* are comparable with other severe heat stress studies. Drooling and open mouth panting was
witnessed by Gaughan et al. (2000) in Bos taurus steers and it was suggested that this was an indication of an animals’ inability to cope with conditions. Similarly panting, hypersalivation, depression of vigour, decreased appetite, and reduced ruminal and intestinal movements were witnessed by Terui et al. (1979) when assessing the effects of acute severe heat stress in Holstein steers. All of these clinical signs were comparable with animals in Bos taurus 1 and Bos taurus 2. Panting or increasing RR is associated with severe heat stress, non adaptation and a last ditch attempt to control body temperature via evaporative cooling (Finch, 1986). The reason animals hyper-salivate or drool is less clear. Ishii (1964, as cited by Terui et al., 1979) suggested that hypersalivation indicated a functional activation of the salivary gland to reduce the body temperature by the evaporation of saliva. Whether there was an increase in saliva production or disturbance of saliva swallowing due to rapid or open mouth breathing was not differentiated (Terui et al., 1979). The reason for excessive licking of hair coat was also unclear. Perhaps it is an attempt by the animal to improve evaporative cooling by wetting the skin or perhaps it is an indication of salt deficiency. Reduction of peristalsis or ruminal movement may be one of the protective reactions to halt excess heat production (Terui et al., 1980).

The daily circadian rhythm of Tc remained approximately 1°C before and during the hottest periods in the Bos taurus 2 experiment. This was in spite of mean daily Tc rising from 38.4°C on day 2 to 41.0°C on day 10, the daily feed intake significantly reducing, the lack of diurnal variation in environmental conditions, and 24 h daylight. A nychthemeral amplitude of 1°C is consistent with some previous studies (Zhang et al., 1994). However, others (Berman & Morag, 1971; Gaughan et al., 1999; Mader et al., 1999) have found differences in diurnal variation of Tc in natural environments to be dependant on climatic conditions. Berman & Morag (1971) found that the range in rectal temperature in dairy cows to be 0.4°C in winter and 1.2°C in summer. Similarly,
Mader et al. (1999), working in climate chambers with Hereford steers fed high energy feedlot diets, reported daily ranges in rectal temperatures to be 0.7°C and 1.3°C under thermoneutral and hot environmental conditions respectively. Daily circadian rhythms of 1.3°C are more consistent with data from Bos taurus 1. The reason for the differences between experiments are unclear; however, care must be taken when interpreting data from Bos taurus 1 as no recordings were made during ambient environmental conditions.

It is likely that increases in nychthemeral amplitude observed by others involved an increase in diurnal maximum $T_c$ under heat stress, while the minimum remained unaffected when nocturnal respite from heat stress was provided. No nocturnal respite was provided in these experiments. An increase in nychthemeral amplitude was observed after the hottest period when WBT was reduced. For Bos taurus 2 animals this was because of an increase in maximum daily $T_c$. Minimum $T_c$ was significantly increased on days 5 to 13 whereas maximum $T_c$ was significantly increased on days 3 to 17 (except day 15). The reason for this is unclear. It would appear that the heat increment of feeding had little impact on the amplitude of $T_c$ since the amplitude was maintained in the absence of feed intake. Furthermore, the daily maximum and minimum $T_c$ for both Bos taurus experiments occurred at approximately 2100 to 2200 h and 0700 to 0800 h respectively, despite the lack of night time cooling and variations in feed intake.

### 3.6.2 Feed and water

A reduction in feed intake is a response to heat stress (Yousef, 1985a; Blackshaw & Blackshaw, 1994) and has been commonly reported in heat stressed cattle (Beede & Collier, 1986). Food intake by the animal is related directly to all aspects of energy metabolism with the release of heat for maintenance, activities and production.
Changes in the quality or quantity of food will alter the intensity of the metabolic heat load (Finch, 1986). A reduction in feed intake is followed by a fall in metabolic rate (Turner & Taylor, 1983) and, therefore, a reduction in heat production (Conrad, 1985). The lowered intensity of heat production is due to decreased maintenance heat production (Finch, 1986). The decrease in feed intake in Bos taurus with increasing WBT during Bos taurus 1 and Bos taurus 2 was more severe and pronounced than expected. Bianca (1965) has reviewed other studies which have cited environmental temperatures, at which feed intake starts to decline, of 21, 24 and 27°C dry bulb for Holstein, Jersey, and Brown Swiss cows respectively. At 32°C dry bulb, feed consumption of lactating Holstein cows was depressed by 20%, and at 40°C dry bulb, feed intake of Holstein and Jersey cows virtually stopped. These figures vary with other studies which have shown that at temperatures of 15 to 25°C dry bulb, normal feed intake will occur (Conrad, 1985; Blackshaw & Blackshaw, 1994). Temperatures between 25 and 35°C dry bulb can be expected to cause a noticeable reduction in feed intake (3 to 10%), but temperatures above 35°C dry bulb can result in a 10 to 35% reduction in feed intake (Conrad, 1985). No indication of relative humidity was provided in these studies so it is difficult to compare with results obtained from Bos taurus 1 and Bos taurus 2 where WBT includes dry bulb temperature and relative humidity. During the hottest periods of the experiments a WBT of 32°C was achieved with a relative humidity of around 80% and dry bulb temperature of 36°C. At this temperature the average feed intakes were reduced by 90 and 78% for Bos taurus 1 and Bos taurus 2 respectively, which varies markedly with the above studies if based on dry bulb temperature alone. It is more than likely that some of the differences between results can be based on the differences in relative humidity. If for instance the relative humidity was 50% in the results obtained by Conrad (1985), then the WBT animals were exposed to would have been much lower than the 32°C WB encountered in Bos
taurus 1 and Bos taurus 2. Other obvious reasons for differences in feed intake when animals are exposed to hot environmental conditions include breed differences and between animal variation as well as time of year and acclimatisation.

The other major reason for the difference in results between Bos taurus 1 and Bos taurus 2 and the studies mentioned above was that the animals in Bos taurus 1 and Bos taurus 2 were exposed to continuous high heat and humidity or high wet bulb temperatures and there was no overnight “cooling off” period. It would appear that the prolonged nature of the heat stress conditions had an impact on the severity of inappetence encountered in these experiments. On day 13, when the WBT was 26°C (30°C dry bulb and 70% relative humidity), feed intakes were still significantly reduced by 56 and 66% for Bos taurus 1 and Bos taurus 2 respectively. A WBT of 26°C at the beginning of the experiments (days 3 and 4) had no significant impact on feed intakes, therefore, the prolonged nature of the heat insult and EHL must have had a cumulative effect to cause the reduced feed intakes on day 13 (and day 14 in Bos taurus 2 when climate rooms were turned off). In both experiments, on days when mean Tc was significantly increased, feed intakes were significantly decreased. So, at 26°C WB on day 5, there was no mean increase in Tc and no reduction in feed intake, whereas at 26°C WB on day 13 (after 11 days of varying continuous heat and humidity), there was an increase in mean Tc and a decrease in feed intake.

The aetiology of the reduced feed intake for the Bos taurus is unknown but could be due to a number of factors including reduced metabolic rate, a direct effect of heat on the brain, changes in rumen temperature or endotoxemia. There is evidence that a reduction in feed intake in cattle is followed by a reduction in metabolic rate (Turner & Taylor, 1983). The reduction in feed intake is an attempt by the animal to bring metabolic heat production in balance with its capacity to dissipate heat (Conrad, 1985). Under chronic heat stress conditions thyroid hormones (T3 and T4) are reduced
A decrease in thyroid hormones will act to decrease metabolic rate and reduce the amount of heat produced by the cells (Beede & Collier, 1986). Whether $T_3$ and $T_4$ decline due to thermal inhibition of the hypothalamus or indirectly because of lowered feed intake and metabolism is not clear (Johnson, 1985). There is evidence that the reduction in feed intake may be a direct effect of heating the hypothalamus as $T_c$ rises. Warming the pre-optic area and rostral hypothalamus of goats with thermodes caused hungry animals, which had just begun to eat with good appetite, to stop eating within 1 minute (Andersson and Larsson, 1961, as cited by Bianca, 1965).

In general, water intake increased for all animals as WBT increased, which agrees with other studies that have also shown water consumption increased when cattle were exposed to hot conditions (Winchester & Morris, 1956; Phillips, 1960; Colditz & Kellaway, 1972). The maximum water intakes during the hottest period were more than double intakes at the beginning, similar to the increased water needs during thermal stress proposed by Beede & Collier (1986). Water intakes remained significantly elevated in spite of reductions in dry matter intake. This disagrees with earlier work by Winchester & Morris (1956) who predicted that water intake per unit of dry matter ingested increases with ambient (dry bulb) temperature. In the Winchester & Morris (1956) study it was concluded that the water intake of cattle is a function of dry matter intake consumption and ambient temperature. When the water intake per unit of dry matter ingested was plotted against ambient dry bulb temperature a curve was obtained which remained level between -12 and 4°C, but which rose at an accelerated rate between 4 and 38°C. No indication of relative humidity was given. In both $Bos$ taurus experiments increasing WBT caused decreases in feed intake and increases in water intake. Therefore, it would appear at these higher WBT that water intake is unrelated to feed intake.
The mechanisms for water consumption increases are not clear. It does not appear that it was related to dry matter intake as suggested by Winchester & Morris (1956). It may be related to an increase in demand due to increased evaporation from the respiratory tract and skin during heat stress conditions (McDowell & Weldy, 1967). These evaporative demands may deplete water stores and induce increased water intake via hypovolemia or hyperosmolarity. Hypovolemia and hyperosmolarity were not evident in either *Bos taurus 1* or *Bos taurus 2*, suggesting a direct effect of heat. This is further supported by Andersson *et al.* (1960) who suggest that it is more likely that the reasons for heat-induced increases in water consumption may involve the hypothalamus since warming the pre-optic area and rostral hypothalamus of the goat with thermodes evoked a large increase in water consumption. A degree of body cooling via conduction may also be achieved if large quantities of cool water are consumed (Bianca, 1965) but a mechanism is still required to drive the behaviour. As well as reducing thermal load, drinking water which is cooler than $T_c$, may have a direct comfort effect by cooling the reticulum (Beede & Collier, 1986).

As already mentioned the increased water consumption may be accompanied by increased water losses due to increases in evaporative cooling from respiratory tract and skin. Water losses via increases in urine production have also been reported during heat stress conditions (McDowell & Weldy, 1967). Although not measured in either *Bos taurus* experiment, it was assumed that urine production and volume was increased during the hottest period as evidenced by a low measured urine specific gravity and increased water intakes. However, what was not as clear was what effect that the above losses have on total body water or overall hydration status of the heat stressed animals. El-Nouty *et al.* (1980) observed that although both water intake and urine output increased in cattle in hot environments, the ratio of water intake to urine output also increased. This, along with a reported two fold increase in antidiuretic hormone (ADH)
may be responsible for the observed increase in total body water (El-Nouty et al., 1980). Theoretically, a reduced plasma osmolality should inhibit water intake, but in pregnant goats heat stress also induced a primary polydipsia (Olsson et al., 1995). It was suggested that stimulating signals from warmth receptors override inhibiting influences from receptors signalling hyponatremia and/or hypo-osmolality at the “thirst centre” in the hypothalamus, leading to polydipsia. A similar mechanism may have operated in both Bos taurus experiments. Although plasma osmolality was not measured in Bos taurus 1 or Bos taurus 2, a hyponatremia and a decrease in PCV was evident during the hottest periods. Hyponatremia can indicate hypo-osmolality (George, 1994) and a reduction in PCV without evidence of blood loss is an indication of over-hydration or increase in total blood volume (Duncan et al., 1994). This agrees with Richards (1985) who also found that lactating dairy cows retained body water during heat stress conditions, which resulted in an increase in total body water. It was suggested that a large proportion of body water retained was of extracellular origin and was useful to the animal by virtue of the high specific heat of water, which meant that the animal could store heat during the hottest part of the day and dissipate it during the cool night in an attempt to ameliorate heat stress (Richards, 1985). In work with humans (Senay et al., 1976), increases in extracellular volume, and especially plasma volume, increases cardiovascular stability (maintains blood pressure) via heat induced vasodilation, a mechanism supported by the observed drop in heart rate in Bos taurus.

The increased total blood volume and subsequent increase in preload to the heart may also be responsible for the apparent decrease in HR observed in the Bos taurus experiments (Ganong, 1997). This was unexpected as others have reported increases in HR when core body temperatures are elevated due to severe heat stress (Bianca & Findlay, 1962; Bianca, 1965; Terui et al., 1979). However, during chronic exposure to mild heat, heart rates of dairy cattle have been shown to decline due to associated
decreases in feed intake and the production of metabolic heat from milk (Bianca, 1965). It was also possible that heart rates at the beginning of both \textit{Bos taurus} experiments were artificially elevated as the animals had not yet become accustomed to the sampling procedures. As the experiments progressed, animals became acclimated to the intensive handling in the rooms and this was reflected in lowered, or more normal, heart rates.

Large individual variations in water intakes were also noted, especially in \textit{Bos taurus 1}, a fact which is supported by Colditz & Kellaway (1972). The maximum amount of water consumed in a day for one animal was 75 L compared with 44 L in another. A reason for the large individual variation in water intakes may be psychogenic polydipsia. The animals in climate controlled rooms have limited space and contact with other animals and boredom may develop. Some animals were seen to develop a habit of playing with water and spilling it over their bodies as WBT increased, a fact also noted by Ragsdale \textit{et al.} (1951, as cited by Bianca, 1965).

\subsection*{3.6.3 Respiratory rate and acid base balance}

An important thermal regulatory reaction to heat stress is increased RR, which aids in heat dissipation via evaporative cooling (West \textit{et al.}, 1991; West \textit{et al.}, 1992; Blackshaw & Blackshaw, 1994). Very high respiratory rates are associated with severe heat stress, non-adaptation and a “last ditch” attempt to control body temperature (Finch, 1986). Both the \textit{Bos taurus} experiments showed that RR increased almost 2-fold as WBT increased from pre heat environmental conditions (day 2) to 26°C WB (day 4). It would appear that the upper limit threshold of RR was achieved in both experiments as RR peaked at around 125 breaths per minute on day 6 for \textit{Bos taurus 1} and day 7 for \textit{Bos taurus 2}. During the hottest period RR declined and only reached peak values again towards the end of the hottest period on day 11 whilst \(T_c\) remained significantly increased throughout the hottest period. This decline or plateau in RR when \textit{Bos taurus}
animals were experiencing heat stress conditions agrees with other work (Bianca & Findlay, 1962; Terui et al., 1980; Gaughan et al., 2000). Bianca & Findlay (1962) first measured this bi-phasic respiratory response to acute heat stress in *Bos taurus* calves. Calves were exposed to 38°C WB and during the first phase RR rose from 20 to 158 bpm, while the tidal volume fell from 1.5 to less than 0.6 L. In the second phase (which occurred after rectal temperature reached approximately 40.5°C) RR fell from 158 to 114 bpm and tidal volume increased from 0.6 to 1.2 L. Throughout these two phases, the resultant ventilation increased from 30 to 120 L/min. In another acute and severe heat stress study it was suggested by Terui et al. (1980) that if the respiratory rate decreased to one half or lower of a maximum rate during heat exposure then death was likely even if animals were removed from heat stress conditions. Gaughan et al. (2000) agreed and suggested that a decreasing RR was not always indicative of an animal coping with hot conditions. It was more likely due to a shift in RR dynamics from rapid open mouth panting to a deep phase open mouth panting at reduced rate.

With increased alveolar ventilation, the expiration of CO₂ exceeds the rate of its formation in the body (Sanchez et al., 1994b). Therefore, pCO₂ of blood declines (Bianca & Findlay, 1962; Schneider et al., 1984), creating a deficit of H₂CO₃ and thus an increase in pH or respiratory alkalosis (Sanchez et al., 1994b). Both *Bos taurus* experiments clearly showed an increase in RR and subsequent decrease in pCO₂. What was not observed in this study was an increase in blood pH as was reported by others (Dale & Brody, 1952; Bianca & Findlay, 1962). Although there was no significant rise in blood pH during the heat stress period, there was a trend towards elevated blood pH towards the end of the hot period in the *Bos taurus* 2 experiment. This, along with the reduced pCO₂ would suggest that animals were experiencing respiratory alkalosis. Presumably blood pH was being maintained through blood buffers and renal compensation, and thus successfully compensating for the loss of CO₂.
The HCO₃⁻ buffering mechanism is the most important buffering system in the blood and under normal conditions blood pH requires a [HCO₃⁻] / [0.03 x pCO₂] ratio of 20:1 (Cunningham, 2002). Thermally induced hyperventilation decreases pCO₂ (Schneider et al., 1988; West et al., 1991). To maintain the ratio of 20:1 and counter alkalosis, HCO₃⁻ is secreted by the kidney and there is a compensatory decrease in renal H⁺ ion secretion which begins within 2 h but is not complete for 2 to 3 days (Rose, 1994). Due to the sustained heat load that the animals were exposed to, renal compensation was able to help maintain blood pH within a normal range during the hottest part of the experiment. This exacerbated the loss of HCO₃⁻ as it was secreted by the kidney. This response, which is presumably mediated at least in part by a parallel rise in renal tubular cell pH, is manifested by a HCO₃⁻ loss in the urine and by decreased urinary ammonia excretion. Both of these effects lower the plasma HCO₃⁻ concentration, the latter by preventing the excretion of the daily H⁺ load, thereby resulting in H⁺ retention (Rose, 1994). It has been hypothesised that this would further decrease blood HCO₃⁻ concentration and increases urine pH (Schneider et al., 1988).

Schneider et al. (1988) characterised the diurnal or ynychthemeral patterns of acid base balance during acute heat stress were over a 24 h period where animals were exposed to heat stress conditions during the day and cool conditions at night. The experiment showed that animals experienced a respiratory alkalosis only during the heat stress hours. During the cooler hours a lower urine pH and higher urine ammonium concentration suggested excretion of H⁺ or compensatory metabolic acidosis. Results from that study were similar to the one reported here, as was the hypothesised metabolic acidosis which occurred during the cooler part of the day, or in this case, once climate controlled rooms were turned off, the difference being that animals had had several days of heat stress conditions before a cooling off period. When the rooms were turned down, animals were no longer panting and blowing off CO₂, so CO₂ rapidly returned to normal
and therefore the $[\text{HCO}_3^-] / [0.03 \times \text{pCO}_2]$ ratio decreased. Without adequate $\text{HCO}_3^-$ to buffer, acidic urine and reduced blood pH resulted. Blood $\text{HCO}_3^-$ concentration took a long time to return to pre-heat concentrations, perhaps due to the more sustained nature of the heat insult. This suggests that there is a requirement for $\text{HCO}_3^-$ to maintain blood pH after a heating period. It would seem that the renal response post heating period was also important. If the kidneys take 2 to 3 days to compensate for decrease in renal $H^+$ secretion, it may take the same time to increase renal $H^+$ retention post heating period. Any delay in renal compensation will further exacerbate the decrease in urine pH post heating period.

The major difference between the Schneider et al. (1988) study and the one reported here was the absence of an increased urine pH during the continuous heat period. The reason for this is not clear. It may be that the dilute nature of the urine, as was indicated by the very low specific gravity, means that any changes in urinary $H^+$ concentration were very difficult to detect. The renal phosphate buffering system has some capacity to buffer urine pH but whether this was enough to maintain urine pH during the hottest period is not clear. The ratio between $\text{HCO}_3^-$ and $\text{pCO}_2$ was maintained throughout the heating period in both experiments, although the values of each were reduced. Post day 11, or after the heating period the ratio was reduced indicating a total body deficit of $\text{HCO}_3^-$ and a metabolic acidosis. Perhaps, during the heating period, animals were experiencing an underlying metabolic acidosis which was partially compensating the respiratory alkalosis and therefore the kidneys were not required to excrete $\text{HCO}_3^-$. At the same time the total body stores of $\text{HCO}_3^-$ were being depleted as it was used as a buffer and not being replaced due to inappetence. This would agree with Bianca & Findlay (1962) who suggested that the exposure of panting mammals to severe heat may have an alkalotic effect due to over ventilation and an acid effect due to accumulation of acid metabolites (i.e. lactic acid) from the respiratory
muscles. Which of these two tendencies prevail would depend on the severity and duration of the heat stress conditions.

It has been noted in the literature review that there are potential problems associated with venous blood sampling of blood gas variables. In panting sheep exposed to a hot environment, it has been shown that cranial blood flow increases and this may potentially reduced pCO$_2$ and increase pH as animals move from a thermoneutral to hot environment (Hales, 1973). In this study there is no way to tell how much of the pCO$_2$ reduction was due to increased blood flow and how much was due to increases in RR as cranial blood flow was not measured. However, when blood gas values were compared during thermoneutral conditions before and after heat stress, when cranial blood flow was assumed to be similar, there was a decrease in pCO$_2$, HCO$_3^-$, and pH suggesting that respiratory changes were affecting acid base status as discussed above.

3.6.4 Electrolytes

Whilst there were significant changes in individual plasma electrolyte concentrations, no electrolyte concentration measured was outside of what is regarded as a normal range for *Bos taurus* animals. Plasma volume increased by up to 31% towards the end of the experiments (see 3.6.5). This led to an increase in the total circulating mass of some plasma electrolytes even though measured plasma concentration was decreased or remained unchanged. The following discussion relies on the measured concentration of circulating electrolytes rather than total circulating mass. This is because regulation of extracellular fluid volume, including plasma, is via the effect of osmolarity of the fluid on release of hormones such as antidiuretic hormone. Osmolarity of extracellular fluid is a function of the concentration of ions in that fluid, and the most abundant of these is Na; thus it is the extracellular fluid concentration
rather than total circulating mass of electrolytes which is important (Guyton & Hall, 1996).

The decline in plasma Na concentration was most likely multifactorial. Firstly, renal HCO$_3^-$ excretion must be accompanied by a cation to maintain electroneutrality. Secondly, body stores of Na were not being replenished due to inappetence. Thirdly, the animals appeared to have increased blood volume towards the end of the experiments and thus a hemodilution effect may be evident. The cattle all had very low fractional excretion ratios for Na during and after the exposure to heat, indicating that they were conserving Na as much as possible. So even though at times towards the end of the *Bos taurus* experiments total circulating Na was increased due to the expanded plasma volume, the animals still had a requirement for Na as concentration was being maintained.

Plasma K was not markedly changed in either of the experiments, which was unexpected, especially for herbivores that are not eating and does disagree with El-Nouty *et al.* (1980) who found that serum concentrations of both Na and K were reduced in Holstein cows during prolonged heat stress. It was possible that the plasma K did not provide an adequate measure for what was happening inside the cells, where the majority of K is held. The fractional excretion ratio for K decreased indicating conservation of K.

Chloride is the major anion of the extracellular fluid. Loss of HCO$_3^-$ causes a relative increase in Cl concentration (George, 1994) which was evident in both *Bos taurus* experiments. Increases in Cl concentration along with decreases in Na concentration will act to decrease the SID (SID = (Na + K) - Cl). Given the law of electroneutrality, a decrease in SID results in acidosis or an increase in H$^+$ (Stewart, 1983). Both *Bos taurus* 1 and *Bos taurus* 2 had decreases in SID towards the end and after the hottest period indicating metabolic acidosis.
Changes to plasma concentrations of Mg and Ca seem to be related to the time of year experiments were conducted, and reductions in feed intake. The reason for the discrepancy in Mg concentration between *Bos taurus 1* and *Bos taurus 2* was most likely due to the time of year the experiments were held. Before the experiment commenced, *Bos taurus 1* animals had access to leguminous pastures which was presumably high in Mg concentration. *Bos taurus 2* animals would have had access to mainly grass species which usually have lower Mg concentrations (Corbett, 1990). Therefore, it was possible that *Bos taurus 1* animals may have started with higher concentrations of plasma Mg. However, plasma concentrations of Mg in *Bos taurus 1* decreased to concentrations similar to those in *Bos taurus 2*, which remained unchanged for the duration of the experiment. The reasons for these reductions are not clear although at no stage did plasma concentrations drop to near the 0.5 mmol/L required to cause clinical signs of hypomagnesaemia.

Calcium concentrations generally decreased during the experiments and were significantly decreased after the hottest period. This was in spite of the ration being very high in Ca concentration due to the 2% lime (calcium hydroxide) used as a binder. It is hypothesised that the reason for the decrease in plasma Ca was related to inappetence and the poor availability or solubility of Ca in calcium hydroxide. However, like Mg, plasma Ca concentration did not decrease to concentrations that would be expected to cause clinical signs of hypocalcaemia. This was not surprising as blood Ca concentration is not a good indicator of calcium status due to plasma Ca being maintained by homeostatic mechanisms such as reabsorption from bone (NRC, 2000). The general decrease in Ca agreed with Bianca & Findlay (1962) who found that with rising body temperature the concentration of Ca in the blood of calves tended to fall due to heat induced over-ventilation. Similarly, Terui *et al.* (1979) reported slight decreases in plasma Ca when *Bos taurus* steers were subjected to severe heat stress. In both
studies, and in the *Bos taurus* experiments described here, the mechanism for the reduction in plasma Ca is unclear.

Both urea and creatinine (Cr) concentrations increased with increasing WBT. This agrees with other reports (Bianca, 1965; Schneider *et al.*, 1988) and suggests catabolism of muscle, possibly resulting from reduced feed intake and stress (Schneider *et al.*, 1988). Terui *et al.* (1979) suggest that increases in blood urea nitrogen during periods of acute intense heat stress was an indication of protein catabolism due to the heat stress and that the rise of blood creatinine was due to the activated endogenous nitrogen catabolism with a decline of feed consumption reinforced by heat stress.

The changes in plasma bicarbonate concentration were related to heat effects or an increase in RR as discussed in the respiratory rate and acid base section above.

### 3.6.5 Haematology

A decrease in PCV indicates a reduction in the percentage of blood composed of erythrocytes, the cause of which can be either due to anaemia or over hydration (Duncan *et al.*, 1994). Both *Bos taurus 1* and *Bos taurus 2* had reductions in PCV whilst other RBC indices measured remained unchanged. Furthermore, there was no clinical evidence of anaemia. Therefore, the most likely cause for the decrease in PCV was over hydration or increase in circulating blood volume due to the increase in water consumption. An increase in plasma volume of up to 25% was confirmed using formulas described in the general materials and methods chapter. In humans, there is evidence to suggest that it is an increase in circulating oncotically active proteins, flushed from the interstitial space by heat induced peripheral vasodilation, that is responsible for retaining the extra plasma in the vascular space (Senay *et al.*, 1976).
Haemoglobin concentration provides the most direct indication of oxygen transport in the blood (Duncan et al., 1994). The reduction in Hb concentration follows the reduction in PCV and again is most likely due to an expanded blood volume.

