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**Water, electrolyte and acid-base balance in transported *Bos indicus*
steers**

Thesis submitted

by

Anthony Joseph PARKER, B.AppSc (Rural Tech)

in April 2004

**For the Degree of Doctor of Philosophy in the
School of Biomedical Sciences at
James Cook University**

ABSTRACT

The objective of these studies was to investigate the physiological mechanisms involved in maintaining water, electrolyte and acid-base balance in *Bos indicus* steers placed under stress. These studies also sought to provide a novel approach to minimize the effects of stress on the physiology of *Bos indicus* steers during long haul transportation in the seasonally dry tropics.

Merino sheep provided a simulated stress response model, to evaluate the effects of the principle stress hormone cortisol on indices of water and electrolyte balance. This study indicated that stressed sheep suffer from a loss of body water in excess of that associated with a loss of electrolytes to support the hypothesis that elevated physiological levels of cortisol induce a diuresis in ruminants that contributes to dehydration.

A second pen study was performed to investigate the effects of excess cortisol on physiological mechanisms that resist dehydration in *Bos indicus* steers. The presence of excess cortisol suppressed the RAA axis but did not markedly affect plasma AVP concentrations. This reflected the complexity of endocrine interactions associated with water balance in *Bos indicus* steers that enabled homeostasis to be maintained.

A quantitative analysis of acid base balance in *Bos indicus* steers demonstrated long haul transportation or extended periods of feed and water deprivation to have no effect on blood pH. The primary challenge to a transported or feed and water deprived animal is a mild metabolic acidosis induced by elevated plasma proteins which may be the result of a loss of body water. The loss of electrolytes has little effect on the acid-base balance of the animals.

The treatment of *Bos indicus* steers prior to long haul transportation with the osmolyte glycerol provided a novel approach to conserving body water, decreasing the energy deficit and preserving muscle quality.

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Anthony Joseph PARKER

April, 2004

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LIST OF ABBREVIATIONS

ACTH	-	adrenocorticotrophic hormone
ADH	-	anti-diuretic hormone
AG	-	anion gap
Ang II	-	angiotensin II
AVP	-	arginine vasopressin
CRH	-	corticotrophin releasing hormone
d	-	day/s
FFA	-	free fatty acids
GIT	-	gastrointestinal tract
h	-	hour/s
HPA	-	hypothalamo-pituitary-adrenocortical
ICF	-	Intra-cellular fluid volume
min	-	minute/s
MSH	-	melanocyte stimulating hormone
POMC	-	pro-opiomelanocortin
RAA	-	renin-angiotensin-aldosterone
SAM	-	sympatho-adrenal-medullary
SID	-	strong ion difference
TBW	-	total Body Water
THI	-	temperature-humidity indices

LIST OF PUBLICATIONS ARISING FROM THIS WORK

Parker AJ, Hamlin GP, Coleman CJ and Fitzpatrick LA (2004) Excess cortisol interferes with a principal mechanism of resistance to dehydration in *Bos indicus* steers. *Journal of Animal Science* **82**: 1037-1045

Parker AJ, Hamlin GP, Coleman CJ and Fitzpatrick LA (2003) Quantitative analysis of acid-base balance in *Bos indicus* steers subjected to transportation of long duration. *Journal of Animal Science* **81**: 1434-1439

Parker AJ, Hamlin GP, Coleman CJ and Fitzpatrick LA (2003) Dehydration in stressed ruminants may be the result of a cortisol induced diuresis. *Journal of Animal Science* **81**: 512-519

“Work, Finish, Publish”

M. Faraday

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..... *"Knowledge once gained casts a faint light beyond its own immediate boundaries. There is no discovery so limited as not to illuminate something beyond itself."* J. Tyndall (1868)

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CHAPTER ONE

INTRODUCTION

Australia has become the largest exporter of live cattle in the world, with the live cattle export trade comprising 6% of annual cattle turn off during 1998 – some 510,000 valued at A\$225 million (FOB). The live cattle export industry has seen a five fold increase in exports since 1998 and is predominately based on *Bos indicus* type cattle sourced from Northern Australia. The majority of these animals are destined for Asian feedlots. Despite the decline in exports in 1999/2000 due to the recent economic downturn in South East Asian economies, the trade in live export cattle offers strong optimism as North African nations enter the market and traditional Asian markets recover. The recent outbreaks in Europe of Bovine Spongiform Encephalopathy and Foot and Mouth disease have increased the demand for Australian cattle as importing nations cease trading with infected European nations.