Although there were reductions in WBC counts and platelet concentrations, values were not outside the normal ranges given for Bos taurus nor were they as severe as those encountered by Terui et al. (1980) who reported greater than 50% reductions in WBC counts in Holstein steers before death due to heat stress conditions.

3.6.6 Conclusions

In general, the physiological responses of Bos taurus animals to prolonged and continuous periods of heat and humidity were more severe and pronounced than those described in other studies which have characterised the responses of Bos taurus to acute heat stress situations. Given the extreme conditions that can occur on board live stock vessels and the now characterised physiological responses of Bos taurus to these conditions, it is no surprise that the physiological strains placed on the animals due to heat stress are of major concern. Of great concern was the larger and more prolonged than expected reduction in feed intake for Bos taurus animals during the experiments. Additionally, the failure of the animals’ heat loss mechanisms to keep up with the continuous heat gains resulted in core body temperatures reaching dangerous levels.

However, these experiments have also demonstrated the remarkable ability of Bos taurus to maintain blood pH during prolonged hyperventilation. It was not until after the heat period that major acid base imbalances occurred. Bicarbonate depletion both during and after the heat exposure appears to be of concern because a rebound metabolic acidosis develops once hyperventilation and respiratory alkalosis ceases.

It was hypothesised that major plasma electrolyte imbalances would become evident given the chronic heat stress conditions, prolonged inappetence and the losses
expected in sweat and urine. Although significant changes were measured in various plasma electrolytes, concentrations did not deviate from what is considered a normal range. However, collectively the small but significant changes in electrolyte concentrations may add to the animals’ inability to cope with the excessive heat load.

These experiments utilised technology that allowed continuous and accurate monitoring of core body temperature. Although there was no diurnal variation in environmental conditions, and relatively little impact from the heat of digestion and metabolism due to inappetence, a 24 h circadian rhythm remained in measured core body temperatures. The reasons for this remain unclear, and it is something that has not been demonstrated before.
Chapter 4: Physiological response of *Bos indicus* to prolonged and continuous heat and humidity

4.1 Introduction

It is well recognised that *Bos indicus* are more heat tolerant than *Bos taurus* (Bianca, 1965; Finch, 1986; Blackshaw & Blackshaw, 1994). The improved heat tolerance in *Bos indicus* has been attributed to their dense smooth coat which has little insulating capacity but reflects solar radiation (Blackshaw & Blackshaw, 1994); lower resistance to internal tissue heat transfer at high levels of heat stress (Finch, 1986); greater length, diameter and volume of sweat glands and their close proximity to the skin surface (Bianca, 1965); and reduced metabolic rate of *Bos indicus* compared with *Bos taurus* (Hansen, 2004).

Although it is clear that *Bos indicus* have improved or greater heat tolerance to hot environmental conditions, physiological responses are still initiated when animals are exposed to heat stress conditions or an EHL (Gaughan *et al.*, 1999). Work in the beef feedlot industry has demonstrated that hot environmental conditions will elicit physiological responses in *Bos indicus* cattle. Responses include elevated rectal temperature, increased RR and increased sweating rate (Gaughan *et al.*, 1999), reduced dry matter intake (Finch, 1986) and increased water intake (Finch, 1986; Blackshaw & Blackshaw, 1994). Little scientific literature exists on the acid base or plasma electrolyte balance response of *Bos indicus* to hot environmental conditions and EHL. Most scientific work in this area has been with dairy cows and other *Bos taurus* breeds.

Environmental conditions vary markedly on board live stock vessels in that animals can be exposed to continuous and prolonged periods of high heat and humidity
when travelling through equatorial regions to the Middle East. Little scientific data exists on the physiological response of *Bos indicus* given these conditions. Norris *et al.* (2003) studied cattle mortalities on 4 voyages to the Middle East between 1998 and 2001. No mortalities of *Bos indicus* were recorded due to heat stroke in this study. No other physiological variables were reported.

### 4.2 Aims

To investigate and characterise the physiological responses of *Bos indicus* to prolonged and continuous periods of high heat and humidity as can occur on livestock vessels travelling to the Middle East during the northern hemisphere summer.

### 4.3 Hypothesis

*Bos indicus* will develop physiological responses to prolonged and continuous periods of high heat and humidity as can occur on long haul voyages to the Middle East during the northern hemisphere summer. The direction of change of these physiological responses will be the same, but less severe, than those encountered by *Bos taurus* given similar environmental conditions as seen in Chapter 3.

### 4.4 Materials and methods

#### 4.4.1 Experimental design

Six individually penned *Bos indicus* heifers were subjected to climatic conditions based on voyage reports collated from voyages to the Middle East during the northern hemisphere summer (MAMIC, 2000a; MAMIC, 2000b). Anecdotal reports suggest that *Bos indicus* are better able to cope with hot humid conditions as may be experienced on board long voyages to the Middle East. Thus the experimental design
was similar to *Bos taurus* 2 (Chapter 3) except set WBT was increased to 33°C on day 11 (Table 4.1). Each day of experiment was from midnight to midnight. Room conditions were set at 0800 h each day. Animals entered the rooms at 0800 h on day 1. Rooms were turned on at 0800 h on day 3 and turned off at 0800 h on day 14. Animals were removed from the rooms at 0800 h on day 16.

### 4.4.2 Animals and management

Six *Bos indicus* heifers were chosen for quiet temperament and similar body weight. Five animals were sourced from New Norcia, Western Australia and 1 from Southern Cross, Western Australia. Both farms were registered Brahman studs and were located in southern Western Australia below the 26th parallel and animals were not considered northern acclimatised. Weights ranged from 289 to 386 kg after 18 h off feed (339 ± 14 kg; mean ± SEM). The total weight of animal in each room was 1022 and 1011 kg. The experiment commenced on the 21st August and ran until the 5th September 2002.

Animals were fitted with temperature telemeters (Datamet, Potchefstroom, South Africa) and temperature loggers - Stowaway XTI (Onset Computer Corp, Massachusetts, USA) 1 week before experiment commenced. Indwelling jugular catheters were fitted the day before experiment commenced.

Animals were given one week adaptation to a commercial dietary shipper pellet which was the same as that used in previous *Bos taurus* experiments; 8.6 MJ of ME, 11.9% CP, and 39.9% NDF per kg of DM (Macco Feeds, Western Australia, Australia) following the same introduction regime as discussed in previous chapter. Full analysis and chemical composition of feed is shown in Appendix 1. On day 1, animals were randomly allocated a pen in one of the 2 climate controlled rooms and remained there throughout the length of the experiment.
Table 4.1 Set wet bulb temperature (°C) of climate control rooms for *Bos indicus* experiment.

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Set WB (°C)</th>
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<tbody>
<tr>
<td>1-2</td>
<td>Ambient</td>
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<tr>
<td>3-4</td>
<td>26</td>
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<td>5</td>
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<td>6</td>
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<td>7-10</td>
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<td>33</td>
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<td>12</td>
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<td>13</td>
<td>26</td>
</tr>
<tr>
<td>14-16</td>
<td>Ambient</td>
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</table>
Feed was offered at 2.5% of bodyweight (as fed) measured after 18 h off feed before the animals entered the rooms. Feed was divided into two equal amounts, given at 0700 and 1300 h daily. Residues were cleaned out and weighed before each morning feed and the previous day’s intake calculated. Water was available ad libitum, in 25 L buckets, topped up throughout the day as necessary. Total amount of water drunk was calculated once daily by subtracting the weighed residue of water each morning from the total amount of water given for the past 24 h.

4.4.3 Sample collection

Body weights were recorded on days 0, 12, 16 and 21 (after 18 h off feed but not water). Heart rate, RR, and RT were measured and recorded four times daily at 0600, 1200, 1800 and 2200 h. Jugular venous blood and voided urine samples were collected at 0600, 1200, 2200 h on days 7 to 11 and once daily at 1200 h on all other days. Daily averages were calculated for variables measured more than once daily.

4.4.4 Statistical analysis

A two way ANOVA with animal and day as fixed factors was used to test for an overall change over days. When the overall effect of days was significant, Dunnett t-tests were used to compare each day with a control or “pre-heat” day. For all variables apart from Tc and BW, the pre-heat day was the average of days 1 and 2 when environmental conditions in the climate control rooms were the same as ambient conditions. The pre-heat day for Tc data was the average of days -3 to -1 when the animals were in the barn under ambient conditions before jugular catheterisation. The pre-heat day for BW was day 0 when animals were weighed prior to jugular
catheterisation. Unless otherwise stated, days on figures which are significantly
different from control days are represented with the symbol “*”.

Dunnett t-tests were performed to alleviate inflated probabilities of type 1 errors
when examining which days were significantly different to normal ambient conditions.
A 5% level of significance was used throughout and all analyses were carried out using
SPSS 11.0 for Windows.

4.5 Results

4.5.1 Temperature

In general, the climate controlled rooms achieved set WBT (Figure 4.1) and
mimicked extreme voyage conditions to the Middle East (MAMIC, 2000a; MAMIC,
2000b). The maximum measured WBT was 34.2°C at 2200 h on day 11.

Data obtained from the temperature loggers ran from the day after they were
surgically implanted on day -4 until when they were surgically removed on day 23
(Figure 4.2.i). Animals were in the climate controlled rooms from day 1 to day 16.
There was a positive linear relationship ($R^2 = 0.71$) between daily mean $T_c$ and daily
mean room wet bulb temperature whilst climate rooms were operating between days 4
and 14 (Figure 4.2.ii). The maximum individual $T_c$ recorded was 41.2°C at 1430 h on
day 11.

The daily mean, maximum and minimum and range in $T_c$ were also calculated
from data loggers. The daily mean $T_c$ increased with increasing WBT and was
significantly elevated on days 7 to 12 (Figure 4.3.i). The daily maximum $T_c$ was
significantly increased during and after the hottest period on days 8 to 14 and day 17
(Figure 4.3.ii). The maximum mean $T_c$ recorded was 40.4°C at 1600 h on day 11. The
daily minimum $T_c$ was significantly increased during the hottest period on days 8 to 11.
Figure 4.1 Measured wet bulb temperature (■) and set wet bulb temperature (Δ) during *Bos indicus* experiment.
Figure 4.2 (i) Mean core body temperature for *Bos indicus*. Means were calculated by averaging core body temperature from temperature loggers every 30 minutes (ii) Relationship between mean daily core body temperature and mean daily room wet bulb temperature for *Bos indicus*.
Figure 4.3 (i) Daily mean core body temperature, (ii) Daily maximum (■) and minimum (○) core body temperature, and (iii) Daily range (maximum minus minimum) in core body temperature. Points show mean ± SEM.
and was significantly decreased on day 19 (Figure 4.3.ii). The daily range in $T_c$ increased after the heating period and was significantly elevated on days 12 to 14 and days 17 to 20 (Figure 4.3.iii).

### 4.5.2 Feed and water intake, body weight

Individual feed and water intakes were converted to a percent of starting BW by dividing intake (kg) by start weight (kg) and multiplying by 100.

In general feed intakes remained unchanged throughout the experiment whilst water intakes increased with increasing WBT (Figure 4.4). One animal became inappetent during the hottest period (was only eating 0.14% of BW on day 11) but the mean daily feed intake was not significantly reduced on any day. Water intakes were significantly increased on days 5 to 12. The maximum mean daily water intake was 9.3% of BW, recorded on day 11. The maximum individual daily water intake was 13% of BW or 49 L.

Body weights were converted to BW as a percent of start weight (start weight = 100%) by dividing measured BW (kg) by starting BW (kg) and multiplying by 100.

Although feed intakes were not significantly decreased, there was a trend towards decreasing BW as the experiment progressed (Figure 4.5). On day 12 the mean live weight was 97.8% of starting weight ($P = 0.08$) and by day 16 it was 96.9% of starting weight ($P = 0.08$). Body weights had surpassed pre-experiment measurements by day 21 (104.4%; $P = 0.02$).

### 4.5.3 Heart rate, respiratory rate and blood gas

Figure 4.6.i shows the daily mean RR and HR over the duration of the experiment. There was a positive linear relationship between RR and measured WBT in the climate controlled rooms (Figure 4.6.ii; $R^2 = 0.75$). The mean maximum recorded
Figure 4.4 Daily feed intake (■) and water intake (○) for Bos indicus. Points show mean ± SEM.
Figure 4.5 Body weight (mean ± SEM), shown as a percent of starting body weight, for *Bos indicus*. 
Figure 4.6 (i) Mean daily respiratory rate (■) and heart rate (○) for *Bos indicus*. Points show mean ± SEM (ii) Relationship between daily mean respiratory and daily mean room wet bulb temperature for *Bos indicus*.

(i)
![Graph showing daily respiratory rate vs. day of experiment with trend line equation y = 3.8038x - 39.304 and R² = 0.8226](image)

(ii)
![Graph showing daily respiratory rate vs. wet bulb temperature with trend line equation y = 3.8038x - 39.304 and R² = 0.8226](image)
RR was 124 bpm at 1200 h on day 11. The daily mean RR was significantly increased on days 5 to 13. There was no significant change in HR over the duration of the experiment.

Mean daily venous blood gas variables are shown in Figure 4.7. The pO₂ was significantly increased during and just after the hottest period on days 7 to 12. The pCO₂ and HCO₃⁻ were similar and decreased as RR and WBT increased. The pCO₂ was significantly decreased on days 6 to 12. Bicarbonate was significantly decreased on day 6 and days 8 to 14. By day 11, pCO₂ had reduced by 16.8% and HCO₃⁻ by 19.9%. Blood pH remained unchanged during the heating period but was significantly decreased after on days 12 to 15.

4.5.4 Urine

Although there was no significant change in urine pH over the length of the experiment, it did follow a similar pattern to blood pH and decreased after the heating period (Figure 4.8). Urine specific gravity decreased with increasing water intake or as WBT increased. Urine SG was significantly decreased on days 10 to 12.

4.5.5 Electrolytes and urea and creatinine

Plasma electrolytes which changed significantly during the experiment are presented in Figure 4.9. There were significant increases in urea and Cr and significant decreases in Na and Ca. Plasma concentrations of K, Mg and Cl did not change significantly (data not presented).

Plasma Na concentrations generally decreased with increasing WBT and were significantly decreased on days 4 to 11. Plasma Ca concentrations were variable. Calcium concentration was significantly decreased on day 12. Plasma urea concentration increased with increasing WBT and remained increased through the
Figure 4.7 Daily venous blood gas parameters for *Bos indicus*; (i) Partial pressure oxygen; (ii) Partial pressure carbon dioxide; (iii) Bicarbonate; and (iv) Blood pH. Points show mean ± SEM.
Figure 4.8 Daily urine pH (■) and specific gravity (○) for *Bos indicus*. Points show mean ± SEM.
Figure 4.9 Daily plasma electrolyte concentrations for *Bos indicus*: (i) Sodium; (ii) Calcium; (iii) Urea; and (iv) Creatinine. Points show mean ± SEM.
hottest period; this was significant on days 5 to 13. Plasma Cr followed a similar pattern and was significantly increased on days 6 to 12, but on day 18 was significantly decreased.

Due to the increased water intakes and dilute nature of the urine, FER were calculated for all electrolytes. The FER of K was significantly reduced after the heating period on days 12 to 16 (Figure 4.10.i). Chloride FER was significantly reduced on day 16 (Figure 4.10.ii). Magnesium FER generally decreased over the length of the experiment and was significantly reduced on days 10, 11, 14 and 18 (Figure 4.10.iii). The FER of Na, Ca and urea did not change (data not presented).

4.5.6 Haematology

Red blood cell concentration, WBC concentration, PCV and Hb concentration all followed a similar pattern and decreased with increasing WBT and remained reduced after the heating period (Figure 4.11). The RBC concentration was significantly reduced on day 6 and days 8 to 16. A similar trend was observed for WBC, Hb and PCV concentrations with significant reductions occurring on days 6 to 18 for WBC, and days 6 to 15 for PCV and Hb. The reduction in PCV resulted in an increase in plasma volume. The maximum increase in plasma volume was calculated to be 38% (day 11; data not presented). Other haematological variables measured included platelets, MCV, PP, and MCH. No measurable changes were detected and data are not presented in results.
Figure 4.10 Daily electrolyte fractional excretion ratios of (i) potassium, (ii) chloride and (iii) magnesium for *Bos indicus*. Points show mean ± SEM.
Figure 4.11 Daily (i) red blood cell concentration, (ii) white blood cell concentration, (iii) packed cell volume, and (iv) haemoglobin concentration for *Bos indicus*. Points show mean ± SEM.
4.6 Discussion

4.6.1 Feed and water

There is evidence to suggest that a reduction in feed intake occurs in response to heat stress (Blackshaw & Blackshaw, 1994) and this has been commonly reported in heat stressed cattle (Beede & Collier, 1986). This experiment indicated that there was no significant reduction in feed intake given the environmental conditions to which the *Bos indicus* were subjected. Therefore, for *Bos indicus* animals, it could be surmised that the environmental conditions were not heat stress conditions. One animal in this experiment showed signs of inappetence during the hottest period, although this did not significantly reduce the mean feed intake. It does show that there was some individual variation in heat tolerance for *Bos indicus* animals during extreme environmental conditions. The reason for the variation is unclear. In the animal which did stop eating there was no problem with adaptation to experimental conditions or feeding regime, as its feed intake during thermoneutral conditions was comparable to other experimental animals. Animals in this experiment were sourced from 2 different Brahman studs. Therefore, genetic background and acclimatisation may have had an impact. The inappetent animal was the only heifer sourced from Southern Cross, Western Australia, so it was possible that the differing backgrounds could be the reason for the recorded differences. Furthermore, the inappetent animal had consistently higher mean, maximum and minimum $T_c$ compared to other animals during the hottest period when it was not eating. This would indicate that its heat loss mechanisms were failing to cope with the EHL, an immediate response to which is a reduction in feed intake in an attempt to reduce metabolic rate and heat generated from metabolism.
Water intakes increased with increasing WBT. On day 11, when WBT reached 34.2°C, the water intakes had increased 2.5 fold and feed intakes had remained unchanged. This would agree with Winchester & Morris (1956) who state that water intakes per unit of dry matter ingested increase with increasing ambient (dry bulb) temperatures. Although increased water consumption was accompanied by increased urine production, as indicated by the very low measured urine SG, total blood volume also appeared to increase, evident by reductions seen in PCV and plasma osmolality. This is supported by others (e.g. Seif et al., 1973; El-Nouty et al., 1980) who also found total body water and extracellular fluid volume increased in ruminants in hot environments. El-Nouty et al. (1980) found that although both water intake and urine output increased in cattle in hot environments, the ratio of water intake to urine output also increased, and this, along with the reported two fold increase in ADH may be responsible for the reported increase in total body water. Theoretically reduced plasma osmolality will inhibit water intake. In work undertaken in pregnant goats heat stress also induced primary polydipsia (Olsson et al., 1995). It was suggested that stimulating signals from warmth receptors override inhibiting influences from receptors signalling hyponatremia and hypo-osmolality at the “thirst centre” in the hypothalamus.

### 4.6.2 Core body temperature

The prolonged and continuous exposure to heat and humidity caused a significant rise in mean daily $T_c$ between days 6 and 13, however; unlike the *Bos taurus* animals, there were no other associated signs of clinical heat stress. This was consistent with other studies which have also shown *Bos indicus* have increased $T_c$ during controlled heat stress conditions (Gaughan et al., 1999). The study by Gaughan et al. (1999) showed a rise of 1.2°C in rectal temperature over 10 h when *Bos indicus* animals in a climate controlled room were subjected to extremely hot conditions (THI > 90).
The animals in the *Bos indicus* experiment were subjected to continuous high heat and humidity resulting in a mean rise of 2.3°C from the lowest recorded mean $T_c$ during ambient conditions (38.1°C at 0900 h on day -3) to the highest recorded mean $T_c$ (40.4°C at 1600 h on day 11). It would also appear that the maximum $T_c$ *Bos indicus* was not reached as there was a continuous increase in daily mean $T_c$ from when the rooms were turned on (day 3) until they were turned down (day 12) i.e. there was no plateau in core body temperature.

The difference of over 1°C between the Gaughan *et al.* (1999) study and the *Bos indicus* experiment described here may also reflect a cumulative effect of the EHL. Environmental conditions of 26°C WB on days 3 and 4 of the experiment did not result in a significant rise in mean daily $T_c$, whereas 26°C WB on day 13, or after 7 days of continuous high heat and humidity, did result in the mean $T_c$ becoming significantly increased.

The daily range in $T_c$ remained approximately 1°C before and during the hottest part of the experiment. This was in spite of mean daily $T_c$ rising from 38.5°C on day 2 to 39.9°C on day 11 and continuous environmental conditions allowing no diurnal or night time cooling. The daily range in $T_c$ increased after the hottest period (after day 11) and remained significantly elevated until the temperature loggers were removed. The reason and mechanism for the increase in daily circadian rhythm of *Bos indicus* after the heat insult is unclear. It is inconclusive as to whether or not the increase is due to an increase in maximum $T_c$ or decrease in minimum $T_c$. Maximum $T_c$ was significantly increased on days 8 to 14 and 17 and minimum $T_c$ was significantly decreased on days 8 to 11 and 19. Finch (1986) suggested that an increase in the diurnal oscillation of $T_c$ in *Bos indicus* was in response to food deficits and was due to a decrease in the lower range of body temperature during the cooler night hours. In this experiment there was no voluntary reduction in food intake and no night time cooling. The daily maximum
and minimum $T_c$ during the experiment occurred at approximately the same time each day (2100 to 2200 h and 0700 to 0800 h for maximum and minimum $T_c$ respectively) in spite of continuous high wet bulb conditions.

4.6.3 Respiratory rate and acid base balance

The RR of *Bos indicus* animals in this experiment showed a positive linear relationship between day 3 and 11 as WBT increased from ambient to 34°C. Given the linear nature of the increase it would appear that the maximum RR may not yet have been reached. The maximum mean RR measured was 125 bpm at 1200 h on day 11 when WBT was over 32°C. This agrees with the maximum RR recorded in Brahman steers under controlled environmental conditions (THI > 90) for 10 h (Gaughan et al., 1999). However, Gaughan et al. (1999) found that the maximum RR of 125 bpm for Brahman steers occurred after 8 h and then there was a levelling out of RR. It was suggested that the levelling out indicates the possibility of a RR threshold. Differences in observed RR ceiling may be due to many factors, including acclimatisation, age, body condition and genetic differences (Hahn et al. 1997, as cited by Gaughan et al. 1999). It was unclear whether a RR threshold was reached in the *Bos indicus* experiment as no levelling out of RR was encountered.

An important thermal regulatory reaction to heat stress is increased RR, which aids in heat dissipation via evaporative cooling (West et al., 1991; West et al., 1992). With increased respiration, the expiration of CO$_2$ exceeds the rate of its formation in the body (Sanchez et al., 1994b). The results of this experiment indicated that RR increased with increasing WBT and this was accompanied by a decrease in pCO$_2$ and HCO$_3^-$.

After the hottest period when the rooms were turned down, animals were no longer panting and blowing off CO$_2$, which then acted as a source of acid. Without adequate HCO$_3^-$, a reduction in blood and urine pH resulted. Similar blood gas changes were
reported and discussed for *Bos taurus* animals in Chapter 3 and will not be repeated here.

### 4.6.4 Electrolytes and urea and creatinine

Whilst there were significant changes in concentrations of five of the six measured plasma electrolytes compared to the pre heat period, none of the values obtained was outside what is considered the normal range for *Bos indicus*. The reduction in plasma Na concentration during heat stress has been described by El-Nouty *et al.* (1980) and Sanchez *et al.* (1994). Sanchez *et al.* (1994b) suggested that renal excretion of HCO$_3^-$ must be accompanied by a cation. Sodium or K are possibilities; however, Na is more likely. El-Nouty *et al.* (1980) reported that Na was excreted via the kidney rather than retained during heat exposure of the bovine animal. It was suggested that low plasma aldosterone concentrations during heat exposure may be responsible for the decrease in plasma Na concentration and the increase in urinary Na excretion and the main factor inhibiting aldosterone release was decreased plasma K concentration (El-Nouty *et al.*, 1980). After the heating period, the Na FER was significantly reduced indicating the animals’ efforts to conserve Na and return plasma concentrations to normal. As noted in the previous chapter, an increase in blood volume may also contribute to an increase in the total mass of circulating plasma constituents.

Potassium concentration was well maintained throughout the heating period and was only significantly reduced on days 12 to 14. Plasma concentration of K is not a good indicator of K deficiency (NRC, 2000). It is possible that the plasma K did not provide an adequate measure for what is happening inside the cells, where the majority of K is held. However, the fractional excretion ratio for K was also decreased after the heating period indicating conservation of K whilst plasma concentration was reduced. This result differs somewhat to El-Nouty *et al.* (1980) who found significant reductions
in serum and urinary K during heat exposure, the reason for which was explained by the greater loss of K in cattle cutaneous evaporation. It was also suggested that decreased plasma K may have been the main factor that inhibited aldosterone release during heat exposure (other factors being higher extracellular fluid volume and higher plasma ADH concentrations).

*Bos indicus* animals had a decrease in SID towards the end and after the hottest period. This was most likely due to increases in plasma Cl and decreases in plasma Na. Reductions in SID suggest metabolic acidosis (Stewart, 1983).

The reason for the slight decrease in calcium is unclear, although it does agree with Terui *et al.* (1979) who also found small decreases in calcium concentrations when cattle were exposed to heat stress conditions. They also were unable to elucidate a reason for the reduction.

It is well known that plasma urea concentration increases with dehydration, renal disorders, cardiac disorders, hyperthermia, pancreatic disorders, severe haemorrhaging, and dysfunction of the thyroid gland (Terui *et al.*, 1979). Results agree with Terui *et al.* (1979) who hypothesised that urea concentrations increased due to increases in protein catabolism from heat stress hyperthermia. Increases in plasma creatinine concentration were also observed by Terui *et al.* (1979) who reported that the rise in blood creatinine was due to the activated endogenous nitrogen catabolism associated with a decline of feed consumption reinforced by heat stress. The same assumptions can not be drawn from the results presented here as the *Bos indicus* maintained feed intake in association with a rise in plasma creatinine concentration.

4.6.5 *Haematology*

A reduction in PCV with concurrent increases in water intake or over hydration indicates an increase in circulating blood volume. Plasma volume in *Bos indicus*
increased by as much as 38% (day 11). This resulted in a net increase in total circulating mass of many of the plasma constituents.

Haemoglobin concentration follows a similar pattern to PCV and should be approximately one third of the packed cell volume if the erythrocytes are of the normal size (Duncan et al., 1994). This was consistent with the data from the Bos indicus experiment.

Reductions in WBC count have been reported in other studies when cattle have been subjected to intense heat stress conditions (Terui et al., 1979; Terui et al., 1980). It was suggested by these authors that the WBC count and pCO$_2$ might be the most changeable blood components during severe heat stress. It was also suggested that when the WBC count reduced to 50% below normal then death was likely to occur. The Bos indicus experiment described here had WBC concentrations reducing from a maximum mean of $11.5 \times 10^9$ L to $6.6 \times 10^9$ L or a reduction of 44%.