Australia's status as a market leader in live cattle exports has required continual improvement in product quality and production efficiency. Industry surveys have identified that the major sectors of the export process where improved management practices are likely to result in increased productivity are from the farm gate to the wharf and during the sea voyage. There is increasing recognition of the need to minimize stress in farm animals both as a result of public concern for the welfare of animals and from the ongoing need to increase the efficiency of animal production. Improved welfare practices that minimize stress during handling and transportation prior to the sea voyage may have a significant effect on the mortality and morbidity of livestock. The timely fashion in which the transportation of export cattle occurs from the property of origin to on board ship and the subsequent ship board transportation to their final destination results in a variable degree of stress on the animals concerned.

There is a lack of data pertaining to the physiological effects of stress on *Bos indicus* genotypes. Ruminants in general respond to stress with an activation of the Sympathetic-Adrenal-Medullary (SAM) axis and the Hypothalamo-Pituitary-

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Adrenal (HPA) axis (Schaefer *et al.* 2001), with long-term stress resulting in pathophysiological effects on the animal such as loss of appetite and body mass (Hutcheson and Cole 1986), a compromised immune system (Murata 1989), and dehydration (Knowles *et al.* 1999).

Matthews and Parrott (1991) suggested a link between stress, dehydration and HPA function using sheep as a model. This evidence gave rise to the hypothesis that transportation stress is likely to be manifest as disturbances of fluid, electrolyte and acid/base balance of these animals.

The objectives of the studies described in this thesis were:

1. To enhance knowledge and understanding of the response of *Bos indicus* cattle to stressors associated with the collection and transportation for live export and their impacts on animal welfare and performance.
2. To study the relationships between stress, dehydration, electrolyte balance and acid/base balance in ruminants.
3. To develop and test protocols to minimize production losses associated with stress in *Bos indicus* cattle for live export.

The fulfillment of these objectives will assist in understanding the physiological mechanisms involved in *Bos indicus* animals maintaining homeostasis during the collection and transportation for live export.

This thesis consists of a literature review, the presentation of four separate but related experiments and, a general discussion and conclusion. The literature review examines the impact of transport and handling in the pre-delivery phase of the export process on cattle. It addresses two stress theories that support a current descriptive definition of stress. The physiology of the neuroendocrine systems are discussed in relation to stimulation by external stressors.

In addressing the hypothesis that stress induces dehydration, body fluid compartments and their measurement are discussed along with the physiological regulation of body fluids and fluid osmolarity. The physiological significance of

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neuroendocrine stimulation on water and electrolyte balance and the use of electrolyte and fluid therapy on relieving transport stress are discussed.

There is anecdotal evidence in cattle to suggest that dehydration has an impact upon the body's metabolism. This may result in a catabolic state that leads to a reduction in body mass. The effects of stress on metabolism and the gastrointestinal tract (GIT) are discussed with respect to stressor effects on liveweight and body composition. The associated effects of rest and recovery periods, injuries and mortalities associated with transport and sexual differences in the coping ability of cattle to transport stress are mentioned.

The following four experimental chapters are the result of four separate experiments each consisting of an introduction, materials and methods, results and discussion and implications. The general discussion chapter is a summary of the main results and conclusions obtained from the experimental results. The appendices at the rear of the thesis outline three small experiments that were completed to support data presented in the main experimental chapters.

CHAPTER TWO

LITERATURE REVIEW

This literature review examines the impact of transport and handling in the pre-delivery phase of the export process on cattle. There is scarce information relating to detailed physiological effects of transportation and handling stress on *Bos indicus* genotypes. Published research on transportation and handling stress in ruminants has largely been concentrated on *Bos taurus* genotypes and sheep.

A definition of stress is chosen and supported by discussion on current stress theories. The physiological effects of stress on the neuroendocrine system of mammals is also discussed.

In addressing the hypothesis that stress induces dehydration, body fluid compartments and their measurement are discussed along with the physiological regulation of body fluids and fluid osmolarity and, acid-base balance. The physiological significance of neuroendocrine stimulation on water and electrolyte balance and the use of electrolyte and fluid therapy on relieving transport stress are discussed.

The effects of stress on metabolism and the GIT are discussed with respect to stressor effects on liveweight and body composition. The increased morbidity and mortality from infectious diseases following transportation stress is addressed. The associated effects of rest and recovery periods, injuries and mortalities associated with transport and sexual differences in the coping ability of cattle to transport stress are mentioned.

2.0 Stress in Cattle

The pre-delivery phase of the export process, which occurs from the property of origin to the wharf, can place cattle under psychological and physical stressors that may result in transient endocrine responses, altered products of energy and protein metabolism, changes in appetite and growth rate, dehydration and a compromised

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immune system. The handling and transport of cattle are known to be significant stress stimuli in cattle (Grandin 1997). Long hauls exacerbate feed and water deprivation, while overcrowding, poor air quality, poor sanitation, novelty, coping ability, climate and unexpected noise also contribute to transport and handling stress. It is the sum of these factors that precipitates into increased morbidity and mortality and causes acute and chronic depression in performance (Loerch and Fluharty 1999).

The management of stress in cattle has two major components: (1) management of the cause of stress and (2) management of the effects of stress – the quantified physiological, immunological and behavioral changes seen in the animals (NRC 2000). Because transport and handling of cattle to be exported is impossible to eliminate, the weight of minimizing the stress in these animals lies with the management of the effects of stress.

Studies have been undertaken with this in mind and have included manipulation of protein (Mader *et al.* 1989), energy, minerals and fiber levels in pre- and post-transit diets (Lofgreen 1983), hydration status post transit (Whythes *et al.* 1980), pre-conditioning cattle to receiving diets (Pritchard and Mendez 1990), and the use of electrolyte fluids post- and pre-transit / stress (Gortel *et al.* 1992; Schaefer *et al.* 1992; Apple *et al.* 1993; Phillips 1997; Schaefer *et al.* 1997). The use of electrolytes in minimizing stress is a topic of interest to the live export industry and is currently being investigated (Alliance 2000).

2.1 Stress Theories

2.1.1 Selye's concept of stress

Hans Selye described biological stress as “*the non specific response of the body to any demand*” (Loerch and Fluharty 1999). Selye's research drew attention to the fact that a wide range of adverse environments apparently evoked a limited range of responses. In particular, he emphasized the secretion of adrenal glucocorticoids as a widespread, non-specific response, as are suppression of the immune system, and gastrointestinal ulcers (Selye 1976). Furthermore, Selye noted similar patterns of physiological response in a range of animal species, which he summarized as:

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1. An 'alarm' reaction
2. A stage of physiological resistance to the disturbance, and if this continues for long enough
3. A stage of exhaustion of the adaptive process leading to death.

2.1.2 Moberg's model for stress response in animals

Moberg (1985) developed a model for the animal stress response. He adapted his model from processes that occur when psychological factors affect pathologic systems in humans. Moberg's concept of a pre-pathological state is a more perceptive attempt to describe criteria for the term "*stress*" (see Figure 2.1- Moberg's model of stress in animals).

In this model, the crucial effect of the environment is to predispose the animal to the development of some pathological state. The pre-pathological state is recognizable by any of a variety of abnormalities and inadequacies in behavior, physiology, immune system function and reproduction and is the threshold at which an animal is said to be 'stressed'.

2.2 Definition of stress

The term stress has been one of the most controversial of biological terms. It is open to wide interpretation and, as a result, research in this area historically has suffered from a lack of firm definitions. Selye (1976) and Moberg's (1985) theories of stress have been utilized by Broom and Johnson (1993) to arrive at the following definition of stress that will be used in this review

"Stress is an environmental effect on an individual which overtaxes its control systems and reduces its fitness or appears to do so" (Broom and Johnson 1993).