4.7 Conclusion

It is clear that the Bos indicus cattle in this experiment experienced a range of physiological responses to the prolonged and continuous periods of high heat and humidity to which they were subjected. However, of interest, was the maintenance of feed intake throughout the hottest period. This was in direct contrast to the Bos taurus heifers described in Chapter 3. The core body temperature and respiratory rate of the Bos indicus increased with increasing wet bulb temperature but it was not clear if they had peaked. These findings suggest that the Bos indicus in this experiment had not yet reached their upper critical limit of heat stress conditions and thus have a superior ability to cope with excessive heat loads. It can be concluded that Bos indicus cattle would be much better suited to travel on board live export voyages to the Middle East.
during the hot, humid northern hemisphere summer months. With no reductions in feed intake the welfare of *Bos indicus* animals would appear not to be compromised.
Chapter 5: Comparing the physiological responses of *Bos taurus* and *Bos indicus* to prolonged and continuous periods of heat and humidity.

5.1 Introduction

The effect of excessive heat on the health and welfare of cattle shipped live from Australia to the Middle East is of major concern. Heat stroke, trauma and respiratory disease are considered the most common causes of mortalities in cattle during live export to the Middle East (Norris *et al.*, 2003). Heat stress is of greatest concern when unacclimatised animals are transported from winter in southern Australia into the northern hemisphere summer (Norris *et al.*, 2003). Both *Bos taurus* and *Bos indicus* breeds are shipped live to the Middle East from southern Australian ports and it has been shown that *Bos indicus* cope with hot humid conditions better than *Bos taurus* (Norris *et al.*, 2003).

*Bos indicus* cattle are better able to regulate body temperature in response to heat stress than *Bos taurus* (Finch, 1986). Superior ability for regulation of body temperature during excessive heat load is the result of lower metabolic rates as well as increased capacity for heat loss (Blackshaw & Blackshaw, 1994). As compared to European breeds, tissue resistance to heat flow from the body core to the skin is lower for *Bos indicus* (Finch, 1986) while sweat glands are larger (Hansen, 2004). Properties of hair coat in *Bos indicus* cattle enhance conductive and convective heat loss and reduce absorption of solar radiation (Hansen, 2004). Furthermore, there is evidence to suggest that *Bos indicus* cattle have evolved with genes for thermo tolerance and that
these genes can protect cells from the deleterious actions of elevated temperature (Hansen, 2004).

Previous studies have assessed some of the physiological responses of both *Bos taurus* and *Bos indicus* to heat stress conditions (Hammond *et al.*, 1998; Gaughan *et al.*, 1999). No other study has defined or compared the physiological responses of either *Bos taurus* or *Bos indicus* to the prolonged and continuous heat stress conditions which may be encountered on board livestock vessels.

### 5.2 Aims

To compare the differences and similarities in the physiological responses to prolonged and continuous periods of heat and humidity observed in *Bos taurus* 2 (chapter 3) and *Bos indicus* (chapter 4).

### 5.3 Hypotheses

The primary hypothesis is that *Bos indicus* will be better adapted to regulate body temperature in response to chronic periods of high heat and humidity. As a result, the physiological responses to excessive heat load conditions will be less severe than those experienced by *Bos taurus*.

### 5.4 Materials and methods

#### 5.4.1 Experimental design

This chapter compares experiments from Chapter 3 (*Bos taurus* 2) and Chapter 4 (*Bos indicus* 1). Animals from both these experiments were in climate controlled rooms for 15 days with an additional 3 days of sampling after they exited. Each day of experiments was from midnight to midnight. Room conditions were set at 0800 h each
day. For both experiments rooms were turned on at 0800 h on day 3 and turned off at 0800 h on day 14. The environmental conditions that animals in each experiment were subjected to were not exactly the same (Table 5.1), the difference being that *Bos indicus* animals were subjected to slightly longer and higher WBT than *Bos taurus* and that *Bos taurus* were offered feed at 2.25% BW and *Bos indicus* at 2.5% BW. For the *Bos taurus* experiment rooms were turned down to 28°C WB at 2200 h on day 11. For the *Bos indicus* experiment rooms were turned down to 28°C WB at 0700 h on day 12. On day 11 the set WBT was 33°C for *Bos indicus* and 32°C for *Bos taurus*.

5.4.2 Animals and management

As described in Chapter 3 (*Bos taurus 2*) and Chapter 4 (*Bos indicus 1*)

5.4.3 Sample collection and measurements

As described in Chapters 3 and 4. Variables measured more than once daily were averaged over the day and means were presented as daily measurements.

Plasma cortisol and aldosterone were analysed for both *Bos taurus 2* and *Bos indicus 1*. Plasma aldosterone was analysed using DPC Coat-A-Count® Aldosterone radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma Cortisol was measured using an extraction tritiated immunoassay (Appendix 5).

5.4.4 Statistical analysis

For each experiment a two way ANOVA with animal and day as fixed factors was used to test for an overall change over days. When the overall effect of days was significant, Dunnett t-tests were used to compare each day with a control day. For all variables apart from core body temperature and BW, the control day was the average of days 1 and 2 when environmental conditions in the climate control rooms were the same.
Table 5.1 Daily wet bulb temperatures that climate controlled rooms were set for during *Bos taurus* 2 and *Bos indicus* 1 experiments.

<table>
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<tr>
<th>Day of experiment</th>
<th>Set wet bulb temperature (°C)</th>
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<th>Bos indicus</th>
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<td>14-16</td>
<td>Ambient</td>
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</table>
as ambient environmental conditions. The control day for \( T_c \) data was the average of days -3 to -1. The control day for BW was day 0, the day before animals entered the climate controlled rooms. Unless otherwise stated, days on figures which were significantly different from control days are represented with the symbol “a” for \( Bos taurus \) and “b” for \( Bos indicus \).

Dunnett t-tests were performed to alleviate inflated probabilities of type 1 errors when examining which days were significantly different to normal ambient conditions. A 5% level of significance was used throughout and all analyses were carried out using SPSS 11.0 for Windows.

The relationship between elevations in \( T_c \) and feed intake, water intake, RR, and HR rate, were analysed by regressing the mean daily feed intake, water intake, RR, and HR for each breed on the mean daily \( T_c \) for each breed. Initially the strength of each relationship was tested with linear regression. If there proved to be a significant relationship between \( T_c \) and any variable for both breed, first the slopes were compared, and if the slopes were not different, the elevations were compared (Zar, 1996).

### 5.5 Results

#### 5.5.1 Environmental conditions and temperature

Measurements of environmental conditions (DB and RH) were taken 2 to 3 times daily. On average, \( Bos indicus \) animals were subjected to slightly longer and higher WB conditions (Figure 5.1). Furthermore, the \( Bos indicus \) were more winter acclimatised than the \( Bos taurus \). This was due to the \( Bos indicus \) experiment taking place later in the year compared to \( Bos taurus \). This also led to differences observed in WBT when CCR were not operating on days 1 and 2. On these days climatic conditions were the same as ambient conditions and it was cooler for \( Bos indicus \) animals.
Figure 5.1 Measured wet bulb temperatures during *Bos taurus* 2 (o) and *Bos indicus* 1 (■) experiments.
compared to *Bos taurus*. When CCR were operating, the maximum WBT measured in the *Bos taurus* experiment was 33.4°C at 1200 h on day 10 and the maximum WBT measured in the *Bos indicus* experiment was 34.2°C at 2200 h on day 11. In general, climatic conditions were comparable with voyage conditions recorded on livestock vessels during the northern hemisphere summer (M. McCarthy, personal communication).

Core body temperature was averaged over 30 min periods and the mean value calculated for both the *Bos taurus* and *Bos indicus* experiments (Figure 5.2.i). The climate controlled rooms were operating from day 3 to 14 with the hottest set period being from day 7 to day 11. Associated with the rise in WBT was a rise in $T_c$ (Figure 5.2.ii). *Bos taurus* had a higher and more prolonged rise in $T_c$ than the *Bos indicus*.

The daily mean $T_c$ for *Bos taurus* was significantly higher on days 5 to 14 and day 17 when compared with the average of days -1 to -3. The mean $T_c$ for *Bos indicus* was significantly higher on days 7 to 12 when compared to the average of days -1 to -3 (Figure 5.3.i). The mean maximum $T_c$ reached for the *Bos taurus* was 41.2°C (day 10 at 0330 h) and for *Bos indicus* 40.4°C (day 10 at 1600 h). The maximum individual $T_c$ for *Bos taurus* was 41.9°C (day 10 at 2200 h) and for *Bos indicus* 41.2°C (day 11 at 1430 h). The mean daily maximum and minimum $T_c$ are shown in Figure 5.3.ii and 5.3.iii respectively.

In general, the daily circadian rhythm of $T_c$ (mean maximum minus mean minimum $T_c$) was maintained throughout the hot period and increased after the heat insult (Figure 5.3.iv). After the heat insult (post day 11), the daily range of $T_c$ was significantly increased on days 12 and 17 for *Bos taurus* and days 12, 13, 14 and 17 for *Bos indicus*. A maximum circadian rhythm of 1.7°C (day 17) and 1.9°C (day 13) were reached for *Bos taurus* and *Bos indicus* respectively. The increase seems due to an
Figure 5.2 (i) Continuous mean core body temperature for *Bos taurus* 2 and *Bos indicus* 1 over the length of the experiments. Means were calculated by averaging core body temperature measurements over 30 minute intervals (ii) Relationship between mean daily core body temperature and mean daily room wet bulb temperature for *Bos indicus* (■) and *Bos taurus* (○).
Figure 5.3 Daily (i) mean; (ii) maximum; (iii) minimum and (iv) range (maximum minus minimum) core body temperature for *Bos taurus* 2 (○) and *Bos indicus* 1 (■). Points show mean ± SEM.
increase in the daily maximum $T_c$. This appears to be more evident in the *Bos indicus* although the same trend was evident in the *Bos taurus*.

### 5.5.2 Feed and water intake and body weight

Figure 5.4 shows the daily feed and water intake for *Bos taurus* and *Bos indicus*, expressed as a percent of starting bodyweight. The feed intake of the *Bos taurus* was significantly reduced from days 6 to 14. For *Bos indicus*, there was no significant mean feed intake reduction measured on any day. Water intake was increased for both breed as WBT increased. *Bos taurus* animals showed significant increases in daily water consumption between days 8 to 11. *Bos indicus* animals showed significant increases in mean daily water consumption on days 5 to 12.

Associated with feed intake reductions was a decrease in the average BW of *Bos taurus* animals. Body weight was significantly reduced ($P < 0.05$) when measured on days 12, 16 and 22 compared with the starting weight of 100% on day 0 (Figure 5.5). Although feed intakes were not significantly reduced in *Bos indicus*, there was a trend towards decreasing body weights as the experiment progressed. On day 12 the mean body weight was 97.8% of starting weight ($P = 0.08$) and by day 16 it was 96.9% of starting weight ($P = 0.08$). By day 22, 6 days after the experiment had finished, body weight losses remained evident in *Bos taurus*. At the same time BW of *Bos indicus* animals was significantly higher than starting weights. By day 35, both *Bos taurus* and *Bos indicus* animals had significantly surpassed starting weights.

### 5.5.3 Respiratory and heart rate

As WBT increased both *Bos taurus* and *Bos indicus* respiratory rates increased (Figure 5.6.i and 5.6.ii). The mean daily RR of *Bos taurus* was significantly increased on days 3 to 12 and day 14 when compared to the pre heating period (days 1 and 2). For
Figure 5.4 Mean daily (i) feed intake and (ii) water intake for *Bos taurus* 2 (o) and *Bos indicus* 1 (■). Points show mean ± SEM.
Figure 5.5 Live weight (mean ± SEM), shown as a percent of starting weight, for *Bos taurus* 2 (○) and *Bos indicus* 1 (■).
*Bos indicus* RR was significantly increased on days 5 to 13. There was a positive linear relationship between mean daily RR and mean WBT in the climate controlled rooms for both *Bos taurus* and *Bos indicus* animals ($R^2 = 0.77$ and 0.82 respectively). There appeared to be a linear increase in mean RR for *Bos taurus* as WBT increased from 26°C (day 3, 75 bpm) to 32°C (day 7, 127 bpm). After day 7, RR declined even though WBT was maintained at 32°C. By day 11, RR had increased to a similar level as seen on day 7 (126 bpm). The RR increase for *Bos indicus* was also linear; however, 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 (126 and 125 bpm respectively). Heart rate was variable and did not change in *Bos indicus* at any time during the experiment. In the *Bos taurus* experiment, HR was significantly decreased on days 7 to 16 (Figure 5.6.iii).

### 5.5.4 Blood gas measurements

In general, blood gas responses were similar in *Bos taurus* and *Bos indicus* but *Bos taurus* were more severely affected for longer periods. Blood pCO$_2$ and HCO$_3^-$ concentration were significantly reduced during and after the hot period (Figure 5.7.i and 5.7.ii respectively) with the changes in *Bos taurus* being more pronounced and prolonged than *Bos indicus*. For *Bos taurus* pCO$_2$ was significantly reduced on days 6 to 13 and for *Bos indicus* days 6 to 12. Bicarbonate concentration took longer than pCO$_2$ to return to pre-heat concentrations in both breed (day 16 for *Bos taurus* and day 14 for *Bos indicus*). Blood pH was maintained in the lead up to and during the hot period but was significantly reduced after the heating period for both *Bos taurus* and *Bos indicus* (Figure 5.7.iii). Blood pH was significantly reduced on days 12 to 16 for *Bos taurus* and days 12 to 15 for *Bos indicus*.
Figure 5.6 Mean daily (i) respiratory rate, (ii) relationship between daily mean core temperature and daily mean wet bulb temperature, and (iii) heart rate for *Bos taurus* 2 (○) and *Bos indicus* 1 (■). Points show mean ± SEM.

(i) Respiratory rate (bpm)

(ii) Relationship between respiratory rate and wet bulb temperature

(iii) Heart rate (bpm)
Figure 5.7 Mean daily venous blood gas parameters; (i) Partial pressure of carbon dioxide; (ii) Bicarbonate concentration and (iii) Blood pH for *Bos taurus* 2 (○) and *Bos indicus* 1 (■). Points show mean ± SEM.

(i) Venous Blood pCO₂

(ii) Bicarbonate (mmol/L)

(iii) Venous Blood pH
5.5.5 Urine pH and specific gravity

For *Bos taurus* the urine pH follows a similar pattern to venous blood pH, and was significantly decreased after the hottest period on days 12 to 18 (Figure 5.8.i). The urine pH for the *Bos indicus* was more stable and was not significantly different on any day. Urine SG was significantly decreased on days 9 to 13 for *Bos taurus* and days 10 to 12 for *Bos indicus* (Figure 5.8.ii).

5.5.6 Plasma electrolytes and urea and creatinine

Significant decreases were observed in plasma Na concentrations for both *Bos indicus* and *Bos taurus* during and after the heating period (Figure 5.9.i). Plasma Na concentration was significantly reduced on days 10 to 15 for *Bos taurus* and days 4 to 11 for *Bos indicus*. There was a general tendency for plasma Cl concentration to increase after the hottest period although there was no significant difference on any day during the *Bos indicus* experiment (Figure 5.9.ii). For *Bos taurus*, plasma Cl concentration was significantly increased on day 10, 15, 17 and 18. Plasma Ca concentration decreased in both experiments and was significantly decreased on days 10 to 18 for *Bos taurus* and day 12 only for *Bos indicus* (Figure 5.9.iii). Significant increases were seen in plasma urea and Cr for both *Bos taurus* and *Bos indicus* experiments as WBT increased (Figure 5.9.iv and 5.9.v). On day 17 urea concentration was significantly decreased in *Bos taurus*. Plasma Cr took longer to return to pre-heat concentrations. Plasma urea was significantly increased on days 7 to 11 (the hottest period) for *Bos taurus* and days 5 to 13 for *Bos indicus*. Plasma Cr was significantly increased on days 7 to 12 for *Bos taurus* and days 6 to 12 *Bos indicus*. Plasma K concentration did not change significantly in either *Bos taurus* or *Bos indicus* experiments (data not presented).
Figure 5.8 Mean daily (i) urine pH and (ii) urine specific gravity for *Bos taurus* 2 (o) and *Bos indicus* 1 (■). Points show mean ± SEM.
Figure 5.9 Mean daily plasma (i) sodium; (ii) chloride; (iii) calcium (iv) urea and (v) creatinine for *Bos taurus* 2 (○) and *Bos indicus* 1 (■). Points show mean ± SEM.
5.5.7 Urine fractional excretion ratios

The fractional excretion ratios of sodium, potassium, chloride, calcium and magnesium were calculated (Figure 5.10). The Na FER was variable between breeds during the heating period (Figure 5.10.i). On days 6 to 8 and day 10, the FER of Na was significantly increased in *Bos taurus*. The FER of Na was not significantly different for *Bos indicus* animals. After the heating period the Na FER for both breeds was decreased and very low. The FER of K decreased during and after the heat period for both *Bos taurus* and *Bos indicus* (Figure 5.10.ii). This change was less marked for *Bos indicus*. The FER of K was significantly decreased on days 9, 11 to 16 and 18 for *Bos taurus* and days 12 to 16 for *Bos indicus*. The FER Cl was variable in both experiments (Figure 5.10.iii). There was no significant differences during the *Bos taurus* experiment and only on day 16 was the FER of Cl significantly decreased in the *Bos indicus* experiment. There were no significant differences detected in the FER of Ca in either experiment (data not presented). The FER of Mg was significantly decreased on days 10, 11, 14 and 18 for *Bos indicus* only (Figure 5.10.iv).

5.5.8 Haematology

Packed Cell Volume (PCV) decreased in both *Bos indicus* and *Bos taurus* during and after the hottest period (Figure 5.11.i). Packed cell volume for *Bos taurus* was significantly reduced on days 12 to 15 and for *Bos indicus* days 6 to 15. Plasma protein concentration was variable throughout both experiments (Figure 5.11.ii). There was no significant difference detected in either breed. Haemoglobin concentration followed a similar pattern to PCV for both experiments (data not presented).
Figure 5.10 Daily mean urine fractional excretion ratios of (i) sodium; (ii) potassium; (iii) chloride and (iv) magnesium for *Bos taurus* 2 (○) and *Bos indicus* 1 (■). Points show mean ± SEM.
Figure 5.11 Mean daily (i) packed cell volume and (ii) total plasma protein for 

*Bos taurus 2* (○) and *Bos indicus 1* (■). Points show mean ± SEM.
5.5.9 **Hormones**

Hormonal assays for both *Bos taurus* and *Bos indicus* included cortisol and aldosterone. Mean aldosterone concentrations were consistently undetectable (< 70 pmol/L) for both *Bos taurus* and *Bos indicus* (data not presented). In general, cortisol concentrations decreased for both *Bos taurus* and *Bos indicus* over the duration of the experiments (Figure 5.12). On days 12 and 13 cortisol concentration for *Bos indicus* was significantly decreased.

5.5.10 **Clinical responses**

There was variation noted in the clinical responses of the 12 animals subjected to continuous high heat and humidity. *Bos taurus* exhibited clinical signs of heat stress with accumulated excessive heat load, while the *Bos indicus* were less affected by the conditions in the rooms. Clinical signs of heat stress such as open-mouthed panting, inappetence, drooling, reluctance or inability to rise, increased licking of coat, and general dullness including neurological signs with staring and glazed eyes were seen in *Bos taurus* animals at WBT above 30°C. The *Bos indicus* animals showed very few of these clinical signs. They did not pant to the same extent, and never showed open-mouthed breathing. It was also apparent that there was obvious between animal variation within both *Bos taurus* and *Bos indicus* groups.

5.5.11 **Regression analysis**

The regression lines for each variable (feed intake, water intake, RR and HR) were significant, except for the HR for *Bos indicus* where the line was not significant (Figure 5.13).
Figure 5.12 Mean daily plasma cortisol concentration for *Bos taurus* 2 (○) and *Bos indicus* 1 (■). Points show mean ± SEM.
Figure 5.13 (a) Daily average feed intake, (b) respiratory rate, (c) water intake and (d) heart rate plotted against daily averages of core body temperature for *Bos taurus* and *Bos indicus*. Significant regressions are indicated by lines in each panel (the thicker line indicating *Bos indicus*). Results of comparisons of relationships given in the text.
Water intake (% BW)

Heart rate (bpm)

Core temperature (°C)
For food intake both breeds showed significant decreases in feed intake as $T_c$ increased ($F_{1, 12} = 38.7, P < 10^{-4}, R^2 = 0.76, F_{1, 12} = 35.2, P < 10^{-4}, R^2 = 0.75$ for *Bos taurus* and *Bos indicus*, respectively). The slopes were not different ($F_{1, 24} = 1.9, P = 0.2$) and the elevation of the *Bos indicus* line was significantly above the *Bos taurus* line ($F_{1, 25} = 25.0, P < 10^{-4}$). This indicates that the *Bos indicus* ate more than the *Bos taurus* at all levels of $T_c$.

For water intake both breeds showed significant elevations in water intake as $T_c$ increased ($F_{1, 12} = 26.4, P < 10^{-4}, R^2 = 0.69, F_{1, 12} = 33.8, P < 10^{-4}, R^2 = 0.74$ for *Bos taurus* and *Bos indicus*, respectively). The slope ($F_{1, 24} = 4.2, P = 0.06$) and elevation ($F_{1, 24} = 1.5, P = 0.23$) did not differ, indicating that the increase in water intake stimulated by $T_c$ increase was the same in both breeds.

For RR both breeds showed significant elevations in RR with increases in $T_c$ ($F_{1, 15} = 34.8, P < 10^{-4}, R^2 = 0.70, F_{1, 15} = 262, P < 10^{-4}, R^2 = 0.95$ for *Bos taurus* and *Bos indicus*, respectively). The slopes were significantly different ($F_{1, 30} = 23.0, P < 10^{-4}$) indicating that the *Bos indicus* began with a lower RR than the *Bos taurus*, but the increase in $T_c$ stimulated larger increases in RR in *Bos indicus* than that observed in the *Bos taurus*.

For HR the *Bos indicus* line was not significant, while the *Bos taurus* showed a significant decrease in HR with increases in $T_c$ ($F_{1, 15} = 13.3, P = 0.002, R^2 = 0.47$).

### 5.6 Discussion

The results of these experiments highlight the animals’ ability to maintain blood pH and acid base homeostasis as well as a circadian rhythm of $T_c$ during prolonged periods of heat stress without diurnal variation in environmental conditions. The consequences appeared after the heat insult when animals experienced a rebound
metabolic acidosis, as seen by the reduction in blood pH, and an increase in the circadian rhythm of $T_c$.

Both breeds were tested separately, but the conditions to which both were exposed were similar. If anything the *Bos indicus* were exposed to slightly longer and more extreme WBT than *Bos taurus* and also the *Bos indicus* experiment was conducted later in winter so they may be less heat acclimated than the *Bos taurus*. Despite these differences, the *Bos indicus* showed better homeostatic responses to the heat challenge than the *Bos taurus* suggesting that *Bos indicus* are better adapted to hot, humid conditions compared to *Bos taurus*.

The prolonged exposure to heat and humidity caused a significant rise in $T_c$ for both *Bos taurus* and *Bos indicus*, indicating that the animals’ heat loss mechanisms could not compensate fully for the excessive heat load. Many other studies have confirmed a rise in $T_c$ when both *Bos taurus* and *Bos indicus* are exposed to hot conditions in natural environments (Hammond *et al.*, 1998; Gaughan *et al.*, 1999), but no other study has assessed their physiological responses to continuous and prolonged high heat and humidity as described here. In *Bos taurus*, mean $T_c$ became significantly elevated on day 5 (WBT 28°C) and remained elevated until day 13 when WBT had been decreased to 26°C. Associated with the rise in WBT and $T_c$ were clinical signs of heat stress in *Bos taurus* animals. These clinical signs included open-mouthed panting, drooling, reluctance or inability to rise, increased licking of coat, and general dullness including neurological signs with staring and glazed eyes. For *Bos indicus* animals the prolonged exposure to heat and humidity caused a significant rise in mean daily $T_c$ between days 7 and 12 of the experiment; however, clinical signs of heat stress were not observed.

The mean daily $T_c$ of *Bos indicus* animals increased steadily when WBT increased. This would suggest that the maximum tolerable $T_c$ for *Bos indicus* was not
reached, since the mean $T_c$ for *Bos taurus* animals appeared to plateau by day 7 at the start of the hottest period. The maximum mean $T_c$ of around 41°C for *Bos taurus* 2 was below the maximum tolerable $T_c$ of 42.7°C reported by Terui *et al.* (1980); however, this was for more acute and extreme heat stress conditions.

The *Bos indicus*’ superior ability for regulation of body temperature during heat stress is the result of lower metabolic rates as well as increased capacity for heat loss (Hansen, 2004). As discussed previously, these mechanisms or attributes to improve heat loss include their dense smooth hair coat which has little insulating capacity but reflects solar radiation (Blackshaw & Blackshaw, 1994); lower resistance to internal tissue heat transfer at high levels of heat stress (Finch, 1986); greater length, diameter and volume of sweat glands and their close proximity to the skin surface (Bianca, 1965); and reduced metabolic rate of *Bos indicus* compared with *Bos taurus* (Hansen, 2004). More recently it has also been suggested that the genetic evolution of *Bos indicus* cattle has seen them acquire genes for thermotolerance. There is evidence that cattle which have evolved in hot climates have acquired genes that protect cells from the deleterious actions of elevated temperature (Hansen, 2004). The finding that there are genetic differences in cellular resistance to high environmental temperatures in cattle is the first example in endotherms of genetic adaptations in cellular resistance to elevated temperature (Hansen, 2004).

The nychthemeral amplitude (mean daily maximum minus mean daily minimum $T_c$) of $T_c$ remained approximately 1°C in both breeds during the hottest period. This was in spite of mean daily $T_c$ rising from 38.4°C to 41.0°C (*Bos taurus*) and 38.5°C to 39.9°C (*Bos indicus*), the daily feed intake of *Bos taurus* animals falling significantly, the lack of diurnal variation in environmental conditions, and 24 h daylight.

It is likely that increases in nychthemeral amplitude observed by others (Berman & Morag, 1971; Gaughan *et al.*, 1999; Mader *et al.*, 1999) involved an increase in
diurnal maximum of $T_c$ under heat stress, while the nocturnal minimum remained unaffected when nocturnal respite from heat stress was provided. No nocturnal respite was provided in either experiment. An increase in nychthemeral amplitude was observed after the hottest period when WBT was reduced. For *Bos taurus* animals this was because of an increase in maximum daily $T_c$. Minimum $T_c$ was significantly increased only on days 5 to 13 whereas maximum $T_c$ was significantly increased on days 5 to 14 and day 17. The reason for this is unclear. For *Bos indicus* animals the increased amplitude after the heating period on days 12 to 14 and 17 was also due to an increase in maximum $T_c$. Maximum $T_c$ was significantly increased on days 8 to 13 while minimum $T_c$ was significantly increased only on days 8 to 11. It would appear that the heat increment of feeding had little impact on the amplitude of $T_c$ since the amplitude was maintained in the absence of feed intake in *Bos taurus* animals. Furthermore, the daily maximum and minimum $T_c$ for both breeds occurred between 2100 to 2200 h and 0700 to 0800 h, respectively, throughout the experiments, despite the lack of night time cooling and variations in feed intake. Finch (1986) suggested that an increase in the amplitude of $T_c$ in *Bos indicus* was in response to food deficits and was due to a decrease in the lower range of body temperature during the cooler night hours. In the *Bos indicus* experiment described here, there was no voluntary reduction in feed intake, no night time cooling, and no decrease in nocturnal $T_c$.

Elevated respiratory rates are part of the repertoire of responses used by cattle to increase heat loss in situations of elevated heat load (Hales & Findlay, 1968; Hales, 1976). Initially the elevated rate is associated with a decreased tidal volume and increases in alveolar ventilation are limited (Hales, 1976). However, in extreme conditions tidal volume increases, which increases minute ventilation and so increases respiratory evaporative water loss, but also leads to elevated alveolar ventilation, elevated CO$_2$ excretion, and alkalosis (discussed previously). *Bos taurus* had increased
core temperatures and respiratory rates at lower WBT temperatures compared to *Bos indicus*. Low RR is a characteristic of *Bos indicus* regardless of ambient temperature (Hammond *et al.*, 1998). This is not surprising as *Bos indicus* apparently have a number of anatomical and physiological features that improve heat loss from the skin (discussed above). However, for both breeds, there were significant increases in RR with increases in $T_c$, and the maximum RR was similar for both breeds, although the pattern of response to increasing WBT was different, reflecting that *Bos taurus* used panting for heat loss at lower WBT than did the *Bos indicus*. There was also an indication, with reduced RR in the middle of the hottest period for the *Bos taurus*, of a shift in RR dynamics from rapid open mouth panting to deep open mouth panting at a reduced rate (Gaughan *et al.*, 2000), which was associated with further blood gas changes (discussed below). Similar maximum RR between *Bos taurus* and *Bos indicus* was inconsistent with Hammond *et al.* (1998) who reported that low RR is a characteristic of Brahman regardless of ambient conditions.

Regression analysis of mean changes in RR with mean $T_c$ confirmed that *Bos taurus* displayed elevations in RR at lower $T_c$ than *Bos indicus*, but that as $T_c$ rose, *Bos indicus* had a larger increase in RR for a given increase in $T_c$ than *Bos taurus*. This suggests that the mechanisms linking RR to $T_c$ are more sensitive in *Bos indicus* and helps explain why the maximum RR were similar in the two breeds despite *Bos indicus* exhibiting smaller increases in $T_c$ over the hot period. The greater sensitivity of the panting mechanism in *Bos indicus* would help them to maintain lower $T_c$ than *Bos taurus* under heat load. Thus, in addition to the greater capacity for heat loss across the skin (outlined above) it appears *Bos indicus* also possess a more sensitive panting response than *Bos taurus*.

Increase in RR is an important thermoregulatory response to heat stress and aids in heat dissipation via evaporative cooling (West *et al.*, 1991; West *et al.*, 1992;
Blackshaw & Blackshaw, 1994). However, increased alveolar ventilation results in the excretion of CO₂ at a rate exceeding its production (Sanchez et al., 1994b), shifting bicarbonate equilibrium toward H₂CO₃ from H⁺ and HCO₃⁻. The net result of these processes is a respiratory alkalosis where pCO₂ decreases, pH increases, and the concentration of HCO₃⁻ decreases and is replaced by other buffers (Cunningham, 2002). When RR increased in *Bos taurus* 2 and *Bos indicus* 1, pCO₂ and HCO₃⁻ decreased, but there was no increase in blood pH. The results agree with Schneider et al. (1988) and suggest that there is a large turnover of HCO₃⁻ to maintain blood pH after a heating period, even more so after such a prolonged and continuous heat stress period. Respiratory alkalosis occurred only when heat stress was present during the day. During the cooler hours at night a lower urine pH and higher urine ammonium concentration were recorded, suggesting excretion of H⁺ in a compensatory urinary acidosis. This pattern of responses was similar to those observed in *Bos taurus* 2 and *Bos indicus* 1, but the respite only occurred when WBT was decreased. Then the animals were no longer panting and so expired less CO₂. Without adequate HCO₃⁻ buffering, a reduced blood pH and acidic urine resulted. Blood HCO₃⁻ concentration was still lower than control values at the end of the experiments, many days after WBT had been reduced.

Unlike Schneider et al. (1988) there was no observed increase in urine pH during the continuous heat period for either *Bos taurus* or *Bos indicus*. If HCO₃⁻ was being secreted by the kidneys a rise in urine pH would be expected. The ratio between plasma HCO₃⁻ and plasma pCO₂ was maintained throughout the heating period in both *Bos taurus* and *Bos indicus*, although the values of both were reduced. After the heating period this ratio was reduced, indicating a total body deficit of HCO₃⁻ and a metabolic acidosis. Total body stores of HCO₃⁻ were depleted as it was used as a blood buffer to counter the loss of CO₂ (for both breeds) and not being replaced due to inappetence (in *Bos taurus* only). The blood gas changes were not as marked in the *Bos indicus* as in the
This was most likely due to *Bos taurus* relying more heavily on evaporative respiratory cooling compared to *Bos indicus*.

Cattle reduce feed intake in response to heat stress (Yousef, 1985a; Blackshaw & Blackshaw, 1994) and this has been commonly reported in heat stressed cattle (Beede & Collier, 1986). A reduction in feed intake is followed by a fall in metabolic rate and, therefore, reduced heat production which helps to maintain heat balance (Turner & Taylor, 1983). Given the WBT achieved in both experiments, feed intake were drastically reduced for *Bos taurus* (days 6 to 14) and remained unchanged for *Bos indicus*. Therefore, it may be hypothesised that the environmental conditions that the *Bos indicus* were subjected to were not heat stress conditions. The decrease in feed intake in *Bos taurus* with increasing WBT was more pronounced than has previously been reported (Bianca, 1965; Conrad, 1985). The major reason for the difference in results probably was that the animals in the experiments described here were exposed to continuous high WBT with no nocturnal “cooling off” period. It would appear that the continuous high WBT had no effect on *Bos indicus* animals.

Regression results confirmed that the decreases in feed intake with elevations in \( T_c \) were less pronounced in *Bos indicus* than *Bos taurus*, but that there was an effect of \( T_c \) on feed intake in *Bos indicus*. This result may have occurred because of the influence of the one *Bos indicus* that did exhibit reduced feed intake during the experiment. Nevertheless, at all levels of \( T_c \) the feed intake of the *Bos taurus* was lower than that of *Bos indicus*. Thus the better maintenance of feed intake for *Bos indicus* during the hot period resulted from the interaction of two factors, the fact that feed intake was higher for a given \( T_c \) in *Bos indicus* than *Bos taurus*, and also that \( T_c \) did not increase as much.

Maximum water intakes for both breeds at least doubled, from 4.8 to 9.8% of starting weight for *Bos taurus*, and from 3.8 to 9.3% of starting weight for *Bos indicus*, agreeing with previous studies (Winchester & Morris, 1956; Phillips, 1960; Colditz &
Kellaway, 1972; Beede & Collie, 1986), although in the *Bos taurus* this increase could not be linked to a higher requirement per unit of feed ingested, as Winchester and Morris (1956) proposed, because of the drastic decrease in feed intake by the *Bos taurus*.

The mechanism for the increase in water consumption may, like feed intake, involve direct effects of the heat, since directly warming the pre-optic area and rostral hypothalamus of the goat caused a large increase in water consumption and hungry goats to stop eating (Andersson *et al*., 1960) (discussed previously). Additionally, although depleted water stores due to evaporative demands could induce increased water intake via hypovolaemia or hyperosmolarity, neither of these changes occurred in this study, suggesting the direct effect of heat. Whatever the mechanism underlying increases in water intake, Figure 5.13 suggests that they operate similarly in both breeds. There was a tendency for the relationship to be more sensitive in the *Bos indicus* ($P = 0.051$) which would account for the similar maximum water intake in both breeds despite smaller increases in $T_c$ in *Bos indicus*. However, on the evidence available we have to conclude that there was no difference in the effect of $T_c$ on water intake. It remains possible that elevations in water intake were driven by something other than elevations in $T_c$, and so there may not be a cause and effect in their relationship.

For both *Bos taurus* and *Bos indicus* the increased water consumption was accompanied by increased urine production, as indicated by the very low urine SG, but total blood volume also appeared to increase because PCV decreased. This has been discussed for both *Bos taurus* and *Bos indicus* in previous chapters with plasma volume increasing by a maximum of 31% (day 16) for *Bos taurus* and 38% (day 11) for *Bos indicus*. Reductions in PCV were contrary to Hammond *et al.* (1998) who reported higher PCV in Brahman compared to Angus. It was suggested that Brahmans had a higher blood oxygen carrying capacity compared to Angus.
The increased total blood volume and subsequent increase in preload to the heart may also be responsible for the apparent decrease in HR observed in the *Bos taurus* experiment. This was unexpected as others have reported increases in HR due to heat stress (Bianca, 1965). Heart rate remained unchanged for *Bos indicus*, again suggesting that animals were not experiencing heat stress conditions.

*Bos indicus* animals maintained plasma Na concentrations whilst *Bos taurus* had significant decreases both during and after the hottest period of the experiment. The reduction in plasma Na concentration during heat stress has been described by El-Nouty et al. (1980) and is caused by an increase in urinary Na excretion due to increased total urinary output. Low plasma aldosterone during heat exposure may be responsible for the increase in urinary Na excretion and decrease in plasma Na concentration. It has also been suggested that renal excretion of HCO$_3^-$ must be accompanied by a cation (Sanchez et al., 1994b). Sodium or K are possibilities; however, Na is more likely. After the heating period, the Na FER was reduced, indicating conservation of Na. For the *Bos taurus* animals inappetence meant that Na stores were not being replenished and this is probably why they had larger and more prolonged reductions in plasma Na concentrations than those observed in *Bos indicus*.

Similar responses in plasma potassium, calcium, urea, and creatinine concentrations as well as PCV were reported for both *Bos taurus* and *Bos indicus* and these changes have been discussed in previous chapters.

Activation of the hypothalamic-pituitary-adrenal axis and the consequent increase in plasma cortisol concentration are the most prominent responses of an animal to stressful conditions (Silanikove, 2000). The effects of heat stress on plasma cortisol concentrations in cattle are somewhat varied. For the *Bos taurus* experiment, plasma cortisol concentrations were highest on days 1 and 2 (when rooms were turned off) and day 3 when WBT was only 26°C. Introduction into the climate controlled rooms may
have been a stressful enough event to cause an initial increase in plasma cortisol concentrations. There is evidence to suggest that plasma cortisol concentrations increase during periods of acute heat stress in cattle (Alvarez & Johnson, 1973). There was no evidence in either the *Bos taurus* or *Bos indicus* experiments that this was the case when climate control rooms were turned on. However, with prolonged or chronic heat exposure, a significant decrease was measured. This agrees with Alvarez & Johnson (1973) who found that Holstein cows exposed to 35°C for 24 days had lower glucocorticoid levels by day 24 compared to cows kept at 18°C. The physiological significance of the depressed plasma hydrocortisone concentration during heat acclimation was not clear. Beede & Collier (1986) suggested that as cattle adapt to chronic thermal stress, their energy metabolism (basal metabolic rate) decreases, while water and electrolyte metabolism increases. These adaptations are reflected in lower concentrations of metabolic hormones such as thyroxine, growth hormone and corticoids. Alvarez & Johnson (1973) suggest that the depression of plasma hydrocortisone they found with chronic heat stress may be related to the fact that glucocorticoids exert a stimulatory effect on heat production in cattle and depressed adrenocortical function during chronic heat stress might contribute to depression in heat production. Roman Ponce *et al.* (1981, as cited by Beede and Collier, 1986) suggested lower glucocorticoid and higher progesterone concentrations in heat stressed animals may be related to reduced conversion of progesterone to cortisol. More recently, Silanikove (2000) has suggested that a decline in plasma cortisol concentration during chronic heat stress indicates adaptation to the stress and an increase in the cortisol concentration over the basal level in animals that are chronically exposed to heat load is an indication that the animals are distressed. Animals in the *Bos taurus* experiment gave every indication that they were clinically distressed after chronic exposure to heat load and yet there was no subsequent increase in plasma cortisol concentration.
5.7 Conclusions

*Bos indicus* animals are much better adapted to physiologically cope with periods of high heat and humidity as highlighted by less significant changes in feed intake, $T_c$, RR and blood gas variables. Both *Bos taurus* and *Bos indicus* have a remarkable ability to maintain blood gas homeostasis during prolonged and continuous high heat and humidity. It would appear that it is after the heating period that animals are no longer able to maintain homeostasis and a metabolic acidosis develops. The directions of changes in blood gas variables were the same for both breeds but *Bos taurus* were more severely affected for longer. Inappetence would appear to be of major concern for *Bos taurus* animals experiencing continuous periods of high heat and humidity. Further research is required to evaluate methods of alleviating or modifying the physiological responses and imbalances that occur when *Bos taurus* animals in particular are subjected to prolonged and continuous periods of high heat and humidity.

Given the *Bos indicus’* superior ability to cope with excessive heat loads, in particular the maintenance of feed intake, it would appear that they are much better suited to travel on board live export vessels to the Middle East during the hot, humid northern hemisphere summer months.
6.1 Introduction

The physiological effects of excessive and prolonged heat load on Bos taurus animals have been described in previous chapters. Results indicated that under the heat stress conditions the cattle encountered, reductions in feed intake were of major concern. Furthermore, excessive heat load is also associated with plasma electrolyte imbalances and acid base disturbances as well as significant increases in water consumption. What was not clear was whether it was the reductions in feed intake, the heat stress conditions, or a combination of both which caused the physiological disturbances. With electrolyte intervention experiments planned, greater knowledge of the physiological and metabolic effects of reduced feed intake and increased water intake without excessive heat load was required.

An experiment was undertaken to assess the physiological responses of cattle to reduced feed intake and increased water intake without heat stress conditions. The physiological and metabolic responses were then compared to the same responses observed in Bos taurus heifers subjected to excessive heat load in Chapter 3.

6.2 Aims

The aim of this chapter was to differentiate between the effects of excessive heat load and the effects of reductions in feed intake and increases in water intake on acid base balance, plasma electrolyte concentrations and energy metabolism in Bos taurus heifers.

Chapter 6: Pair feeding Bos taurus heifers to heat stress conditions.
6.3 Hypotheses

1. It is the excessive heat load, rather than the reductions in feed intake and increases in water consumption, which influences acid base homeostasis.

2. It is the excessive heat load, rather than the reductions in feed intake and increases in water consumption, which influences plasma electrolyte concentrations.

3. It is the reduction in feed intake, rather than excessive heat load, which is responsible for changes in energy metabolism in *Bos taurus* heifers.

6.4 Materials and methods

6.4.1 Experimental design

Six *Bos taurus* heifers were randomly allocated to individual pens in one of 2 experimental rooms at Murdoch University for 15 days under ambient climatic conditions. Three heifers (Control; “C”) were fed at 2.25% of BW (as fed) (weighed after 18 h feed curfew but not water) with water available *ad libitum*. The other 3 heifers (Pair Fed; “PF”) had their feed intake reduced for 10 days between days 3 and 12, so as to match the average feed intake of the 6 *Bos taurus* 2 heifers (Hot; “H”) measured in Chapter 3. The 10 days of reduced feed intake corresponded to days 4 to 13 in Chapter 3. The PF animals with reduced feed intakes were also drenched with water once or twice daily, for 10 days, so that their total daily water intake matched the average daily intake of the animals between days 4 and 13 in Chapter 3 (Table 6.1). Animals entered the experimental rooms on day 1 at 0800 h and were removed on day 15 at 0800 h.
Table 6.1 Daily feed and water intake, expressed as a percentage of body weight, of pair fed heat animals. The intakes of the “pair fed” animals were calculated by averaging the daily intakes of the “hot” animals (*Bos taurus* 2). Control animals received 2.25% body weight (as fed) feed intake daily and *ad libitum* water throughout.

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Feed intake (PF)</th>
<th>Water intake (PFH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.25</td>
<td><em>Ad libitum</em></td>
</tr>
<tr>
<td>2</td>
<td>2.25</td>
<td><em>Ad libitum</em></td>
</tr>
<tr>
<td>3</td>
<td>1.61</td>
<td>7.23</td>
</tr>
<tr>
<td>4</td>
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<td>8.66</td>
</tr>
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<td>8.21</td>
</tr>
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<td>0.72</td>
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</tr>
<tr>
<td>13</td>
<td>2.25</td>
<td><em>Ad libitum</em></td>
</tr>
<tr>
<td>14</td>
<td>2.25</td>
<td><em>Ad libitum</em></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>Animals removed at 0800 h</td>
</tr>
</tbody>
</table>
The physiological and metabolic responses of C animals and PF animals were compared to the responses of H animals used in experiments described in Chapter 3 (*Bos taurus 2*).

### 6.4.2 Animals and management

Six 18 to 24 month old Angus cross heifers (343 ± 19 kg BW; mean ± SEM) were chosen from the Murdoch University farm herd based on temperament and body weight. The total weight for each room was 1041 kg (C) and 1022 kg (PF). The experiment ran from 13\textsuperscript{th} September to 27\textsuperscript{th} September 2002.

Animals were given one week adaptation to a commercial dietary cube; 8.6 MJ of ME, 11.9% CP, and 39.9% NDF per kg of DM (Macco Feeds, Western Australia, Australia) as described in Chapter 3. Full analysis and chemical composition of feed is shown in Appendix 1.

Indwelling jugular catheters were inserted the day before the commencement of the experiment.

On day 1, the C animals were randomly allocated a pen in room 1 and the PF animals randomly allocated a pen in room 2. The climate controlled rooms were not operating so environmental conditions were the same in both rooms. Feed was offered at 2.25% BW daily for the C animals and as per Table 6.1 for PF animals. Feed was divided into two equal amounts, given at 0700 and 1300 h daily. Residues were cleaned out and weighed before each morning feed. Total daily intakes for the previous day were then calculated. Water was available *ad libitum* for C animals and as per Table 6.1 for PF animals. For C animals the total amount of water drunk was calculated once daily by subtracting the weighed residue of water each morning from the total amount of water given for the past 24 hours. Water buckets were topped up as necessary. Pair Fed
animals were drenched either once or twice daily so as they received the required intake as indicated in Table 6.1.

The experimental design for the H animals was described in Chapter 3 (*Bos taurus* 2).

### 6.4.3 Sample collection and analysis

Animals were weighed on days 0, 12 and 15 (after 18 h off feed but not water). On day 10 one animal in the PF group escaped from the room and gorged on pelleted feed. Consequently this animal was removed from the experiment and PF animals were n = 2 from day 10 onwards. Body weights were converted to BW as a percent of start weight (start weight = 100%) by dividing measured BW (kg) by starting BW (kg) and multiplying by 100.

Heart rate, RR and RT were measured twice daily at 0600 and 1200 h on days 4 to 14 and once daily at 1200 h on all other days. Jugular venous blood and voided urine samples were collected daily at 1200 h except on days 3 and 10 when no samples were taken. Blood and urine were also collected at 2200 h on days 6, 7 and 8 and at 0600 h on days 7, 8 and 9. For those samples which were measured more than once daily, a single daily average was calculated.

Sample collection for H animals was described in Chapter 3.

Blood gas variables and plasma electrolytes were analysed as described in the general materials and methods Chapter 2.

### 6.4.4 Metabolite analysis

Plasma glucose and lactate concentrations were measured by using specific Sigma-Aldrich kit assays (Sigma-Aldrich, Sydney, Australia) on a Titertek Multiscan ELISA reader. Plasma insulin concentrations were measured using a Porcine insulin kit
Plasma NEFA concentrations were measured using a Wako NEFA kit assay (Wako Pure Chemical Industries, Japan) on a Titertek Multiscan ELISA reader.

6.4.5 Statistical analysis

For all measured variables a two way ANOVA with treatment and time as main effects and time as a repeated measure, was used to statistically analyse data (Statistica Mac software; StatSoft Inc, Tulsa, OK, USA). The 3 levels of treatment were C (n = 3), PF (n = 2) and H (n = 6). When a significant interaction was detected by the ANOVA a post hoc test (Student Newman Keuls) was used to determine the difference between the three treatments on each day. The small number of animals tested, especially in the PF group where one animal was withdrawn, limited the power of the statistics.

On all figures, days on which a 5% level of significance was detected between treatments are represented by the symbol “a” for difference between C and PF on that particular day, “b” for differences between PF and H on that particular day and “c” for differences between C and H on that particular day.

6.5 Results

Overall results of ANOVA’s from measured variables are presented in Table 6.2. Only variables for which treatment was different on a particular day are presented in results. It was accepted that the low number of animals tested limited the power of the statistics. Therefore, the means of the measured variables are discussed in general terms.

The feed intake of the C animals remained at around the amount offered (2.25% of BW) for the duration of the experiment (Figure 6.1.i). Over the period the H animals were exposed to heat stress conditions, their feed intakes decreased and were
Table 6.2 Table of P values from ANOVA for main effects of time and treatment and the interaction between treatment and time. Treatments were control (n = 3), pair fed (n = 2) and hot (n = 6).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect of day</th>
<th>Effect of treatment</th>
<th>Interaction effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake</td>
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<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Water intake</td>
<td>&lt; 0.0001</td>
<td>0.45</td>
<td>0.04</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.04</td>
<td>0.74</td>
<td>0.003</td>
</tr>
<tr>
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significantly less than the C animals on days 3 to 13. As expected the PF animals mirrored closely the H animals and compared to C their intakes were significantly less on days 4 to 12. Only on day 13 was there a difference between PF and H animals. On day 13 PF animals had a significantly higher feed intake than H animals. Results for feed intake for day 14 were not included as feed was removed at 1400 h in order to obtain curfew weights on day 15.

Water consumption increased for H as WBT increased (Figure 6.1.ii). As expected the water intakes of PF animals followed a similar pattern to H animals as drenching took place between days 3 to 12. The water consumption of C animals remained relatively constant throughout the experiment. However, statistically there were no days on which treatments differed.

Control animals maintained body weight throughout the experiment (Figure 6.2). For both PF and H animals the reduction in feed intake led to a reduction in body weight and this was significant on both day 12 and day 15 compared to C. On day 12, PF had lost significantly more BW compared to H. On day 15, H had lost significantly more BW than PF.

In general plasma Mg concentration of all treatments remained relatively unchanged throughout the experiments. Magnesium concentration started higher and remained higher in H compared to C and PF suggesting that Mg concentration had more to do with animal backgrounding and variation due to time of year rather than feed intake or heat (Figure 6.3.i). Other measured plasma electrolytes included Na, K, Cl and Ca. Although there were significant interactions between treatment and time for Na, K and Ca, treatments did not differ on any days (data not presented).

Excessive heat load caused an increase in plasma urea concentration for H animals (Figure 6.3.ii). In general, urea concentration increased over the duration of the experiment in C animals and decreased in PF animals. On days 8 to 12 plasma urea
Figure 6.1 Daily (i) feed and (ii) water intake for control (∆), pair fed (x) and hot animals (o). Points show mean ± SEM. Days on which treatments are different (P < 0.05) are represented by the letters “a” for difference between C and PF, “b” difference between PF and H and “c” difference between C and H.
Figure 6.2 Live weight change (mean ± SEM) expressed as a percent of starting weight for control, pair fed and hot animals. Days on which treatments are different (P < 0.05) are represented by the letters “a” for difference between C and PF, “b” difference between PF and H and “c” difference between C and H.
concentration was significantly higher in H animals compared to PF animals. On days 11, 12 and 15, C had higher plasma urea concentrations compared to PF. On day 15 H had a higher urea concentration compared to C.

Plasma creatinine concentration in H increased with increasing WBT (Figure 6.3.iii) and was significantly higher than both C and PF on days 7 to 11 and higher than PF only on day 12. For C and PF plasma creatinine concentration did not appear to change over the duration of the experiment.

The RR of the H animals increased as WBT increased (Figure 6.4). The RR of the C and PF animals remained relatively constant throughout the experiment and did not differ on any day.

Changes in the blood gas variables pH, pCO\(_2\), and HCO\(_3^-\), are shown in Figure 6.5. As reported in previous chapters, the venous blood pH of H remained unchanged during the hottest period and decreased after the climate controlled rooms were turned down. The blood pH also decreased for PF animals but this occurred earlier and coincided with reductions in feed intake. The PF animals had significantly lower blood pH compared to H animals on days 4, 5, 8, 9 and 11. On days 1, 12 and 13 the PF had significantly lower pH compared to C. On day 14 the H animals had significantly lower pH compared to C animals (Figure 6.5.i).

For the H animals, pCO\(_2\) decreased as respiratory rate increased during the hot conditions (Figure 6.5.ii). The pCO\(_2\) for both the C and PF remained unchanged. The pCO\(_2\) for H was significantly less than the PF on days 5 to 14 and significantly less than C on days 6 to 11.

Plasma HCO\(_3^-\) concentration followed a similar pattern to pCO\(_2\) (Figure 6.5.iii). Bicarbonate concentration for H was significantly less than PF and C on days 7 to 12 and 14.
Figure 6.3 Daily plasma (i) magnesium; (ii) urea and (iii) creatinine concentrations for control (△), pair fed (x) and hot animals (o). Points show mean ± SEM. Days on which treatments are different (P < 0.05) are represented by the letters “a” for difference between C and PF, “b” difference between PF and H and “c” difference between C and H.
Figure 6.4 Daily respiratory rate for control (△), pair fed (x) and hot animals (o).

Points show mean ± SEM. Days on which treatments are different (P < 0.05) are represented by the letters “a” for difference between C and PF, “b” difference between PF and H and “c” difference between C and H.
Figure 6.5 Daily plasma (i) pH; (ii) partial pressure carbon dioxide and (iii) bicarbonate concentration for control (△), pair fed (x) and hot animals (○). Points show mean ± SEM. Days on which treatments are different (P < 0.05) are represented by the letters “a” for difference between C and PF, “b” difference between PF and H and “c” difference between C and H.
Urine pH for H animals followed a similar pattern to venous blood pH and decreased after the heating period. Urine pH for PF animals appeared to decrease as feed intakes were reduced. Urine pH for C animals remained unchanged throughout (Figure 6.6). Hot animals had significantly lower urine pH compared to C animals on days 12 to 15. Pair Fed animals had significantly lower urine pH compared to C animals on days 11 and 13. Hot animals had significantly lower pH than PF animals on days 14 and 15.

Plasma lactate concentrations were variable. The trend for both C and PF was for lactate to decrease early in the experiment and then plateau (Figure 6.7.i). However, for PF animals there was a sharp increase in lactate concentration on days 14 and 15. For H animals, plasma lactate tended to increase slightly during the hot period. On days 1 and 14 PF animals had significantly higher lactate concentrations than H animals. Pair Fed animals also had higher lactate than C animals on day 14.

The mean plasma concentration of NEFA did not appear change throughout the experiment for C animals. Non esterified fatty acid concentration increased for both H and PF animals (Figure 6.7.ii). The PF animals had significantly higher NEFA concentrations than C animals on days 7 to 11. The H animals had significantly higher NEFA concentrations than C animals on day 11 and 12. On days 11 and 12 PF had significantly higher NEFA concentrations than H.

Other metabolic variables measured included glucose and insulin. Although there were significant interactions between treatment and time for these variables there were no differences in treatments on any days (data not presented).
Figure 6.6 Daily urine PH for control (△), pair fed (x) and hot animals (o). Points show mean ± SEM. Days on which treatments are different (P < 0.05) are represented by the letters “a” for difference between C and PF, “b” difference between PF and H and “c” difference between C and H.
Figure 6.7 Daily plasma (i) lactate and (ii) non esterified fatty acid concentration for control (△), pair fed (x) and hot animals (○). Points show mean ± SEM. Days on which treatments are different (P < 0.05) are represented by the letters “a” for difference between C and PF, “b” difference between PF and H and “c” difference between C and H.
6.6 Discussion

The loss of one animal from the pair fed group resulted in the power of the statistical analysis being reduced. However, the figures presented in results highlighted the trends in means for the variables measured and these means will be discussed in general terms where appropriate.

6.6.1 Feed and water intake and body weights

As expected the daily feed intake of PF animals closely matched that of the H animals. The only exception was on day 13. Day 12 was the last day of pair feeding so on day 13 the starved pair fed animals returned to full feed intake. The prolonged nature of the heat exposure on H animals meant that their feed intake did not return to normal or pre-heat levels on day 13 even though environmental conditions were the same as ambient environmental conditions. The reasons for the delay in return to full feed intake for previously heat stressed animals remains unclear, although was most likely linked to elevated core body temperatures which were still evident after heat stress conditions subsided. There is also evidence to suggest that physiological stressors such as heat stress and starvation may cause endotoxemia which may further inhibit feed intake after heat stress conditions subside (Hungerford, 1990). In humans, hyperthermia or an increase in Tc, may cause leakage of endotoxins from the gut into the blood stream. This may contribute to the pathophysiology of heatstroke (Hales et al., 1996). Reducing core temperature is not sufficient therapy as the patient is not only suffering from hyperthermia, but also endotoxemia (Hales et al., 1996).

It proved more difficult to successfully match the water intakes of PF to H animals. Even though there was no significant difference on any days between the PF and C animals there was also no difference on any days between H and C animals.
However, the daily means suggested that PF animals were consuming more water than C animals. It was considered that the water intakes of the PF animals were sufficiently increased to match those that may be expected when animals are exposed to prolonged and continuous EHL.

Given the reductions in feed intake by H and PF animals it was not surprising to see reductions in BW compared to C animals. Reductions in dry matter intake during heat stress conditions have been reported in other studies (Collier et al., 1982; Blackshaw & Blackshaw, 1994). The decrease in BW for PF and H animals was evident on day 12 and day 15. Of interest was the fact that on day 12, PF animals had a greater reduction in BW change than H animals (91.0 versus 97.1% respectively). The reason for this is not clear as daily feed and water intakes remained much the same and it has been reported that daily maintenance requirements for heat stressed cows increase due to elevated body metabolism in an attempt to alleviate excess heat load (Collier et al., 1982). An explanation for the greater loss in BW for PF compared to H on day 12 may be because PF animals were more active, resulting in an increase in metabolic rate and greater energy expenditure. Although there was not a lot of room for animals to move around in the climate rooms, it was evident that H animals spent more time standing still or lying down in an attempt to reduce elevations in core body temperature from over exertion. Pair Fed animals were more active or restless. This would agree with Beede & Collier (1986) who suggest that as cattle adapt to chronic thermal stress their energy metabolism (basal metabolic rate) decreases due to decreases in the activity and concentration of T₃ and T₄. Whether T₃ and T₄ decline due to thermal inhibition of the hypothalamus or indirectly because of lowered feed intake and metabolism is not clear (Johnson, 1985) and neither T₃ or T₄ were measured in this experiment.

The result was reversed on day 15 although the difference between the two groups was not as great (96.5 versus 94.8% of starting weight for PF and H
respectively). It may be that by day 15 the PF animals had become accustomed to the lack of food offered and activity slowed accordingly (perhaps due to reductions in thyroid hormone concentrations). The H animals were continuing to expend energy as heat loss mechanisms such as increased RR and sweating were being utilised to try and alleviate the excessive heat load. For example accelerated panting may increase maintenance requirements by 7 to 25% (NRC, 2000).

6.6.2 Plasma electrolytes

Previous experiments reported in this thesis have indicated that heat stress conditions such as those which may be encountered by cattle on long haul voyages to the Middle East may cause reductions or imbalances in essential plasma electrolytes such as sodium and potassium. This has also been noted in heat stressed dairy cows and steers, with losses in sweat (Collier et al., 1982) and urine (Beede & Collier, 1986) being suggested as reasons for reductions. Furthermore, as cattle adapt to chronic thermal stress, water intake and electrolyte metabolism increase and are associated with adaptation to thermal stress as evaporative cooling requirements increase (Beede & Collier, 1986). Increased water turnover requires associated increases in electrolyte turnover to move water through various fluid pools to the evaporative surfaces (Beede & Collier, 1986). Decreases in feed intake result in the intake of absolute amounts of essential nutrients, electrolytes and ME being decreased, unless their density is increased proportionally in the diet at lower intakes (Collier et al., 1982). Results from the Bos taurus 2 or H animals (Chapter 3) showed that plasma Na concentration was reduced towards the end and after the heating period compared to initial pre-heat concentration. Furthermore, both the FER of Na and K were very low after the heating period indicating renal conservation of these electrolytes. For the pair feeding experiment described here there were no days on which either Na or K plasma
concentrations were different between PF, C and H animals. Therefore, it can not be elucidated whether it was the reduced feed intake and increased water intake or the continuous heat stress conditions which influence plasma sodium and potassium concentration. However, as stated earlier, plasma concentrations of Na and K are not great indicators of total body deficiency (Duncan et al., 1994) and with the limited number of animals tested in these experiments and the small changes expected, care should be taken in interpreting results.

It is interesting to note that plasma magnesium concentration was generally higher in the H group than both the C and PF animals. This was largely explained by the different time of year the experiments were conducted. Pastures that the H were grazing prior to experiment would have been higher in Mg content than later in the year when the pair feeding experiment took place. Generally the plasma concentration of Mg did not change a great deal over the duration of the experiments and so was not influenced by either reduced feed intake or excessive heat load. Furthermore, there were no days during any experiment when mean plasma Mg concentrations were outside what is considered the normal range for cattle.

6.6.3 Blood gas

The changes in blood gas homeostasis due to increases in RR from EHL have been described previously, with reductions in bicarbonate and pCO₂ occurring during hot periods, and acidosis, as indicated by reduced blood pH, occurring after heat stress conditions subside. As expected there was no change or difference in RR between PF and C animals. The H animals increased RR as WBT increased and their RR was different to both C and PF whilst climate rooms were operating. Therefore, it can be concluded that RR was influenced by heat and humidity rather than reductions in feed intake. It appeared that HCO₃⁻ and pCO₂ were also influenced by heat only, as
concentrations of both were reduced for H animals and remained relatively unchanged for PF and C animals with no differences observed on any day between these two groups.

For H animals, blood pH remained relatively constant throughout the hottest period but decreased once heat stress conditions subsided. Results appear to indicate that reductions in feed intake without heat also alter blood pH. Pair Fed animals had significant reductions in blood pH compared to H animals on days when CCR would have been at maximum WBT. It has already been established that starvation can be a cause of metabolic acidosis (Cunningham, 2002) and the results described here agree. It is hypothesised that the metabolic acidosis would have also been evident in H animals (due to starvation); however, a simultaneous respiratory alkalosis, as indicated by reduced pCO₂ and HCO₃⁻, meant that blood pH homeostasis was maintained. It was only after the heating period when the respiratory alkalosis subsided that the metabolic acidosis caused a reduction in blood pH for H animals. The decrease in pH accompanying metabolic acidosis is a stimulus to ventilation which eliminates CO₂ thereby decreasing pCO₂ and restoring the [HCO₃⁻/0.03 x pCO₂] ratio and pH towards normal (Cunningham, 2002). An increase in RR accompanying metabolic acidosis was not seen in PF animals. This was most likely due to the small number of animals tested and the fact that RR was only measured one to two times daily and not consistently over the entire day. Therefore, subtle changes in RR were unlikely to be detected.

The daily means of urine pH followed similar trends to blood pH; however, significant differences were only detected towards the end of the experiments. In general, PF animals produced acidic urine compared to other groups whilst feed intakes were reduced. This is in response to the metabolic acidosis caused by starvation. The kidneys excrete non volatile fatty acid by the secretion of hydrogen ions (Cunningham, 2002). A greater reduction in urine pH was evident in H animals; however, this was
only present after heat stress conditions subsided when not only was the metabolic acidosis still evident, but there was also a reduction in $\text{HCO}_3^-$ available for blood buffering due to the chronic respiratory alkalosis animals had encountered.

### 6.6.4 Urea and creatinine

It was established in earlier *Bos taurus* experiments that both plasma urea and creatinine concentrations increased with increasing WBT, and the reasons for this have been discussed. Increased protein catabolism secondary to starvation or fever may cause mild increases in blood urea via increased hepatic synthesis of urea (Duncan *et al.*, 1994). This is supported by others such as Terui *et al.* (1979) who reported that increases in plasma urea concentrations in heat stressed cattle were due to an increase in protein catabolism from heat stress hyperthermia. Reduction in feed intake in PF animals did not cause increases in urea concentration; therefore, it appears that hyperthermia may influence urea concentration more than starvation. Interestingly, Srikandakumar & Johnson (2004) reported decreases in blood urea nitrogen in heat stressed lactating cows which may have been associated with decreased feed intake. Why there were significant differences on day 11 and 12 between C and PF animals remains unclear. The daily mean of the C animals appeared to follow a similar pattern to H animals and increase over the duration of the experiment. It can only be assumed that the small number of animals tested and individual variation were responsible for this finding.

Plasma creatinine concentration was clearly affected by heat only, because creatinine in both PF and C animals remained unchanged for the duration of the experiment and were significantly lower than H animals when they were experiencing the hottest conditions. Earlier discussion on increases in plasma creatinine concentration centred around decreases in feed intake, with Terui *et al.* (1979) reporting increases in
plasma creatinine concentrations being due to the activated endogenous nitrogen catabolism with a decline of feed consumption reinforced by heat stress or cell degeneration in skeletal muscle. Similar conclusions were made by Schneider et al. (1988) who suggested that creatinine concentrations increased due to catabolism of muscle, possibly resulting from reduced feed intake and stress. From the results described here, it would appear that a decline in feed consumption alone has little impact on creatinine concentration. This is supported by Duncan et al. (1994) who state that creatinine concentration is not significantly affected by diet and catabolic factors but is affected by muscle mass which may be altered by muscle disease, generalised wasting, and conditioning. Therefore, the combination of starvation and excessive heat load along with chronic inactivity in H animals led to reductions in muscle mass and large increases in creatinine concentration. This was not evident in either C or PF animals.

6.6.5 Metabolites

During metabolism, there is a continuous production of fixed acids. An increase in their production or failure of hydrogen ion elimination by the kidneys causes metabolic acidosis (Cunningham, 2002). As discussed previously, starvation can cause increased production of fixed acids due to protein catabolism or ketone production, leading to metabolic acidosis. Increased production of fixed acids can also be a result of anaerobic metabolism which in turn leads to lactic acidosis (Cunningham, 2002). Studies have indicated that lactic acid accumulation in the blood and the excretion of alkali from the kidneys may be responsible for counteracting the respiratory alkalosis during heat stress conditions (Bianca & Findlay, 1962). Contrary to Bianca & Findlay (1962) there was no evidence to suggest that either heat stress conditions, reductions in
feed intake or a combination of both was responsible for increasing plasma lactate concentration.

Dale and Brody (1954, as cited by Collier et al., 1982) suggested that heat stressed cattle may experience metabolic ketosis as energy input would not satisfy energy requirements and thus accelerated body fat catabolism would result. Incomplete fatty acid oxidation would produce ketones, and if these ketones are produced more rapidly than excreted, they would accumulate in the blood and deplete blood alkali reserves, possibly potentiating metabolic acidosis (Collier et al., 1982). Dale and Brody (1954, as cited by Collier et al., 1982) conducted experiments which identified differences in energy metabolism of animals experiencing similar dramatic reductions in feed intake and carbohydrate oxidation. One group of animals had reduced feed intake due to heat stress and the other due to enforced starvation. Blood and urine ketone concentrations increased only in starved animals, suggesting increased fat depot mobilisation and incomplete fat oxidation. Blood and urine ketones were not measured in either H or PF animals, but a similar mechanism may have operated in PF animals as indicated by the metabolic acidosis encountered during starvation. The acidosis encountered post heating by H animals may be due to a combination of factors such as starvation (and increased ketone production) and reduction in buffering capacity due to depleted blood alkali reserves post respiratory alkalosis.

Starvation and lipase stimulation leads to the release of fatty acids from adipose tissue into the blood. Fatty acids in blood are reversibly bound to albumin and are usually referred to as non-esterified fatty acids (Cunningham, 2002). Non-esterified fatty acids in blood may be used directly for energy by many tissues. However, a large portion of the fatty acids are taken up by the liver and used for ketone body production (Cunningham, 2002). Results from the experiment indicated that NEFA concentration was driven by reductions in feed intake rather than excessive heat load and in fact PF
animals had larger increases in NEFA concentration than H animals. The reason for this remains unclear because feed intakes for both H and PF animals were reduced to the same extent. Prolonged starvation in dairy cows has also been shown to increase NEFA concentrations (Brumby et al., 1975).

Other measured metabolic variables included glucose, lactate and insulin. There were no significant differences detected between groups on any given day.

### 6.7 Conclusion

It is accepted that the small numbers of animals involved in the experiment described here reduced the power of the statistics and thus the validity of some measured variables. However, there were still significant changes in many measured variables. Most notable were the changes in blood gas variables where it appeared that blood pH was influenced by both starvation and excessive heat load, thus confirming the presence of an underlying metabolic acidosis in conjunction with a respiratory alkalosis during prolonged and continuous heat exposure. The cause of the metabolic acidosis was most likely multifactorial. It did not appear that lactic acid accumulation in the blood had any influence on acid base balance, as had been reported in some previous studies (Bianca & Findlay, 1962). It was assumed that some other fixed acid or ketone bodies were responsible for the acidosis encountered.

It appeared that NEFA increases were driven by decreases in feed intake rather than excessive heat load.

Collectively the findings from this experiment were important when considering electrolyte supplementation for cattle subjected to prolonged and continuous periods of high heat and humidity. Supplementation of electrolytes must occur via the drinking water as large decreases in feed intake may be expected during periods of prolonged and continuous high heat and humidity. Also, attempts to increase feed intake via electrolyte
supplementation may not necessarily prove beneficial as many variables were not influenced by feed intake reductions. Instead supplementation should focus on maintaining acid base homeostasis during and after periods of high heat and humidity.
Chapter 7: Efficacy of electrolyte supplementation in *Bos taurus* during prolonged and continuous periods of heat and humidity

7.1 Introduction

Information about animal husbandry and electrolyte supplementation on board livestock vessels is based largely on anecdotal reports. There is evidence to suggest that large amounts of money are spent each year on electrolyte supplementation of cattle on board long haul voyages to the Middle East (M. McCarthy, personal communication). Most electrolyte supplementation occurs during heat stress conditions as cattle are shipped from southern Australia into the northern hemisphere summer. It has been noted on some voyages that animals have survived initial periods of prolonged high heat and humidity but mortality rates have subsequently increased if the same animals have been subjected to subsequent heat stress events, even if these are not as prolonged or extreme as the primary heat insult (M. McCarthy, unpublished data).

Electrolyte formulation and dose rates are variable and little is known of the welfare, physiological or economic benefits electrolyte supplementation has for cattle during live export (Alliance Consulting & Management, 2001). What is known is based largely on anecdotal evidence. No scientific research has been undertaken to assess the efficacy of electrolyte supplementation on board livestock vessels during periods of heat stress.

Few studies have evaluated the use of electrolyte supplements in cattle. Recent research has been based on balancing Dietary Cation Anion Difference (DCAD) in an
attempt to improve transport stress (Schaefer et al., 1992; Schaefer et al., 1997), increase DMI and milk yield in dairy cows (Tucker et al., 1988; Sanchez et al., 1994a), improve performance in growing steers (Ross et al., 1994) and reduce the effects of heat stress in dairy cows (Schneider et al., 1984; Schneider et al., 1986; West et al., 1991; West et al., 1992; Sanchez et al., 1994b). The theory behind these improvements is that the acid-base balance of animals is dependant upon the balance between anions and cations in the blood and this can affect animal performance (West et al., 1991). Studies have been based on manipulating dietary Na, K and Cl in an attempt to increase DCAD, and results would indicate that increasing the DCAD may prove beneficial to heat stressed cattle (Schneider et al., 1986; West et al., 1991). However, the mechanisms of action remain unclear and the environmental conditions for these land based studies are vastly different to those that may be encountered on long haul voyages to the Middle East.

Results presented in previous chapters have shown that heat stress conditions similar to those encountered on board livestock vessels cause significant reductions in DM intake, acid-base disturbances and plasma electrolyte imbalances. These changes are more pronounced and severe for Bos taurus heifers than for Bos indicus heifers.

Homeostatic mechanisms attempt to maintain core body temperature during heat stress conditions resulting in reduced voluntary feed consumption. A reduction in feed intake results in a reduction in the intake of essential mineral elements. Furthermore, K is lost through sweating at high ambient temperatures (Johnson, 1970) and Na and K are the major regulators of body water balance (Guyton & Hall, 1996). During heat stress conditions Na is required for renal conservation of K and to balance HCO$_3^-$ excretion (Sanchez et al., 1994b).

Significant decreases in the FER of Na and K after heat stress conditions reported in the preceding chapters indicate renal 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 and that any form of electrolyte supplementation would have to be delivered almost entirely via drinking water. It was shown that heat stressed animals could drink 10% of body weight in a 24 h period. The suggested daily intake of sodium and potassium for stressed young cattle (250 kg BW) is 10 to 14 g of Na and 57 to 67 g of K. This equates to 0.2 to 0.3% Na and 1.2 to 1.4% K in the total ration (NRC, 2000). The concentrations of Na and K in the standard shipper pellet that was used in *Bos taurus 1* and *Bos taurus 2* (chapter 3) were 0.065 and 0.486% respectively, which was well below suggested intakes even when the animals were eating well.

Panting in both *Bos taurus 1* and *Bos taurus 2* animals was associated with decreases in blood carbon dioxide concentrations during heat stress conditions indicating that there was excessive alveolar ventilation. During the hottest period, animals appeared able to compensate for the tendency to respiratory alkalosis, and maintained blood pH, via blood buffers and renal compensation. As a result $\text{HCO}_3^-$ was excreted in the urine and blood $\text{HCO}_3^-$ 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. It has also been hypothesised by Schneider *et al.* (1984) that the increased loss of carbon dioxide may decrease the bicarbonate pool available for buffering in the rumen via salivary secretion, resulting in lowered ruminal pH.

Based on the abovementioned discussion, an electrolyte supplement was formulated and experiments were designed and undertaken in an attempt to alleviate the physiological responses which may result due to heat stress conditions such as those encountered on voyages to the Middle East.
7.2  Experiment A and B: Climate controlled room experiments

7.2.1  Aims

1. Formulate a palatable, economical and easily administered electrolyte supplement for cattle subjected to prolonged and continuous periods of high heat and humidity as can be experienced on board livestock vessels travelling from Australia to the Middle East during the northern hemisphere summer.

2. In climate controlled rooms at Murdoch University, test and assess the physiological response of *Bos taurus* animals supplemented with electrolytes during periods of prolonged and continuous high heat and humidity.

3. Following the prolonged heat and continuous heat and humidity insult, provide treated and control animals with a short period of respite then a second shorter heat period.

7.2.2  Hypotheses

1. Treated animals will eat more and as a result will be better able to maintain live weight compared to control animals.

2. Treated animals will be better able to maintain acid base homeostasis as indicated by maintaining blood and urine pH during and after prolonged heat stress conditions.

3. Treated animals will maintain or have significantly greater plasma sodium concentration compared to control animals.

4. A second heat insult, following prolonged heat stress conditions will further exacerbate the abovementioned differences between treatment and control animals.

5. There will be a positive animal welfare benefit for treated animals.
7.2.3 Methods

7.2.3.1 Experimental design

Two replicate experiments (A and B) were conducted in order to test an electrolyte formulation on the physiological responses of cattle during periods of prolonged heat and humidity. Six *Bos taurus* heifers, housed in climate controlled rooms at Murdoch University, were used for each experiment. In each experiment animals were randomly allocated to treatment (n = 3) and control (n = 3) groups. Treatment animals were offered electrolyte supplements in both feed and water. In experiment A, animals were randomly allocated to individual pen position within climate controlled rooms. In experiment B, to control for any pen and rooms effects, assignment of treatment and control animals to pens within each room was the opposite to experiment A. The duration of each experiment was 18 days and the environmental conditions to which animals were exposed are shown in Table 7.1. The set wet bulb temperatures in the climate control rooms were based on conditions which may be encountered on board live stock vessels during the northern hemisphere summer. A second heat period was added in order to test the hypothesis that the physiological responses of control animals to the heat stress conditions would be more pronounced after a second heat period. Animals entered the climate controlled rooms at 0800 h on day 1 and exited at 0800 h on day 19. Each day ran from midnight to midnight and climate controlled rooms were turned up or down at 0800 h.

Experiment A ran from 30th January to 17th February, 2003 and experiment B from 12th May to 30th May, 2003.
Table 7.1: Set wet bulb temperature (°C) of climate control rooms for replicate electrolyte experiments A and B.

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7.2.3.2  *Electrolyte formulation*

The formulated electrolyte supplement for the experiments in this chapter was as follows;

Feed: 1% sodium bicarbonate

Water: 1.8 g/L sodium bicarbonate
3.5 g/L potassium chloride

The 5.3 g/L total salt content (0.53%) in the drinking water is well below maximum tolerable concentrations for ruminants (NRC, 2000) whilst up to three times more concentrated than available commercial products. Supplementation of sodium bicarbonate at around 1% DM in the feed has been proven to be well tolerated and palatable to feedlot cattle (Schneider *et al.*, 1984; Ross *et al.*, 1994). The majority of electrolytes were administered via drinking water as results from previous experiments indicated that animals maintained or increased water intake and reduced feed intake. Each litre of water contained 0.5 g sodium, 1.3 g bicarbonate, 1.8 g potassium and 1.8 g chloride.

7.2.3.3  *Animals and management*

*Bos taurus* heifers for electrolyte replicate experiments A and B were selected from the Murdoch University breeding herd (n = 12, approximately 2 yr of age, 338 ± 8.7 kg mean BW). All animals were Murray Grey cross coming from the same Murray Grey sire.

Surgical techniques and management of the animals was as described in Chapter 2. Two weeks before the experiments started animals were surgically implanted with temperature loggers and telemeters. The day before experiments began, animals had
indwelling jugular catheters inserted. Animals entered the climate controlled rooms at 0800 h on day 1. Individual animals were only removed from the rooms if a jugular catheter needed replacing (2 h procedure) or on welfare grounds. One animal in experiment A (control group) was removed on the morning of day 11 as it was unable to rise, was weak, and had a core body temperature over 43°C. Data from that animal is included up to day 11 as is its weight on day 12.

Animals had one week adaptation to a standard shipper pelleted feed (as described in Chapter 3). During the experiment feed was offered at 2.5% BW (as fed after 18 h off feed) daily in equally divided feeds at 0700 and 1200 h. A standard shipper pellet was used (Glen Forrest Feeds, Western Australia; Appendix 2), with treatment animals receiving the same feed which had been reprocessed with an additional 1% sodium bicarbonate (Advanced Feeds, Western Australia, Australia). Feed residues were weighed and recorded daily before the morning feed and the previous days total intake recorded. Water was offered ad libitum in the 25 L buckets, filled manually from the 20 L reservoirs above all the pens. The treatment animals received supplementation with 1.8 g/L sodium bicarbonate and 3.5 g/L potassium chloride per litre of water throughout the experiment. The electrolyte supplement was thoroughly mixed and dissolved in the 20 L reservoir buckets above the treatment animal pens before topping up treatment and animals as necessary. Water intake was calculated daily before the morning feed, when the buckets were washed out and refilled.

7.2.3.4 Sample collection and measurements

In each climate controlled room dry bulb temperature and relative humidity were recorded and logged every 10 min during both experiments using T-Tec 6 Dataloggers (Temperature Technology, South Australia, Australia). Wet bulb temperature was
calculated from these measurements (Hemp, 1989). A 2 h average of WBT was used for analyses.

Body weights were measured on days 0, 15, and 19 after 18 h off feed but not water. Body weights were converted to BW as a percent of start weight (start weight = 100%) by dividing measured BW (kg) by starting BW (kg) and multiplying by 100.

Heart rate, RR and RT were measured and recorded 3 to 4 times daily at 0600, 1200, 1800 and 2200 h. Venous blood from jugular catheters and voided urine samples were taken 4 times a day at 0600, 1200, 1800 and 2200 h on days 2, 11, 15 and 18 and once daily at 1200 h on every other day.

For detailed description of measurements refer to general materials and methods (Chapter 2).

7.2.3.5 Statistical analysis

For all variables measured, the trends in experiments A and B were similar and so the two experiments were combined and analysed as one. This was not unexpected as experiments were undertaken in climate controlled rooms (CCR) and every attempt was made to keep the conditions the same in both experiments. Once combined n = 6 for both treatment and control groups. After day 10, n = 5 for control group. For all blood samples as well as urine pH, urine SG, HR and RR a repeated measures analysis was used to test for significant interactions between treatment and day using the software package SPSS 11.0 for Windows. Four days were used in the analysis. A daily average was calculated for days 2, 11, 15 and 18. These were considered the key days in the experiment which would highlight treatment effects. Day 2 was the control day when CCR were off and animals were at ambient environmental conditions. Day 11 was the end of the first hot period, day 15 the end of the second hot period and day 18 the end of the experiment and again at ambient temperature. If there was a significant interaction
between treatment and these days (P < 0.05), then an unpaired t-test (Microsoft Excel) was used to see if the difference between day 11 and day 2 was significantly different for the treated and control groups. This t-test was repeated for the difference between day 15 and day 2, and the difference between day 18 and day 2. Only those variables where there was a significant interaction between treatment and days are presented in results. The subscript “*” on figures indicates that on that particular day there was a significant change in the mean between treatment and control groups from day 2.

For urine fractional excretion ratios, an unpaired t-test (Microsoft Excel) was used to detect significant differences between treatment and control animals on days 2, 11, 15 and 18. The subscript “*” on figures indicates that on that particular day there was a significant difference in the mean between treatment and control groups.

For feed and water intakes, the total feed and water consumed (as a percent of body weight) for treatment (n = 6) and control (n = 5) was calculated. An unpaired t-test (Microsoft Excel) was used to compare the means of the totals.

Body weights of treatment and control animals on days 12 and 19 were expressed as a percent of starting weight (starting weight 100% on day 0). An unpaired t-test (Microsoft Excel) was used to see if the difference between day 0 and day 12 was significantly different for treatment (n = 6) and control (n = 6) animals. This t-test was repeated for the difference between day 0 and day 19 (n = 5 for control animals on day 19). The subscript “*” on figures indicates that on that particular day there was a significant change in the mean between treatment and control groups from day 0.
7.2.4 Results

7.2.4.1 Temperature

Within each experiment, there were no differences between climate controlled rooms (data not presented). When climate controlled rooms were operating, wet bulb temperatures were similar between each experiment (Figure 7.1). The maximum mean WBT recorded was 30.58°C at 1400 h on day 7 and 29.51°C at 0200 h on day 10 for experiments A and B respectively. Differences between experiments were seen when climate controlled rooms were turned off and during the hottest periods on days 7 to 11 and day 15. Experiment A was an average of 1°C WB hotter than experiment B on days 7 to 11 and day 15. When climate controlled rooms were turned off, ambient weather conditions were hotter during experiment A resulting in differences of up to 6°C WB.

Rectal temperatures for both treatment and control animals increased with increasing WBT; however, there was no difference between treatment and control (data not presented).

7.2.4.2 Clinical response

The clinical responses of all animals in both replicate experiments were similar to those described for *Bos taurus* animals subjected to heat stress conditions in Chapter 3. During the hottest period open-mouth panting, drooling, lethargy, licking of coats, and neurological signs were observed. There were no observed differences in the clinical responses of treatment and control animals during both replicate experiments. However, one control animal in replicate A was removed from the rooms on day 11 as it was weak, unable to rise and had a core body temperature over 43°C.
Figure 7.1 Calculated wet bulb temperature (°C) during experiment A and experiment B. The wet bulb temperature was calculated every 10 minutes in each room and a 2 hour average determined.
7.2.4.3 Feed and water intake and body weight

There was a tendency for treatment animals to drink more water than control animals. However, the mean total amount of water consumed over the duration of the experiment, as a percent of BW, was not significantly different between treatment and control animals ($P = 0.1$; Figure 7.2.i). The average amount of water consumed for each treatment animal, over the duration of the experiment, was 175% of BW and for control animals was 136% of BW. This equated to treatment animals drinking an average 581 L and control animals an average of 447 L over the 18 days. There was no difference in total feed intakes between treatment and control animals ($P = 0.59$; Figure 7.2.ii). Feed intakes for both treatment and control animals decreased as WBT increased.

Body weights were measured on days 0, 12 and 19 of the experiment (Figure 7.3). No significant differences were detected between treatment and control animals on either day 12 or day 19 when compared to day 0 ($P = 0.27$ and 0.50 respectively). However, there was a trend suggesting that treatment animals lost less body weight compared to control animals.

7.2.4.4 Plasma protein and packed cell volume

A significant interaction was detected between day and treatment for total plasma protein and PCV ($P = 0.02$ and 0.05 respectively). After the hottest period the change in PCV and total protein concentrations tended to be increased for control animals and decreased for treatment animals. Unpaired t-tests indicated that the difference between day 2 and day 11 was significantly different for treatment and control animals for both total protein and PCV (Figure 7.4.i and 7.4.ii). By day 11, the mean total plasma protein had increased by 0.2 g/L for treatment animals and 4.0 g/L
Figure 7.2 Mean daily (i) water intake and (ii) feed intake, expressed as a percent body weight, for treatment (■) and control (○) animals during experiments A and B. Points show mean ± SEM.
Figure 7.3 Live weights measured as percent of starting weight for treatment and control animals during experiments A and B. Bars show mean ± SEM.
Figure 7.4 (i) Total protein concentration and (ii) packed cell volume for treatment (■) and control (○) animals in experiments A and B. Points show mean ± SEM.
for control animals. By day 11, the mean PCV had decreased by 5.2 L/L for treatment animals and increased by 0.7 L/L for control animals.

7.2.4.5 Blood gas

In general, the blood gas changes were similar to those seen in *Bos taurus* animals described in Chapter 3. During the hottest period blood pH was maintained whilst pCO₂ and HCO₃⁻ were decreased as RR increased. After the heating period, pCO₂ returned to pre-heat concentrations quickly whilst HCO₃⁻ took longer to return to pre-heat concentrations. As a result blood pH decreased. Statistical analysis indicated significant interactions between days and treatment for venous HCO₃⁻ concentration and actual base excess (P = 0.003 and 0.005 respectively). The difference between day 2 and day 18 for mean HCO₃⁻ concentration was significantly different for treatment and control animals (Figure 7.5.i). On day 18, mean HCO₃⁻ concentration was decreased by 3.1 mmol/L for control animals and had remained unchanged for treatment animals. Similarly, the difference between day 2 and day 18 for mean ABE concentration was significantly different for treatment and control animals (Figure 7.5.ii). On day 18, the ABE of control animals was decreased by 3.8 mmol/L and for treatment animals 0.4 mmol/L when compared to the control day (day 2). No significant interactions between treatment and day were detected for blood pH, pCO₂ or pO₂ (data not presented). Changes due to increases in WBT for these variables were the same as for previous *Bos taurus* experiments.

7.2.4.6 Urine

There was a significant interaction between days and treatment for urine pH (Figure 7.6). The urine pH of control animals tended to be more acidic than that of treatment animals after the hottest period. Control animals had significantly larger
Figure 7.5 (i) Venous blood bicarbonate concentration and (ii) venous blood actual base excess for treatment (■) and control (○) animals in experiments A and B. Points show mean ± SEM.
Figure 7.6 Urine pH (mean ± SEM) for treatment (■) and control (o) animals in experiments A and B.
reductions in urine pH between days 2 and 15, and days 2 and 18 compared to treatment animals. On day 15, mean urine pH was reduced by 0.3 for treatment animals and 1.0 for control animals when compared to day 2. On day 18, mean urine pH was reduced by 0.3 for treatment animals and 1.7 for control animals when compared to day 2.

There was no significant interaction between treatment and days for urine SG. For both treatment and control animals, urine SG was similar to *Bos taurus* heifers in Chapter 3 and decreased with increasing water intake and WBT (Figure 7.7).

A significant interaction between treatment and days was detected for the FER’s of Na, K, and Cl. On days 2, 11, 15 and 18 there was a significant difference between treatment and control animals for the FER of Na, K and Cl (Figure 7.8). There was no significant difference detected between treatment and control animals on days 2, 11, 15 and 18 for the FER of Mg or Ca (data not presented).

7.2.4.7 Plasma electrolytes

There was no significant interaction detected between treatment and days for plasma Na, K, Cl, Mg, Ca, Urea, and Creatinine (data not presented). The changes in these plasma electrolytes for both treatment and control animals were similar to those described in Chapter 3.

7.2.5 Discussion

The climate controlled rooms did not reach the set WBT in experiment A. For this reason set WBT for experiment B was modified so as to replicate experiment A. The set WBT achieved were high enough to cause excessive heat load and clinical signs of heat stress were observed in both treatment and control animals during the hottest periods on days 7 to 11 and day 15. When the CCR were operating, only minor differences in daily mean WBT were detected (1°C on days 7 to 11 and day 15). The
Figure 7.7 Urine specific gravity (mean ± SEM) for treatment (■) and control (○) animals in experiments A and B.
Figure 7.8 Fractional excretion ratios of (i) Sodium; (ii) Potassium; and (iii) Chloride for treatment (■) and control (○) animals in experiments A and B. Points show mean ± SEM.
large differences in WBT (up to 6°C) when CCR were not operating (days 1, 2, 12, 16 and 19) were considered inconsequential as ambient WBT were not high enough to cause heat stress conditions or differences in physiological responses between the 2 experiments on these days. The success in achieving replication was reflected in the animals’ physiological responses and meant that experiments A and B were analysed as one.

With respect to dietary electrolyte composition, the control diet used for experiments A and B was of a higher quality than the diets used for experiments in earlier chapters. The Na content of the ration used in earlier experiments was 0.065%. The Na content of the control diet for experiments A and B was 0.259%. With 1% NaHCO₃ added to the treatment diet, the Na content was increased to 0.602%. The difference in Na content between the control diet and the diets used in earlier experiments meant that the addition of 1% NaHCO₃ was less important than envisaged. Instead of increasing the Na content from 0.065% to somewhere above the recommended level of 0.3%, the control diet was already 0.259% Na which is close to the 0.3% Na recommended for stressed young cattle (NRC, 2000).

In both replicate experiments, as WBT increased during the hottest periods, feed intakes became significantly decreased for both treatment and control animals, which is a normal response to excessive heat load in cattle (Blackshaw & Blackshaw, 1994) and was reported in previous Bos taurus experiments. The reduction in feed intake, with the corresponding decline in heat generated by ruminal fermentation and body metabolism, aids the maintenance of heat balance (Sanchez et al., 1994b).

No other previously published work has reported supplementing cattle with both dietary and water based electrolyte supplements. Results from previous chapters indicating large reductions in feed intake during the extreme conditions, meant that electrolyte supplements in the water were essential. During the hottest periods,
treatment animals were receiving virtually all their electrolytes from drinking water. The drinking water for treatment animals contained 0.05% Na, 0.13% HCO$_3^-$, 0.18% K and 0.18% Cl, which was obviously palatable as treatment animals tended to drink more than controls.

There are few published studies where electrolytes are provided in the drinking water of cattle. Gortel et al. (1992) provided bulls with electrolyte supplemented drinking water to try and ameliorate transport stress. Their supplement contained approximately 0.04% Na, 0.04% K, 0.06% Cl and 0.06% HCO$_3^-$ as well as smaller proportions of magnesium sulphate and amino acids. Registered commercial electrolyte replacement products available in Australia for use in the transport of cattle include Selectrolyte® (Controlled Medications Pty. Ltd., Victoria, Australia) and Solulyte Concentrate® (Adisseo Australian Pty. Ltd., Queensland, Australia). At the dose recommended both of these products would contain in the drinking water 0.03% Na, 0.045% HCO$_3^-$, 0.023% Cl and 0.0032% K. The differences in concentrations of electrolytes, between the supplements mentioned and the specifically formulated supplement used in experiment A and B, highlights the lack of knowledge of electrolyte supplementation for shipped cattle and the direction for further research. The electrolyte supplement formulated was more concentrated in all supplemented electrolytes compared to commercial products available. However, as these products have not been tested under the same environmental conditions, there is little scope for comparison between supplements. It was assumed that by utilising reported acid base imbalances from previous chapters and recommended daily electrolyte intakes for stressed young cattle (NRC, 2000), that the formulated supplement would provide the maximum benefit for shipped cattle under heat stress conditions.

Dietary electrolyte supplementation, through the manipulation of the DCAD, has been investigated extensively in the dairy industry in an attempt to improve feed intakes.
and milk yields in hot environments. Heat stress induced reduction in feed intake affects
digestive function as well as quantities of minerals consumed if dietary concentrations
are not increased (Sanchez et al., 1994b). As a consequence of altered digestive
function, and particularly reduced ingesta flow, ruminal acid production per unit of
fermentable feed increases, and ruminal pH decreases (Niles et al., 1980). Also,
recycling of salivary buffers into the rumen may be reduced because heat stressed cows
ruminate less and slaver more than cows in normal conditions (Terui et al., 1980).
Furthermore, accelerated RR causes respiratory alkalosis and compensated metabolic
acidosis, changing the demand for Na and K during heat stress (Sanchez et al., 1994b).
Sanchez et al. (1994b) suggested that there was an increased demand of Na for renal
excretion and of K for sweating resulting in an increase of dietary requirements of each
during heat stress.

Increasing the DCAD has been shown to increase DMI in heat stressed dairy
cows (Schneider et al., 1984; Mallonee et al., 1985; West et al., 1991; West et al.,
1992). However, in experiments A and B, supplemented animals did not improve DMI
compared to control animals, and both groups reduced DMI as WBT increased. This is
contrary to work with heat stressed dairy cows supplemented with 0.85% NaHCO₃ in
the feed which resulted in a 7.2% greater daily feed intake compared to those not
supplemented NaHCO₃ (Schneider et al., 1984). However, unlike experiments A and B,
animals in the abovementioned study were exposed to heat stress conditions (average
black globe temperature of 41°C) only during daylight hours. It was hypothesised that
sodium bicarbonate concentration may have aided ruminal buffering mechanisms
during stressful daylight hours resulting in higher daytime feed intake. In the same
study, feed intake during the cooler night time hours was not influenced by NaHCO₃
and both treatment and control animals increased feed intake during the cooler night
time hours. West et al. (1992) also found a positive response to DMI in heat stressed
dairy cows by increasing the DCAD of the ration. The increase in feed intake was shown to be independent of the cation source (Na or K).

Contrary to Schneider et al. (1984), West et al. (1992) hypothesised that it was the greater blood buffering capacity, indicated by blood base excess and a higher blood pH and HCO$_3^-$ content may be responsible for the increased feed intake. Treatment animals in experiments A and B also had significant elevations in blood base excess and HCO$_3^-$ concentration but this was only detected on day 18 or after the second hot period. Treatment animals at this stage had had 18 days of supplemented feed and water and had not shown any difference in feed intake compared to controls. Schneider et al. (1984) found no change in blood pH, HCO$_3^-$, pCO$_2$, total CO$_2$ or ratio of HCO$_3^-$/pCO$_2$ when heat stress cows were supplemented with 0.85% NaHCO$_3$.

In both the Schneider et al. (1984) and West et al. (1992) studies, the heat stress conditions were diurnal in nature with nocturnal cooling allowing animals respite from heat stress conditions and a subsequent reduction in T$_c$. The prolonged and continuous nature of the heat stress conditions in experiments A and B meant that T$_c$ did not return to normal for the whole of the heat period, and thus feed intakes remained depressed in spite of dietary increases in DCAD by the addition of 1% NaHCO$_3$ and the addition of electrolytes in the drinking water. The extreme heat stress conditions nullified any affects that electrolyte supplementation may have had on DMI. A night time cooling off period allows animals to lose stored body heat, reduce T$_c$ and thus increase feed intake. If no night cooling period exists, then the animal continues to gain heat and T$_c$ remains elevated or continues to rise, and it would appear that this is the ultimate mechanism which reduces feed intake.

There was an indication that treatment animals in experiments A and B drank more water than control animals. It was hypothesised that this led to treatment animals losing less weight than controls. Schaefer et al. (1992) demonstrated a positive effect of
electrolyte therapy on live- and carcass- weight loss in transported and handled cattle. Schaefer et al. (1992) suggested that animals receiving the electrolyte treatment retained an average of 1.6% more live body weight (P = 0.01). However, control animals in the Schaefer et al. (1992) study had no access to drinking water; therefore, it was most likely that dehydration alone was responsible for the live weight loss.

Gortel et al. (1992), in assessing the effects of transport stress and electrolyte supplementation on body fluids and weight of bulls, suggested that the difference in extracellular fluid resulting from electrolyte treatments was a possible basis for the observed differences in body weight and carcass characteristics. A similar mechanism may have been evident in experiments A and B. As there was no measured difference in feed intake, it is hypothesised that the increase in water intake of treatment animals was responsible for the apparent differences in body weight. On day 11, the change in PCV had decreased significantly for treatment animals. Decreases in PCV can indicate overhydration (Duncan et al., 1994). It would appear that the decrease in PCV was due to increased water intake leading to increased hydration. Therefore, it is hypothesised that any weight improvement apparent in treatment animals was due to the retention of water.

Schaefer et al. (1990) suggested that the improved carcass weight in electrolyte supplemented bulls was not simply gut fill or hydration but actually a retention of fluid in muscle tissue. However, control animals in that study had no access to water. Gortel et al. (1992) examined body fluids and weights of bulls given no water, water alone and electrolyte treatments. Control animals, having no access to fluids, had vastly lower extracellular fluid volumes compared to animals with access to water alone or electrolyte supplements. It was suggested that the extracellular fluid space is most prone to being decreased because intracellular fluid is conserved at the expense of extracellular fluid. The plasma part of this component is vital to the maintenance of
normal vascular circulation, and is thus conserved, leaving most of the fluctuations in fluid volumes to occur in the interstitial pool (Gortel et al., 1992). In the study by Gortel et al. (1992), animals given water alone drank more than animals given electrolyte solution and subsequently bulls offered electrolytes had larger live weight losses than animals offered water alone. However, carcass yield was higher in electrolyte treated animals. The explanation given for this was that the bulls given electrolytes could have lost weight in compartments that were not part of the carcass yield such as the alimentary tract which was heavier in bulls given water compared to electrolyte solutions. It was further hypothesised that the electrolyte solution was more readily absorbed across the wall of the alimentary tract leading to the increase in carcass yield. Contrary to Gortel et al. (1992), treatment animals in experiments A and B did tend to drink more than control animals. This was not unexpected as animals in experiments A and B were subjected to prolonged periods of heat stress conditions and had vastly different physiological responses to transported animals and thus different electrolyte requirements. If the hypotheses from the above studies hold true, then it would be expected that treatment animals in experiments A and B would also have improved balance between extracellular and intracellular fluids, as well as between the contents of the reticulo-rumen and the bloodstream, and improved body weight as well as carcass yield.

The results of experiments A and B indicated possible improved acid base buffering capacity of treatment animals. It has been noted in previous studies that a concern may exist in supplementing a potentially alkalotic animal during heat stress conditions with more alkalising ions, as would be the case if supplementing with increased DCAD (Schneider et al., 1984). However, in previous chapters it has been shown that given the specific environmental conditions that animals have been subjected to, the main disturbances in blood pH occur after the heat stress or respiratory
alkalotic conditions have subsided. At this time a metabolic acidosis was evident. During and after the heat stress conditions blood HCO₃⁻ was reduced and so supplementing throughout may in fact prove beneficial. In experiments A and B a compensatory metabolic acidosis developed after the heating periods in both treatment and control animals as evidenced by a decrease in venous blood pH. However, although there was no change in venous blood pH between treatment and control animals, there was a difference in the change of HCO₃⁻ and ABE on day 18 and urine pH on days 15 and 18 when compared to control day 2. On these days treatment animals showed significant improvements in returning to pre heat values whilst control animals continued to have decreased values. For every hydrogen ion secreted into the renal tubules, a HCO₃⁻ ion is reabsorbed (Guyton & Hall, 1996). As the venous blood concentration of HCO₃⁻ improves in electrolyte supplemented animals, less hydrogen ions are excreted in the urine and so urine pH increases or becomes less acidic. However, the significant increase in HCO₃⁻ concentration in electrolyte supplemented animals did not correlate with a significant improvement or increase in venous blood pH. Blood pH for both groups of animals remained decreased on day 18, although trends in venous blood pH would suggest that treatment animals are maintaining homeostasis better than control animals. Being a log scale, small changes in blood pH represent major changes to acid base homeostasis. It may be that the small number of animals tested meant that significant differences were unable to be detected.

The benefits to the animal of the improved acid base homeostasis remain unclear. The results obtained in experiments A and B agree with some of the studies in heat stressed dairy cows where there are improvements in blood buffering capacity, as indicated by improved blood base excess and HCO₃⁻ concentration and these have been linked with improvements in DMI and milk yields (West et al., 1992). Due to the vastly different climate conditions and the fact that improvements were only seen in blood
base excess and \( \text{HCO}_3^- \) concentration after the heat stress conditions, no improvements in DMI were noted in electrolyte supplemented animals at any time during experiments A and B. It may be that if electrolyte supplementation had continued after day 18 differences in feed intakes may have been observed. Furthermore, the improvements in the blood buffering capacity of treatment animals noted late in the experiments may have meant that these animals would be better able to cope with subsequent heat stress conditions or dietary acidosis post heat stress as suggested by Sanchez et al. (1994b).

No significant differences were detected between treatment and control animals in any measured plasma electrolytes. This was consistent with Gortel et al. (1992) who also found no difference in serum Na, K or Cl in electrolyte supplemented bulls during transport stress when compared with animals given water alone. This was not surprising considering that plasma concentrations of Na and K are not good indicators of dietary intake (NRC, 2000) and that homeostatic mechanisms ensure that extracellular fluid concentrations of electrolytes and minerals are maintained within a narrow range during all but severe disease states (King, 1994). Fractional excretion ratios of plasma electrolytes were calculated in order to give a measure of renal conservation of each electrolyte. The FER calculates the proportion of each electrolyte that is excreted in the urine, compared to the excretion of creatinine, which is presumed not to be reabsorbed at all by the kidney tubules. The increased FER indicates that there was renal excretion of electrolytes by the treatment animals presumably because they were receiving the additional supplement, while the control animals were reabsorbing and therefore conserving electrolytes as much as possible. That the plasma electrolytes were not different, and did not change markedly, indicates that the kidneys were successful in maintaining a constant plasma value. We have no information about tissue concentrations of electrolytes, but we interpret the difference in FERs to mean that the
treatment animals had sufficient body reserves of the electrolytes for extra to be excreted, while the control animals did not.

There were no additional problems associated with the second short heat insult. This second heat period was shorter than the first. As a result, mean core temperatures did not increase to the same extent for either treatment or control animals. Without as severe or sustained increase in $T_c$, feed intake did not decrease as much during the second heat period compared to the first, and remained around 1.0% of body weight for both treatment and control animals. Similarly, RR increased during the second heat period but not to the same extent and again there was no difference between treatment and control animals. The reason for the decrease in severity of changes in these measured variables may be due to the fact that the accumulated excessive heat load was less severe, or some degree of acclimation was evident. There is evidence to suggest that acclimation occurs after 9 to 10 days (Singh & Newton, 1978; Senft & Rittenhouse, 1985). Singh & Newton (1978) reported that young calves kept at 30.5°C WB for 12 h each day showed signs of acclimation after day 2, and most of the acclimation was completed after the first 9 to 10 days. Animals in experiments A and B may have acclimated to the heat to some extent following the first heat stress period.

Of interest was the change in $\text{HCO}_3^-$ and acid base concentration on day 18 or after the second heat period. On day 18 the change in $\text{HCO}_3^-$ and ABE concentration was significantly less for treatment animals. This result was not present on day 15 or after the first sustained heating period. It would appear that the electrolyte supplement was helping these blood gas variables return to pre-heat values. Whether this can be considered a potential health or welfare benefit is questionable.

One of the primary hypothesis for experiments A and B was that electrolyte supplementation would provide a positive welfare benefit to cattle experiencing heat stress conditions or prolonged periods of high heat and humidity. Animal welfare is a
difficult variable to measure and quantify based on behavioural and physiological responses, largely because of the difficulty of deciding what amount of deviation from normal values is acceptable (Warriss, 2004). For the experiments discussed here, the behavioural and clinical response of animals gave a very subjective measure of animal welfare. The one animal removed from the experiments was a control animal which was unable to cope with the environmental conditions. However, no one measured physiological variable gave an indication that there was a welfare benefit to electrolyte supplemented animals. Collectively, there were enough changes in the physiological responses between treatment and control animals to suggest that there may be welfare or health benefits to electrolyte supplemented animals.

7.2.6 Conclusion

There was a concern with supplementing potentially alkalotic animals with bicarbonate but results agreed with Schneider et al. (1984) and suggest that heat stressed animals are extremely effective at withstanding dietary changes to acid-base homeostasis. There was some evidence that electrolyte supplemented animals were better able to maintain acid base homeostasis as indicated by increases in venous bicarbonate, blood base excess and urine pH towards the end of the experiments. The welfare or physiological benefits of these changes remain unclear. However, the combined benefits of acid base homeostasis and the increase in water intake and subsequent hypothesised increase in total body fluid in treatment animals may be of benefit.

Both treatment and control animals had decreases in feed intake and live weight when exposed to prolonged and continuous heat stress conditions. This, along with increases in core body temperature are indicators of poor animal welfare (Silanikove, 2000). There was no significant difference in feed intake or live weight change between
treatment and control groups, suggesting that there was no improvement or benefit associated with electrolyte supplementation. Also, there was no improvement in plasma electrolyte concentrations in treatment compared to control animals.

Due to the small number of experimental animals, detecting significant changes in measured variables was difficult and it may be that more and/or greater physiological changes could be detected if greater numbers of animals were able to be tested. Furthermore, only one supplement was tested at one dose rate. The formulation tested was based on scientific data and previous measured responses but there is no way of telling if a different composition or different dose rates would further improve or inhibit welfare and physiological responses. Further research is required in this area to maximise the potential benefits electrolyte supplementation may have on the physiological and welfare of *Bos taurus* animals during prolonged and continuous periods of high heat and humidity. Further research is also required to establish more definitive welfare and physiological responses by building on the results obtained in experiments A and B.

### 7.3 Experiment C: On board ship experiment

#### 7.3.1 Introduction

No scientific literature exists on the economic and welfare benefits of electrolyte supplementation of cattle on long haul voyages to the Middle East. Furthermore, information about electrolyte formulations and dose rates are based largely on anecdotal reports (M. McCarthy, personal communication).

Experiments A and B indicated a possible live weight advantage for electrolyte supplemented animals. Any live weight advantage would represent a significant economic benefit for the live export industry and perhaps also suggest a welfare benefit.
for electrolyte supplemented animals. However, this result was not conclusive as only a small number of animals were able to be tested in the climate control rooms. The small number of animals tested in experiments A and B meant that there may also have been other physiological and welfare parameters which were different between treatment and control animals.

7.3.2 Aims

The aim of experiment C was to assess possible economic and welfare benefits as well as the practicality of electrolyte supplementation on large numbers of *Bos taurus* during a long haul voyage from southern Australia to the Middle East during the northern hemisphere summer.

7.3.3 Hypotheses

The primary hypothesis was that *Bos taurus* animals supplemented with specific electrolytes in drinking water would have a significant weight advantage compared to control animals and this weight advantage would be due to increases in water intake.

7.3.4 Methods

7.3.4.1 Experimental design

An area on board a commercial livestock ship was identified as suitable to carry out experiment C. Six pens were chosen on a ventilated closed deck which had good environmental symmetry. Three pens were on the starboard side of the ship and three on the port side. Due to the water delivery system, the three pens on the starboard side of the ship were allocated as treatment pens and the three on the port side control pens (Figure 7.9). Total pen area available to experimental animals was 61.1 m² and 63.6 m²
Figure 7.9 Pen lay out for treatment and control animals on board live stock vessel.
on the starboard and port side respectively. Eighty *Bos taurus* crossbred steers were selected for the experiment. Due to the difference in pen area, 39 steers were randomly allocated to treatment and 41 to control groups. The resulting stocking density was similar at 1.57 and 1.55 m$^2$ per head for treatment and control respectively.

Animals were loaded onto the ship in Fremantle, Western Australia (day -2) and given 3 days acclimatisation to shipboard conditions as the ship sailed from Fremantle to Port Hedland, Western Australia. The experiment began on day 1 whilst docked in Port Hedland, ran for 18 days, and finished after weighing the animals on the day of discharge in the Middle East.

7.3.4.2 *Animals and management*

Two groups of *Bos taurus* crossbred steers were sourced from southern Western Australia for the voyage; 26 animals came from Geraldton, Western Australia (28.5° S, 114.3° E) and 54 from Gingin, Western Australia (25.0° S, 151.6° E). The prior origin of these cattle was unknown, but had spent the winter at these sites, and therefore were assumed to be winter-acclimatised.

The Gingin steers were randomly allocated into 2 of the treatment pens (T1 and T2) and two control pens (C1 and C2). The steers from Gingin and Geraldton were not mixed and the Geraldton steers were randomly assigned to the remaining treatment and control pens (T3 and C3). Table 7.2 shows the number of steers allocated to each pen and the resulting stocking density.

Feed was available *ad libitum* on board the ship in approximately two equally divided feeds at 0600 to 0700 h and 1300 to 1400 h daily. This was standard throughout the cattle decks on board the vessel. Each experimental pen had two feed troughs of equal size (1000 x 300 x 300 mm). Water was available *ad libitum* via two 1000 L water tanks (MH45025, Silverlock Packaging, Western Australia, Australia), located two
decks above the experimental animals, which gravity fed the two water troughs per pen (650 x 250 x 250 cm). One tank supplied the treated animals and one the controls. The 1000 L tanks were filled as necessary via the ship’s drinking water supply and electrolytes were added to the tank for the treatment group at a rate of 1.8 g/L sodium bicarbonate and 3.5 g/L potassium chloride, as per experiments A and B. Electrolytes were added to the water of the treatment animal pens from day 1 to day 18.

Washing events in the experimental pens occurred on days 7, 10, 15 and 17. The washing of pens constituted seawater being hosed through pens at high pressure to remove faecal and bedding material. Sawdust was used as bedding and was replaced after each washing event.

Table 7.2 Mean body weights of treatment and control animals in pens.

<table>
<thead>
<tr>
<th>Pen</th>
<th>Pen area (m²)</th>
<th>No. animals</th>
<th>Mean before (kg)</th>
<th>Mean end (kg)</th>
<th>Percent change</th>
<th>Change (kg)</th>
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<td>428</td>
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<td>19</td>
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<tr>
<td>T2</td>
<td>20.16</td>
<td>13</td>
<td>408</td>
<td>423</td>
<td>104</td>
<td>15</td>
</tr>
<tr>
<td>T3</td>
<td>20.76</td>
<td>12</td>
<td>388</td>
<td>401</td>
<td>104</td>
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<td>400</td>
<td>405</td>
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</tr>
</tbody>
</table>
7.3.4.3 Sample collection and measurements

Cattle were weighed on day 1 and day 18 of the experiment using an FX 1 electronic weighing system (Iconix, New Zealand). Morning feed was withheld on these days and weighing commenced at 0600 h and was finished by 1000 h. Treatment animals were weighed first on each occasion. After weighing on day 18 the percent change in BW was calculated by dividing start weight (start weight = 100%) by end weight and multiplying by 100.

Three animals were randomly identified from each of the experimental pens (n = 9 for both treatment and control) and the respiratory rates and respiratory character or panting score (Gaughan, 2004) of these 18 animals were recorded three times daily at 0600, 1200 and 1800 h for the duration of the experiment.

Urine was collected on days 8 and 16 of the experiment. Voided samples were collected into a 50 ml urine container attached to a metal rod when an animal was seen to urinate in the pen. Urine pH and specific gravity were measured within 1 h of collection. Urine pH was measured using a Shindengen ISFET pH meter (Japan) and specific gravity with a Leica VET 360 Veterinary Refractometer (USA).

Ten samples from each group were obtained on day 8 and 15 samples from each group were obtained on day 16. Animals could not be individually handled or identified and so urine was randomly collected from any animal when it was seen urinating.

Environmental conditions were monitored three times daily at 0600, 1200 and 1800 h at two symmetrically similar locations on the port and starboard side of the ship within the experimental area. Measurements of dry bulb temperature, relative humidity, and CO₂ were made using a Testo 445 VAC measuring instrument (Testo Australia, Victoria, Australia) and ammonia concentrations were monitored using a Neotox MK5 ammonia meter (Nutech Australia, Western Australia, Australia). Dry bulb temperature
and relative humidity was logged every 30 min using T-TEC 6 Dataloggers (Temperature Technology, South Australia, Australia) which were permanently suspended in both locations. Bridge dry bulb temperature, wet bulb temperature and wind speed and direction were obtained from the ship’s log and recorded every 4 h. Seawater temperature was recorded daily at 1200 h and was also obtained from the ship’s log. Drinking water temperature was monitored three times daily at 0600, 1200 and 1800 h. Water temperature was taken from a treatment and control water trough as well as a drinking water trough from a pen that was part of the main water system. At the same times bedding was assessed and a subjective score of 1 to 5 was given to each pen. A bedding score of 1 was considered normal with a light cover of dry manure and 5 was deep wet manure above the level of the dew claw.

7.3.4.4 Statistical analysis

Body weight changes during the experiment were analysed with a multiple regression analysis. The dependent variable was the end weight as a percent of starting weight (change in body weight). Independent variables were treatment and initial weight. Initial weight was included as an independent variable as it was found that approximately 10% of weight improvement over the duration of the experiment was due to starting weight (see results). Analyses were performed using Microsoft Excel (Version 11.1).

Statistical analysis of water intake was not possible as individual animals could not be identified. Results show daily intake for the group of treatment and control animals.

Urine pH was analysed using a 2-way factorial ANOVA on Statistica Mac software (StatSoft Inc, Tulsa, OK, USA) with treatment and day as main effects.
The bridge, control and treatment pen WBT were analysed using a repeated measures ANOVA on Statistica Mac software (StatSoft Inc, Tulsa, OK, USA) for the three different sites across time. Only the times where the temperature was measured for all three sites were included.

7.3.5 Results

7.3.5.1 Environmental conditions

Dry bulb temperature and relative humidity measured in the treatment and control pens, as well as the bridge, were converted to WBT (Hemp, 1989). Figure 7.10 shows the 2 h average of control and treatment pens WBT (for the duration of the experiment) and the bridge WBT (taken every 4 h from day 2 to 18). Averages were calculated every 4 h for treatment and control pens from days 2 to 18 and these values, along with the bridge WBT, were subjected to a repeated measures ANOVA. The main effect of site was significant (P < 0.0001). Post Hoc analysis indicated that bridge was different to control and treatment (P < 0.0001 for both) and treatment was different to control (P < 0.0001). On average, the bridge was approximately 1°C WB cooler than control pens and the control pens were approximately 0.4°C WB cooler than treatment pens. The maximum WBT recorded for treatment pens was 31.4°C at 0200 h on day 17 and for control pens 30.8°C also at 0200 h on day 17. It was suspected that the spike in bridge WBT on day 12 was due to human error in recording data and was ignored for statistical analysis.
Figure 7.10 Measured wet bulb temperature (°C) for treatment pens, control pens and bridge. For treatment and control pens WBT was calculated every 10 minutes and a 2 hour mean plotted. Bridge WBT was recorded and plotted every 4 hours.
7.3.5.2 Body weights

One steer from treatment group (pen T3) was removed on day 3 due to illness. At the same time one steer was randomly selected and removed from the corresponding control pen C3 to maintain similar stocking densities. The weights of all animals in each pen at the beginning and end of the experiment were recorded and used to calculate the percent change in body weight over the length of the experiment (Table 7.2). There was no significant difference in mean starting weights between treatment and control animals (T-Test, P = 0.2).

On day 18 the mean liveweight (compared to 100% start weight) for treatment animals was 104.0% and for control animals 100.8%. A regression analysis (or two sample T-test) comparing the mean weight change for treatment and control groups showed that treatment animals had a $3.3 \pm 1.7\%$ (95% confidence interval from 1.5 to 5.0%) weight advantage compared to control animals (Table 7.3). That is, treatment animals increased in weight by about 3.3% of initial weight more than control animals. However, because treatment animals tended to have lighter start weights compared to control animals it was decided to further analyse data taking into account start weight. Figure 7.11 shows the percent change in weight plotted against initial starting weight. Regression lines for treatment and control animals show the effect of initial weight. There was a significant effect of both treatment and starting weight ($P = 0.001$ and 0.01 respectively; Table 7.4). Further analysis indicated that there was a difference of 0.008 between the slopes of the treatment and control regression lines (Table 7.5). This difference was not significant ($P = 0.72$). Therefore, the effect of treatment on end weight did not depend significantly on initial weight. Treatment animals had a $2.9 \pm 1.7\%$ (95% confidence interval from 1.2 to 4.6%) weight advantage compared to control animals ($P < 0.001$). Therefore, approximately 10% of the weight advantage was due to
Table 7.2 Mean body weights of treatment and control animals in pens.

<table>
<thead>
<tr>
<th>Pen</th>
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<th>No. animals</th>
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Table 7.3 Regression analysis using treatment to predict end weight of treatment and control animals. “Treatment” defined to be 0 for treatment and 1 for control.

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<td>Adjusted R Square</td>
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<tr>
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Table 7.4 Multiple regression analysis using treatment and initial weight to predict end weight of treatment and control animals. “Treatment” defined to be 0 for treatment and 1 for control.

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Table 7.5 Multiple regression analysis using treatment, initial weight and their interaction to predict end weight of treatment and control animals. “Treatment” defined to be 0 for treatment and 1 for control.

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<td>0.720713</td>
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Figure 7.11 Scatterplot showing the relationship between starting weights (kg) and finishing weights (as percent starting weight) for treatment and control animals.
treated animals tending to have slightly lower starting weight and with lighter animals tending to increase weight more than heavier animals.

7.3.5.3 Urine analysis

An attempt was made to monitor the urine pH of treatment and control animals on day 9 and day 17 (Figure 7.12). There was no control over which animal was sampled on either day. Both the main effects of treatment and day were significant ($F_{1,55} = 10.5$, $P = 0.002$ and $F_{1,55} = 9.0$, $P = 0.004$ respectively). There was no significant interaction between treatment and days ($F_{1,55} = 0.12$, $P = 0.73$). Treatment animals had a more alkaline or higher urine pH than control animals on both days (day 9; 8.6 vs 8.2 and day 17; 8.2 vs 7.9) and mean urine pH decreased for both treatment and control animals from day 8 to 16. The decrease within each group was similar for both treatment and control animals (0.36 and 0.28 for treatment and control respectively).

7.3.5.4 Feed and water intakes

It was not possible to measure individual daily water intakes. The total amount of water intake consumed by treatment and control animals was measured daily. The total daily water intake per kg of BW for each group is shown in Figure 7.13. On all days except day 6, the total daily water intake for the treatment animals was higher than the control animals. Furthermore, the daily water intake for the treatment group appeared to increase over the duration of the experiment, whilst that of the control group remained relatively constant. The mean daily consumption of water was 102 and 85 ml/kg of BW for treatment and control groups respectively. The total amount of water consumed between day 2 and day 16 was 1532 and 1278 ml/kg for treatment and control groups respectively.
Figure 7.12 Measured urine pH for treatment (■) and control (○) animals on day 8 and day 16. Ten samples were collected from each group on day 8 and 15 samples on day 16. Points show mean ± SEM. ** indicates P < 0.01.
Figure 7.13 Measured daily water consumption for treatment (■) and control (○) groups. Points show the amount of water consumed by each group, in millilitres, divided by the total body weight of the group.
7.3.5.5 Bedding

In general, the average daily bedding score was higher for treatment animals (Figure 7.14). After each washing event, the average bedding score decreased for both treatment and control animals.

7.3.5.6 Drinking water temperature

The drinking water temperature did not differ between treatment, control and the main drinking water system (data not presented). There was a strong correlation between seawater temperature and drinking water temperature ($R^2 = 0.82$) with the mean drinking water temperature and seawater temperature increasing over the duration of the experiment. The ambient WBT also increased over the course of the voyage in a similar manner. The maximum mean drinking water temperature measured was 32.3°C on day 16 and the maximum seawater temperature was 31°C recorded on days 14 to 17.

7.3.5.7 Respiratory rate and character

Mean respiratory rates of the individually selected animals monitored over the duration of the experiment did not differ between treatment and control animals, and as with previous experiments, there was a positive correlation between RR and WBT (Figure 7.15). There was no sign of open mouth panting in either treatment or control animals and the panting score did not get above 2 for either treatment or control groups (data not presented).
Figure 7.14 Mean daily bedding score for treatment (■) and control (○) animals.
Figure 7.15 Relationship between daily mean respiratory rate and deck wet bulb temperature for treatment (■) and control (○) animals.

Treatment (light line)
\[ y = 4.086x - 38.994 \]
\[ R^2 = 0.6854 \]

Control (heavy line)
\[ y = 4.0478x - 39.738 \]
\[ R^2 = 0.7501 \]
7.3.6 Discussion

Experiment C ran for 18 days which was one day longer than experiments A and B in the climate controlled rooms at Murdoch University. Apart from the increase in RR during the hotter periods of the experiment, there were no visible clinical signs of heat stress observed in any of the experimental animals. Although not accurately measured, there appeared to be no observed decrease in feed intake or any difference in feed intake between treatment and control groups. This was in spite of WBT reaching over 31°C which in previous experiments would be sufficient to cause clinical signs of heat stress such as inappetence, open mouth panting, drooling, reluctance or inability to rise and general dullness. It is hypothesised that the greater ventilation or air flow over the animals on board the livestock vessel meant that animals were better able to dissipate heat via conduction and convection and therefore maintain body temperature homeostasis. Air flow rates were not measured on board the livestock vessel but the deck and area where the experiment was conducted was known to have the highest pen air turnover of the ship (M. McCarthy, personal communication). Also supply air fans were located on the port and starboard side of the ship, adjacent to control and treatment pens respectively, thus creating direct air movement over all experimental animals. Furthermore, animals in experiment C were not subjected to prolonged periods of WBT above 30°C. There were only 2 to 3 days when WBT was at or over 30°C and so animals did not develop as high an accumulated heat load as in the CCR experiments. There were other areas on board the ship where ventilation was not as efficient and WBT were as high as 34°C and clinical signs of heat stress, such as those encountered in CCR experiments, were evident.

As heat stress conditions were not encountered, there were no reductions in feed intake and no reductions in BW for either treatment or control animals over the length
of the voyage. This was contrary to results obtained in experiments A and B in which animals had reductions in BW. However, the significant finishing weight advantage of approximately 3% for treatment animals in experiment C was consistent with the trend observed in experiments A and B. The weight advantage was apparent in spite of no observed clinical signs of heat stress. Therefore, there appeared to be a consistent weight advantage of 3% for treatment animals regardless of heat stress conditions and whether animals maintained or lost body weight. Given that there were no differences in feed intake between treatment and control animals in experiments A and B and the assumption that there was no reduction in feed intake in experiment C, the weight advantage can largely be explained by the difference in amount of water consumed. In the climate control room experiments, animals were clinically heat stressed and became virtually inappetent and lost weight. In those experiments, the weight advantage of the treated animals was that they lost less weight than the control animals (5.1% compared to 7.9% of starting weight, respectively). However, the presence of a significant weight advantage in the shipped cattle, in the absence of clinical heat stress, indicates that electrolyte supplementation may be beneficial 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. It is unknown whether 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. Of note in this regard was the observation that the Bos indicus on this particular voyage were on decks experiencing more extreme conditions, and did show clinical heat stress with open-mouhted panting.

The electrolyte supplementation in the water increased water intake similar to the amounts drunk in experiments A and B. There was no obvious decrease in water intake even when drinking water temperatures were over 30°C. Furthermore, there did
not appear to be any apparent problems with aggression or dominance preventing animals from drinking.

Further research and investigation into the metabolic advantages of high fluid intake due to electrolyte supplementation is required. No metabolic variables could be measured during this experiment, but it is suggested that maintaining good hydration would be helpful in assisting animals to cope with high heat and humidity and secondary stressful events that may be encountered on long haul voyages. Animals may be better equipped to maintain acid base homeostasis and fluid losses due to sweating and increased alveolar ventilation.

Similarly it was very difficult during this experiment to evaluate welfare benefits of electrolyte supplemented animals. The environmental conditions encountered over the duration of the voyage did not cause clinical signs of heat stress and animal welfare did not appear to be compromised.

The increased water intake of the treated animals led to an observed increase in urination and increased daily bedding score. Washing effectively alleviated this potential problem. The greater deterioration in bedding of treated animals needs to be taken into consideration when supplementing animals with electrolytes. Environmental ammonia has been shown to increase with increasing WBT and wet bedding (Accioly et al., 2003). There was no difference in ammonia concentrations between treatment and control monitoring points on this shipboard experiment. However, as WBT did not remain above 30°C for long periods of time and washing events removed faecal material and wet bedding, maximum ammonia concentrations were low (less than 12 ppm). Higher WBT with less frequent washing may lead to increases in ammonia concentrations. Treatment animals, with greater urine production and higher bedding scores, may exacerbate this problem.
Results indicated an improved acid base buffering capacity in the treatment steers despite no obvious signs of clinical heat stress. It has been noted in previous studies that a concern may exist in supplementing a potentially alkalotic animal during heat stress conditions with more alkalising ions (Schneider et al., 1984), as was the case with electrolyte formulation described here. However, results from earlier experiments have shown that the main disturbances in blood pH occur after heat stress conditions subside. At that time a metabolic acidosis, which was probably masked by the respiratory alkalosis during heat stress, became evident. During and after the heat stress conditions blood $\text{HCO}_3^-$ was reduced and so supplementing throughout may in fact prove beneficial. The urine pH of treatment steers supports this hypothesis. No pre-supplement sample was taken for logistical reasons, and the samples that were obtained were randomly collected from unidentified animals, over the period of a day, so it is difficult to interpret the changes from day 8 to day 16. On both days that urine pH was analysed, the urine pH of treatment steers was higher than that of control steers. For every hydrogen ion secreted into the renal tubules, a $\text{HCO}_3^-$ ion is reabsorbed (Guyton & Hall, 1996). As the blood concentration of $\text{HCO}_3^-$ improved in the electrolyte supplemented animals, less hydrogen ions would have been excreted in the urine, and so urine pH increased and became less acidic. The benefits to the animal of the altered acid base homeostasis remain unclear.

Regardless of welfare or metabolic variables, experiment C has shown that there appears to be the potential for a large economic benefit associated with the use of the electrolyte supplement. The electrolyte supplement for treatment animals cost approximately AUD $4 per head. This did not include infrastructure for delivery. Treatment animals had a live weight advantage of at least 11 kg. At AUD $2 per kg this would equate to a net profit of AUD $18 per head.
The effects of different electrolyte formulations, commercial electrolyte products and different dose rates can not be predicted from this work.

7.3.7 Conclusion

There was a significant weight advantage of approximately 3% for shipped animals supplemented with electrolytes in drinking water. This was present even without experimental animals showing clinical signs of heat stress. However, the 3% weight advantage was consistent with previous experiments in which *Bos taurus* animals were suffering from heat stress conditions. It appears that the weight benefit was due to treatment animals drinking more water. Where and for how long this body water is retained by the animal is unclear. It is clear that the weight improvement could provide a large economic advantage.

The apparent increase in water intake not only resulted in a weight improvement but also an increase in urine output as highlighted by an increase in bedding score.

Welfare benefits for electrolyte supplemented animals remain unclear. As clinical signs of heat stress were not observed in experiment C, it is not known if supplementing animals will reduce or alleviate the clinical response to heat stress. Logistics did not allow blood, urine or metabolic variables to be obtained, therefore, it can not be determined if there was any improvement or response for electrolyte supplemented animals.
Chapter 8: Feeding roughage versus concentrate pelleted feeds to *Bos taurus* during mild heat stress conditions

8.1 Introduction

*Bos taurus* cattle travelling to the Middle East during the northern hemisphere summer can be subjected to prolonged and continuous periods of high heat and humidity. It has been demonstrated that these heat stress conditions can cause significant and prolonged reductions in feed intake. With severe heat and reduction in feed consumption, metabolic heat production and productivity are reduced (NRC, 2000).

Lower dry matter intake during hot weather reduces nutrients available for absorption, and absorbed nutrients are used less efficiently (West, 1999). Mineral losses via sweating and changes in blood acid base chemistry resulting from hyperventilation reduce blood bicarbonate and blood buffering capacity and increase urinary excretion of electrolytes (West, 1999). Heat stress conditions also decrease rumination in dairy cows (Collier *et al.*, 1982) and Holstein steers (Terui *et al.*, 1980). Decreases in roughage intake contribute to decreases in volatile fatty acid production and may contribute to an alteration in the ratio of acetate/propionate (Collier *et al.*, 1982). Therefore, it would appear that during prolonged heat stress conditions, a balance is required between heat production and energy and mineral requirements.

The digestion and metabolism of nutrients creates heat. Heat increment is defined as the increase in heat production following consumption of food by an animal
in a thermoneutral environment (Conrad, 1985). Included in heat increment is the heat of fermentation and energy expenditure in the digestive process as well as heat produced as a result of nutrient metabolism (Conrad, 1985). Feed ingredients influence metabolic heat by way of their individual characteristic heat increment and their influence on DMI (Sparke et al., 2001). There is greater heat production associated with metabolism of acetate compared with propionate; therefore, fibrous ingredients, which ferment in the rumen to produce acetate, have a higher heat increment than concentrates which produce a greater proportion of propionate. Ingredients with the lowest heat increments are fats and oils. This is followed by carbohydrates and then proteins, with increasing heat increments associated with decreasing digestibility (Conrad, 1985).

During periods of hot weather where cattle are able to satisfactorily thermoregulate their body temperature by dissipating excess body heat, theoretical heat production assessments favour the use of feed ingredients with a lower heat increment or diets with a lower roughage content and higher fat or concentrate content (West, 1999). However, increasing the concentrate content of a diet increases the metabolisable energy content of the diet. High energy diets contribute to elevated metabolic heat load (Mader et al., 2002) and also increase the risk of ruminal or metabolic disorders such as a reduction in rumen pH and metabolic acidosis (West, 1999).

The question of heat of digestion of roughage diets versus heat derived from metabolisable energy (ME) has arisen with regard to the most appropriate feed for cattle exposed to high environmental heat. There is a concern that either one type or other of a feed will increase the amount of heat in the animal and potentially worsen their condition. West (1999) reviews work on dairy cows that suggests advantages to feeding rations with a lower heat increment in hot weather; that is, feeds containing less forage fibre and more grain. Forage fibre is assumed to have a higher heat increment because there is greater heat production associated with its digestion and assimilation, compared
to grains (West, 1999). However, recent work has shown that it is the metabolisable energy intake of the diet that influences body temperature of feedlot cattle under hot conditions (Mader et al., 1999).

The question of what the pelleting process does to individual feed heat increments also arises. Pelleting roughages results in lowering heat increment so that the net dietary energy from these roughages is often higher than for the parent product (NRC, 2000). No research has been undertaken to assess rumen heat production and core body temperature when cattle are fed different types of pelleted feedstuffs with the same ME content. Furthermore, little is known of feed intakes when cattle are fed different pelleted feeds with the same ME content and whether or not feed intakes are affected by high environmental temperatures as can occur on board live stock vessels travelling to the Middle East. This experiment was undertaken to assess rumen heat production, core body temperature and feed intake when cattle are fed different pelleted feedstuffs, in particular high roughage versus high concentrate pellets with similar ME and crude protein contents, during periods of mild heat stress.

8.2 Aims

Using pelleted feed of the same metabolisable energy and crude protein, but different roughage content and digestibility, determine whether there are differences in feed intake, core temperature and rumen temperature in Bos taurus heifers subjected to increased environmental heat.

8.3 Hypotheses

1. It is the metabolisable energy intake of the pelleted feed rather than the heat increment of the feed that is responsible for reductions in dry matter intake under heat
stress conditions. Therefore, it is expected that there will be no difference in feed intakes between pelleted feeds under heat stress conditions.

2. The pelleting process will effectively nullify the individual heat increments of the high roughage pellet so as there will be no difference in rumen temperature or core body temperature between the high roughage pellet and high concentrate pellet.

3. The heat stress conditions that the cattle will be exposed to will cause increases in core and rumen temperature and decreases in feed intake. These changes will be the same for both diets.

8.4 Materials and methods

8.4.1 Experimental design

A controlled cross over experimental design was used to investigate differences in feed intake, rumen temperature ($T_r$) and core body temperature ($T_c$) when *Bos taurus* heifers were fed two different pelleted diets. The diets differed in the amount and quality of roughage content, but were of similar metabolisable energy and protein content. Diet 1 was a 40% straw based pellet and diet 2 was an 80% hay based pellet (Table 8.1). Both pellets were a standard 9 mm in diameter and varied in length between 25 and 45 mm depending on durability and handling. The length of roughage for both pellets was similar being approximately 10 mm.

Six *Bos taurus* heifers were given 7 days gradual introduction onto a standard shipper pellet (diet 1; 40% straw based pellet, Appendix 4a) in the Murdoch University Veterinary barn (as described in Chapter 3). The experiment started on day 1, when following this introductory period, animals were randomly allocated into one of two pens. Pen 1 animals were offered a normal shipper pellet (diet 1) and pen 2 animals were offered a hay-based pellet (diet 2; 80% hay based pellet, Appendix 4b). At 0800 h
Table 8.1 Feedstuff content of diet 1 and diet 2 used in replicate experiments 1 and 2. Full mineral analysis of diets is shown in Appendix 4a and 4b.

<table>
<thead>
<tr>
<th></th>
<th>Units</th>
<th>Straw pellet</th>
<th>Hay pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>%</td>
<td>-</td>
<td>80 (60% IVD)</td>
</tr>
<tr>
<td>Straw</td>
<td>%</td>
<td>40 (38% IVD)</td>
<td>-</td>
</tr>
<tr>
<td>Barley</td>
<td>%</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>Lupin</td>
<td>%</td>
<td>32</td>
<td>18</td>
</tr>
<tr>
<td>Lime</td>
<td>%</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dry matter</td>
<td>%</td>
<td>90.8</td>
<td>91.6</td>
</tr>
<tr>
<td>Ash</td>
<td>% DM</td>
<td>5.1</td>
<td>7.8</td>
</tr>
<tr>
<td>ADF</td>
<td>% DM</td>
<td>24.5</td>
<td>24.9</td>
</tr>
<tr>
<td>Crude protein</td>
<td>% DM</td>
<td>12.4</td>
<td>11.0</td>
</tr>
<tr>
<td>DM digestibility</td>
<td>% digestible DM</td>
<td>63.6</td>
<td>64.6</td>
</tr>
<tr>
<td>Metabolisable energy</td>
<td>MJ / kg DM</td>
<td>9.1</td>
<td>9.3</td>
</tr>
</tbody>
</table>
on day 4, animals were moved into individual pens in the two climate controlled rooms (CCR) at Murdoch University. Two animals on the hay diet and one on straw diet were randomly allocated pens in room one and two animals on the straw diet and one on the hay diet were randomly allocated pens in the room two. Animals spent a total of 9 days in the climate controlled rooms. The first four days (days 4 to 7) the CCR were turned off and so climatic conditions were the same as ambient. At 0800 h on day 8 CCR were turned on and animals had 3 days (0800 h day 8 to 0800 h day 11) at 25°C WBT and 2 days (0800 h day 11 to 0800 h day 13) at 27°C WBT. At 0800 h on day 13, animals were removed from the CCR and returned to the barn. The experiment was replicated with animals swapping diets and starting again at day 1. Each day ran from midnight to midnight.

8.4.2 Animals and management

Six *Bos taurus* Angus cross heifers (357 ± 15 kg BW; mean ± SEM) were selected from the Murdoch University breeding herd for temperament and body weight. Nine days before the commencement of the experiment animals had temperature loggers - Stowaway XTI (Onset Computer Corp, Massachusetts, USA) surgically implanted into the abdomen via an incision in the right paralumbar fossa (Chapter 2). Animals then had a 7 day gradual introduction onto a standard straw based shipper pellet (diet 1) as described in chapter 3. The day before the commencement of the experiment animals had an Elanco AH0315 Rumensin Capsule (Elanco Animal Health, New South Wales, Australia) with the monensin core removed and replaced with a temperature telemeter (Datamet, Potchefstroom, South Africa) orally inserted into the rumen.
Surgical retrieval of the abdominal data logger was described in the general materials and methods chapter. For the retrieval of the temperature telemeters, a routine rumenotomy was performed (Oehme, 1988).

The experiment started on day 1 when animals were randomly allocated into one of two pens. Pen 1 animals were fed diet 1, and pen 2, diet 2. All animals were fed at 2.5% of BW (as fed), based on the total weight of the pen, in two equally divided feeds at 0700 and 1300 h. Residues were removed each morning before the morning feed and the previous day’s intake recorded for the pen. On day 4 animals were moved into the climate controlled rooms and individually fed their respective diets at 2.5% (DM) of BW twice daily at the 0700 and 1300 h. Residues were removed, weighed and previous days intake recorded each morning before the morning feed. Throughout the experiment animals were offered water *ad libitum*. Individual daily water intakes were recorded from day 4 when animals were individually penned in the climate controlled rooms. Each animal had access to 25 L buckets which were topped up from 20 L reservoirs above each animal as necessary throughout the day. Residues were weighed, buckets were cleaned and refilled and the previous days water intake was recorded each morning at 0700 h.

**8.4.3 Sample collection and analysis**

Identical samples were collected during both replicate experiments. Animals were weighed on days 1, 4, 8 and 13 after 15 h off feed but not water. On days 1, 8 and 13 at 0700 h, blood samples for blood gas analysis were taken via jugular venipuncture and voided urine samples were taken for measurement of pH and SG. Heart rate, RR, and rectal temperatures were taken once daily at 0700 h on days 7 to 12 as previously described. Dry bulb temperature and relative humidity were logged every 10 min using T-Tech 6 data loggers (Temperature Technology, South Australia, Australia) suspended...
in the middle of each room. Wet bulb temperature was calculated dry bulb temperature and relative humidity (Hemp, 1989) and a 2 h average determined.

Preparation and analysis of samples was as described in the general materials and methods chapter.

8.4.4 Statistical analyses

Data were analysed using a 3-way ANOVA on Statistica Mac software (StatSoft Inc, Tulsa, OK, USA) with main effects of replicate (between group effect), diet (within animal effect) and days (within animal effect). Significant interactions were investigated further with a Student Newman Keuls post hoc test. Days of significant difference (P < 0.05) were represented on figures by the following letters;

a = difference between hay and straw in replicate 1
b = difference between hay and straw in replicate 2
c = difference between replicate 1 and 2 for hay
d = difference between replicate 1 and 2 for straw

8.5 Results

8.5.1 Environmental conditions

Climate controlled rooms were operating between 0800 h on day 8 and 0800 h on day 13. Late on day 10 during the first replicate, a humidifier in one of the climate controlled rooms malfunctioned. Therefore, both humidifiers were shut down and the rooms relied on their heaters to reach a maximum WBT of 22°C between day 11 and 13. The maximum WBT achieved was 25.6°C for both replicates 1 and 2 and was achieved on day 8 and day 10 respectively. The mean WBT achieved when climate
controlled rooms were turned on between day 8 and 13 was 22.6°C for both replicates (Figure 8.1).

After the breakdown of the humidifier in one of the climate controlled rooms, great difficulty arose trying to replicate the first experiment after day 10 and 11. Coinciding with day 10 and 11 of the second replicate, ambient dry bulb temperatures were 40°C with high relative humidity. Therefore, on days 10 and 11, conditions were not the same between replicate experiments and animals during the second replicate were exposed to more extreme conditions. Statistical analysis reflected this in that there was a main effect of replicate for most variables measured. Therefore, replicates were not combined and analysed as one. Data were analysed with a 3-way ANOVA with replicate included as a main effect. Each replicate was treated as an individual experiment and differences were determined between diets in each replicate and the same diets between replicates. Instead of a potential n = 6, as would have been the case if replicates were combined, n was reduced to 3.

The other major problem arising due to equipment failure was that the maximum WBT achieved of 25.6°C in both replicates was not able to be maintained and thus feed intake was not compromised until the end of the hot period in replicate 2. The initial experimental design aimed to increase WBT in order to partially reduce dry matter intake. This was to be achieved by maintaining WBT at 25°C between days 8 and 11 and then at 27°C from day 11 to day 13. Only on days 11 and 12 in replicate 2 did feed intake appear to be compromised.

8.5.2 Core and rumen temperature

Due to the malfunction of one of the data loggers, n = 2 for core body temperature data for animals on hay diet in replicate 1 and straw diet in replicate 2. For all rumen temperature data as well as core temperature data for animals on straw diet in
Figure 8.1 Two hour average wet bulb temperature for both climate controlled rooms during replicate 1 and replicate 2.
replicate 1 and hay diet in replicate 2, n = 3. Mean $T_c$ for animals in both replicates on both diets increased when the climate controlled rooms were turned on after day 8 (Figure 8.2.i). Significant differences in $T_c$ were seen between diets in replicate 1 on days 11 and 12. On days 11 and 12 animals on the straw diet had a significantly lower $T_c$ than animals on the hay diet. On days 9 to 11, animals on the straw diet in replicate 1 had a lower $T_c$ when compared to animals on the straw diet in replicate 2. On day 10, animals on the hay diet in replicate 1 had a significantly lower $T_c$ when compared to animals on the hay diet in replicate 2.

Within replicates the mean daily maximum $T_c$ was different between diets on days 11 and 12 in replicate 1 and day 10 in replicate 2 (Figure 8.2.ii). On days 11 and 12 during replicate 1, animals on the straw diet had significantly lower maximum $T_c$ compared with animals on the hay diet. On day 10 during replicate 2, animals on the straw diet had significantly higher maximum $T_c$ compared with animals on the hay diet.

For both diets there were also differences between replicates. On day 6 animals on the hay diet in replicate 1 had significantly higher maximum $T_c$ compared to animals on the hay diet in replicate 2. However, on day 10, animals on the hay diet in replicate 2 had significantly higher maximum $T_c$ than animals on the hay diet in replicate 1. On days 8 to 10, animals on the straw diet in replicate 1 had significantly lower maximum $T_c$ compared with animals on the straw diet in replicate 2 (Figure 8.2.ii).

The mean daily maximum rumen temperature did not differ between diets in either replicate experiment. On days 9 and 10, the animals on the straw diet in replicate 1 had a significantly lower maximum rumen temperature than the animals on the straw diet in replicate 2 (Figure 8.2.iii).

On any day during either experiment the maximum rumen temperature was between 1.5 and 2.2°C hotter than the maximum core body temperature for both diets. The mean daily difference between maximum rumen temperature and maximum core
Figure 8.2 (i) Daily core body temperature, (ii) Maximum core body temperature, and (iii) Maximum rumen temperature for animals on straw and hay diets during replicate 1 (■) and replicate 2 (■). Points show mean ± SEM.
body temperature was between 1.7 and 1.8°C (Figure 8.3). There was no significant difference between diets or replicates.

8.5.3 Feed and water intakes

During natural conditions diet did not affect daily feed intake in either replicate, but during the heating periods there were effects on feed intake. Animals on the straw diet in replicate 2 ate less on days 11 and 12 than animals on the straw diet in replicate 1, coincident with the hotter conditions on those days in replicate 2. The feed intake of the straw diet on day 12 of replicate 2 was also less than that of the hay diet in replicate 2 (Figure 8.4.i). There was no difference in water intakes between diets or replicates (Figure 8.4.ii).

On day 13, animals on the straw diet in replicate 1 weighed significantly more than the animals on the hay diet in replicate 1 and animals on the straw diet in replicate 2. No changes were evident between replicate or diet when animals were weighed on day 8 (Figure 8.5).

Acid base variables (pH, pCO\(_2\), HCO\(_3^-\)) measured on venous blood samples taken on days 1, 8 and 13 were not different between diets in either replicate 1 or 2 (data not presented). Respiratory rates increased when climate controlled rooms were operating resulting in decreases in blood bicarbonate concentration and pCO\(_2\). However, blood and urine pH remained unchanged for both diets before and after the hot period for both replicate experiments (data not presented).

Apart from the increase in RR there were no other clinical signs of increased heat load in either experiment.
Figure 8.3 Maximum daily rumen temperature minus maximum daily core body temperature (mean ± SEM) for straw and hay diets in replicate 1 and replicate 2.
Figure 8.4 (i) Daily feed intake and (ii) Daily water intake for animals on hay and straw diets during replicate 1 (■) and replicate 2 (■). Points show mean ± SEM.
Figure 8.5 Body weight, as a percent of starting weight, for animals on straw and hay diets during replicate 1 (■) and replicate (■). Points show mean ± SEM.
8.6 Discussion

8.6.1 Feed intake

The pelleted feeds tested in the experiment reported here were of very similar ME and overall dry matter digestibility, but contained quite different amounts and types of roughage. There was no evidence of a difference in intake of these two diets for the majority of the experiment; however, those animals on the straw based pellet in replicate 2 did eat significantly less than the animals on the hay based pellet on day 12. It is unclear whether this is a real effect of continuing exposure to higher heat in replicate 2 or an influence of different diet on feed intake. Furthermore, on days 7 and 12, animals only had access to feed for 9 hours due to the 15-hour feed curfew required prior to weighing. There may also be between animal differences and effects on feed intake. For example, animal No. 949 was the heaviest and tended to have higher respiratory rates, and was assumed to be more greatly affected by the hot conditions. Unfortunately that animal was implanted with the core temperature logger that failed, so we cannot confirm our assumption that she may have had a higher $T_c$ resulting in a lower feed intake of the straw based pellets by day 12 in replicate 2, biasing the results. Given that there were no real differences in feed intake between the hay and straw based pelleted diets, mean metabolisable energy intake (MEI) for animals in each replicate was approximately the same.

8.6.2 Core and rumen temperature

Apart from animal number 949, the temperature telemetry and logger technology functioned well to record both core and rumen temperatures. There were large reductions in rumen temperature when animals drank, which in turn influenced the
daily mean rumen temperature. In order to overcome this problem, daily maximum rumen temperatures gave a more realistic and unbiased picture of rumen temperature and were thus used for statistical comparisons. In the first replicate the animals on the straw diet had a significantly lower mean core body temperature than those on the hay diet for the last two days of that replicate. It may be that animals on the hay diet were producing more heat from rumen digestion compared to the straw diet, but this was not reflected in maximum rumen temperatures. There were no significant differences in core temperatures between animals on the different feeds in the second replicate. However, core temperatures increased quickly on exposure to heat in the second replicate even though the environmental conditions were similar between the replicates for the first few days, and this rapid core temperature response may indicate a carry-over effect of the heat exposure in replicate 1, and may mask any effects of dietary heat. Mader et al. (1999) fed 2 diets with a similar MEI (a 28% roughage diet and a 6% roughage diet fed 90% of ad libitum) to feedlot steers in a hot environment. Animals on the high roughage diet reduced feed intake to a greater extent than the animals on the high concentrate diet, and as a result decreased their MEI. This resulted in decreases in core body temperature for animals on the high roughage diet. Results from replicates 1 and 2 show no difference in feed intake or MEI. If anything, results would indicate that animals on the high roughage diet in replicate 1 had higher core body temperature than the low roughage diet. There was no evidence to suggest that animals on the high roughage diet had a greater reduction in feed intake resulting in a reduction in metabolisable energy intake.

The lower core temperature of the animals on the straw based diet in replicate 1 corresponded to the same animals having a significant improvement in weight gain compared to those animals on the hay diet. However, there were no significant differences in feed or water intake during the first replicate. The reason for the
differences in core temperature and body weight cannot be determined. It is possible that the small difference in crude protein between the hay and straw based pellet (12.4 versus 11.0%) may have contributed to the improved body weight for those animals on the straw based diet. However, it is more likely that the small sample size may simply indicate between animal variability in response, rather than an effect of diet.

There was no difference in mean maximum rumen temperatures between diets in either replicate experiment. Given that the feed intakes were the same, differences in heat increment and the hypothesised changes in rumen temperature between feeding high roughage diet versus low roughage and high concentrate diet would appear to be minimal. The results indicate that there is no difference in rumen heat production between the two diets. This is contrary to West (1999) and others who suggest that lower fibre, high grain diets may reduce metabolic heat production and contribute to lower heat load in the animal. The results of replicate experiments 1 and 2 agree with NRC (2000) and suggest that the pelleting of roughages results in lowering heat increment so that the net dietary energy from these roughages is often higher than for the parent product. If the ME content of both diets was the same and the MEI was the same (due to similar feed intakes) then it was hypothesised that the heat increments of both pelleted diets were similar due to the fact that maximum rumen temperatures between diets were not different.

8.7 Conclusions

Each replicate experiment was analysed separately resulting in small numbers of animals tested. Therefore, the small changes expected in measured variables meant that testing for significant differences between diets was difficult. At the wet bulb temperatures achieved in these experiments, it made no difference to feed intake in Bos taurus animals, if they were fed a high quality hay-based pellet or a standard straw-
based shipper pellet. Whether there would be significant differences at more extreme conditions was not tested. Replicate 1 showed that feeding the hay pellet may increase core body temperature when animals are already subjected to high heat and humidity and this may lead to a reduction in weight gain, but this difference was not seen in the second replicate. Maximum rumen temperatures were not different between diets suggesting that the effects of heat increment on pelleted feeds are minimal.

Further research is required to fully understand the effects of pelleted diets and prolonged and continuous heat and humidity.
Chapter 9: General conclusions and future research

This thesis has assessed and defined the physiological responses of cattle to prolonged and continuous high heat and humidity similar to that which may be encountered on board livestock vessels travelling from southern Australia to the Middle East. The thesis has defined these physiological responses for both *Bos taurus* and *Bos indicus* animals. Particular emphasis was placed on feed and water intakes as well as blood gas homeostasis and plasma and urine electrolytes, hormones and metabolites. It was concluded that *Bos indicus* animals were better able to physiologically cope with continuous periods of high heat and humidity as highlighted by smaller changes in feed intake, core body temperature, respiratory rate and blood gas variables. Both *Bos taurus* and *Bos indicus* showed a remarkable ability to maintain blood gas homeostasis during prolonged and continuous high heat and humidity. It would appear that after the heating period the animals were not able to maintain homeostasis and a metabolic acidosis developed. The directions of changes in blood gas variables were the same for both breeds, but *Bos taurus* were more severely affected for longer durations.

Inappetence would appear to be of major concern for *Bos taurus* animals experiencing continuous periods of high heat and humidity. This concern was not reflected in the *Bos indicus* animals.

These findings led to the design of electrolyte and nutritional intervention experiments in an attempt to alleviate some of the physiological changes observed and improve animal welfare. An electrolyte replacement was designed to be supplemented via the drinking water and this was tested both in climate control rooms at Murdoch University and on board a livestock vessel to the Middle East. The end result was that there was a significant weight advantage of approximately 3% for animals
supplemented with electrolytes in drinking water. This was present even without experimental animals showing clinical signs of heat stress in the ship experiment. It appears that the weight benefit was due to treatment animals drinking more water. Where and for how long this body water was retained by the animal was unclear. It was clear that the weight improvement could provide a large economic benefit for electrolyte supplemented animals.

Welfare benefits for electrolyte supplemented animals remain unclear. There were measurable differences in urine pH between treatment and control animals in both climate controlled room studies and on board ship suggesting that animals treated with electrolytes were better able to maintain acid base homeostasis. However, this benefit was not reflected in venous blood gas variables measured in the climate controlled room experiments, perhaps due to the small sample size. Furthermore, supplementing with electrolytes did not improve plasma electrolyte concentrations or alleviate clinical signs of heat stress in the climate controlled room studies where heat stress conditions were evident. It was envisaged that the larger numbers of animals tested on board the livestock ship would give an indication of potential welfare benefits for supplemented cattle. Unfortunately for the experimental outcome, clinical signs of heat stress were not observed in the ship board experiment, so it is still not clear if supplementing animals will reduce or alleviate the clinical response to heat stress.

There are many directions future research could take with electrolyte supplementation of heat stressed cattle during live export. Firstly, only one electrolyte formulation was tested. This was carefully formulated and provides a starting point for further research with different compositions and dose rates of electrolytes likely to show different results. Furthermore, only *Bos taurus* animals were tested with this particular supplement. There is scope to test *Bos indicus* animals as well as animals in a variety of other situations, both on board ship as well as on farm or feedlot. There is also potential
to supplement cattle with electrolytes in feed. This may be more practical for the live export industry but requires animals to be eating satisfactorily and so would not be possible if severe heat stress conditions were present.

Animal welfare parameters proved difficult to assess. Research is required to further define and standardise animal welfare on board live export ships. It is envisaged that no one measurable variable will be able to accurately determine animal welfare. Instead, a number of variables, clinical, physiological and environmental, will need to be combined to give a measure of animal welfare.

The thesis also touched on nutritional interventions or manipulation to try and help alleviate some of the physiological imbalances which occurred during prolonged and continuous high heat and humidity, in particular reductions in feed intake and increases in core body temperature. The effect of a high quality hay based roughage pellet versus a normal straw based shipper pellet was tested.

The small numbers of animals in each replicate experiment and the small changes expected in measured variables meant that testing for significant differences between diets was difficult. At the wet bulb temperatures achieved in these experiments, it made no difference to feed intake in Bos taurus animals, if they were fed a high quality hay-based pellet or a standard straw-based shipper pellet. Whether there would be significant differences at more extreme conditions was not tested. Replicate 1 showed that feeding the hay pellet may increase core body temperature when animals are already subjected to high heat and humidity and this may lead to a reduction in weight gain, but this difference was not seen in the second replicate. Maximum rumen temperatures were not different between diets suggesting that the effects of heat increment on pelleted feeds are minimal.

It would have been useful to test a non pelleted hay diet ration with similar ME and crude protein content to assess the affects of the pelleting process on heat
increments. Introducing a third diet would have meant either increasing the number of replicate experiments or reducing the number of animals tested. Financial and time restraints meant that this was not possible. Furthermore, feeding non pelleted feedstuffs on board long haul voyages is not a feasible option due to limited storage space for feedstuffs.

Further research is required to fully understand the effects of pelleted diets and prolonged and continuous heat and humidity. Larger numbers of animals need to be assessed before any definitive conclusions can be made about the feeding of hay versus straw based pelleted feeds under mild heat stress conditions. There is scope for research into feeding different types of diets during different levels of heat stress conditions. It was noted in initial experiments that *Bos taurus* affected by excessive heat load (not eating) would eat if offered hay. Whether or not this was a sign of a roughage deficiency was not elucidated. Furthermore, it is not known whether manipulating diets to try and increase feed intake would be beneficial or detrimental as further increases in rumen and/or core body temperature may result with increases in feed intake especially under excessive heat load conditions. The technology now available to measure core and rumen temperature simultaneously means that there is scope to manipulate diets and feedstuffs to assess animals’ thermoregulatory response during changes in climatic conditions.
Chapter 10: Appendices

Appendix 1: Feed analysis

**Results of analysis:**

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>%</td>
<td>9.7</td>
</tr>
<tr>
<td>Dry matter</td>
<td>%</td>
<td>90.3</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>7.6</td>
</tr>
<tr>
<td>Crude Protein (N x 6.25)</td>
<td>% of DM</td>
<td>11.9</td>
</tr>
<tr>
<td>Crude Protein (N x 5.83)</td>
<td>% of DM</td>
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<tr>
<td>Acid Detergent Fibre</td>
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<tr>
<td>Neutral Detergent Fibre</td>
<td>% of DM</td>
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<tr>
<td>Phosphorus</td>
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<tr>
<td>Potassium</td>
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<td>Sulphur</td>
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<tr>
<td>Sodium</td>
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<tr>
<td>Calcium</td>
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<tr>
<td>Nitrate</td>
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NB. Metabolisable energy not supplied but assumed to be between 8 and 9 MJ
## Appendix 2: Feed analysis

**Results of analysis: Electrolyte Control diet**

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<thead>
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<tr>
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<td>91.1</td>
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<tr>
<td>Ash</td>
<td>%</td>
<td>6.2</td>
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<tr>
<td>Crude Protein (N x 6.25)</td>
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<td>Acid Detergent Fibre</td>
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<td>Digestibility</td>
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<td>Metabolisable Energy</td>
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<tr>
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**Results of analysis: Electrolyte Treatment diet**

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### Appendix 3: Feed analysis

**Results of analysis: Ship trial diet**

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### Appendix 4: Feed analysis

**Results of analysis: Feed straw diet**

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<td>Ash</td>
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<td>Crude Protein (N x 6.25)</td>
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<td>Metabolisable Energy</td>
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## (b) Results of analysis: Feed hay diet

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<td>Moisture</td>
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<tr>
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<td>%</td>
<td>91.6</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>7.8</td>
</tr>
<tr>
<td>Crude Protein (N x 6.25)</td>
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<td>11.0</td>
</tr>
<tr>
<td>Acid Detergent Fibre</td>
<td>% of DM</td>
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</tr>
<tr>
<td>Digestibility</td>
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<td>Nitrate</td>
<td>mg / kg</td>
<td>38.0</td>
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</table>
Appendix 5: Cortisol assay

Plasma cortisol was measured using an extraction tritiated immunoassay developed in the University of Western Australia physiology laboratory by Mrs M Blackberry.

Day 1

Each assay included one standard curve and up to 200 unknown samples in duplicate. The standard curve included triplicate tubes for total counts (TC) and NSB, 9 replicates of zero standard and 6 replicates for each of three quality controls. 50μl of sample was added to glass test tubes (12x75mm) and supernatant containing cortisol was extracted using 2 ml dichloromethane by vortexing for 3 minutes and freezing in a dry ice/acetone bath, allowing the solvent to be poured into a new test tube (10x75). 100μl of standards in triplicate and samples were dried under heat and air. Tracer (100μl), standard buffer (100μl) and antibody (50 μl) were added to all tubes except no antibody was added to the NSB tubes. Tubes were vortexed and incubated at 4°C for approx 40 hours.

Day 2

0.5ml of dextran-coated charcoal was added to all tubes except total count (TC) tubes that received 0.5ml of standard buffer. Tubes were incubated for 15 minutes at 4°C then centrifuged for 10 minutes at 3000rpm (2000g). 0.6ml of supernatant was removed and dispensed into counting vials with 2ml of scintillant (Starcint, Packard Chemical Operations). Vials were capped, inverted 5 times and allowed to stand for 24 jours before counting in a liquid scintillation counter (Packard Tri Carb 1500) for 3 minutes.
Cortisol Standards

A stock solution of cortisol was prepared in ethanol 100ng/ml. Standards were made by serial dilution to concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.195, 0.095 and 0.047 ng/ml in ethanol.

Antiserum

The antiserum (rabbit) against cortisol was raised in the UWA laboratory. The antigen was raised against cortisol-21 hemisuccinate (ARH-270) donated by Dr. R Cox (CSIRO, Prospect, NSW, Australia). Cross-reactions were 100% with cortisol, 3% with corticosterone, 0.6% with progesterone, 20% with 17OH-progesterone, 6.5% with testosterone and 0.1% with 4-androstene-3, 17-dione. Sensitivity of the assay was 0.5ng/ml with xx% non-specific binding and extraction efficiency of 94%. Intra-assay coefficient of variation was xx% and inter-assay coefficient of variation xx%

Buffers

1. 0.1 M Phosphate Buffer (stock phosphate buffer): 122.6g Na₂HPO₄ + 21.2g NaH₂PO₄.2H₂O + 10g sodium azide, dissolved to 10 litres double distilled water (DDW) (pH 7.5).

2. Phosphate buffered saline: 1 litre 0.1M stock phosphate buffer (1); 0.14 M sodium chloride (89g); 0.1% sodium azide (10g); DDW to 10 litres (pH 7.5).

Standard buffer: Buffer (2) with 0.1% bovine serum albumin (BSA, Fraction V, Sigma; pH 7.5).

3. Gelatin Phosphate Buffer (GPB): Buffer (2) with 0.1% gelatin (pH 7.5).

Tracer

The tracer (1,2,6,7-)³ H-cortisol (Amersham) with a specific activity of 47Ci/mM was diluted to give 12,000 DPM per 100μl.
Charcoal

Charcoal solution was made 24 hours before use with 1.5 grams of refined charcoal, 0.15 grams of dextrin and 300 ml of GPB. Solution was mixed thoroughly for 1 hr prior to use.
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