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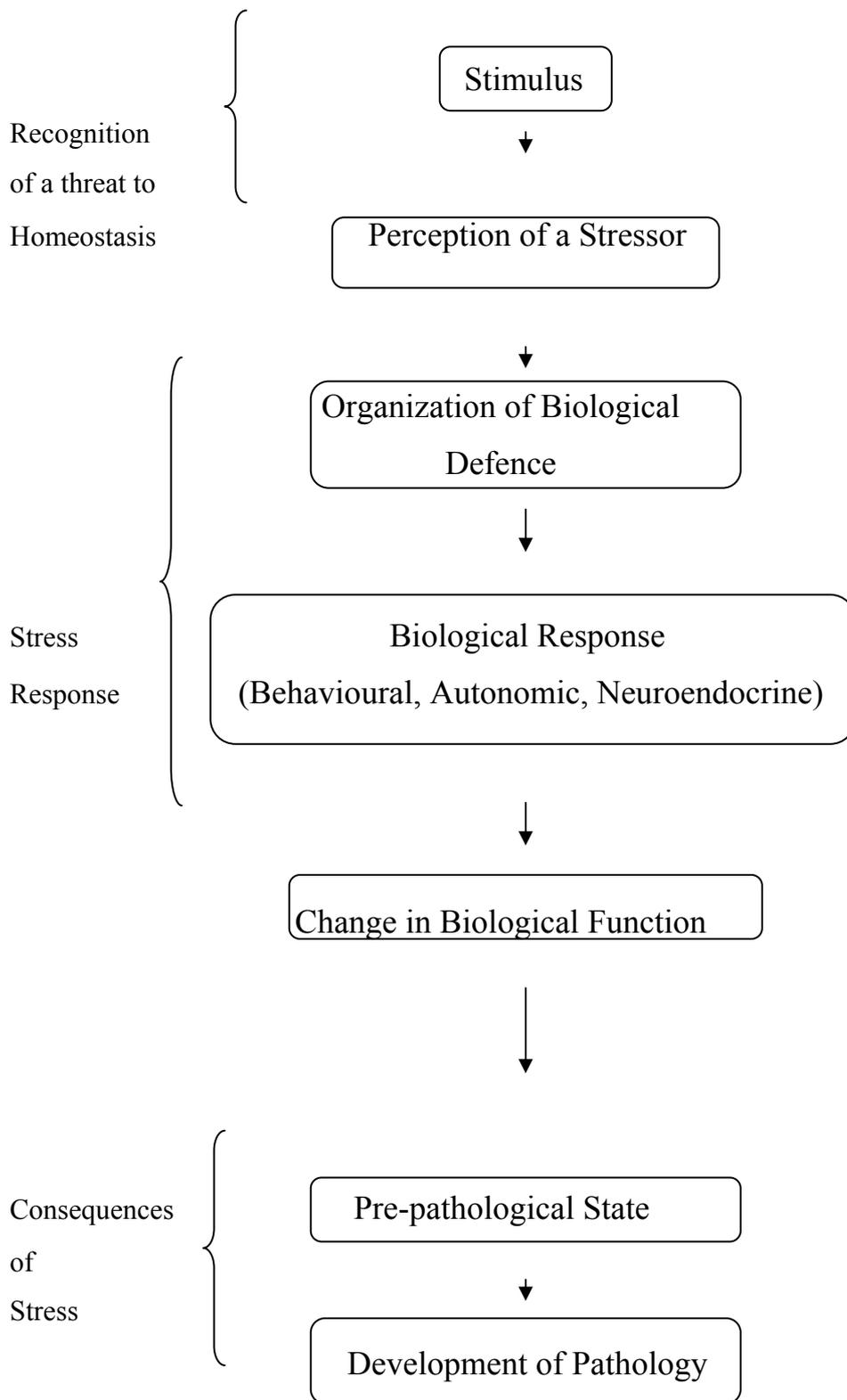


Figure 2.1. Moberg's model of stress in animals.

2.3 Sympatho-adrenal-medullary (SAM) axis

The major effects of a stress stimulus lie within the neuroendocrine systems. Cognitive stimuli received by the cerebral cortex of the brain produce neurological impulses that cause hypothalamic stimulation of the autonomic nervous system with an associated production of neurotransmitters (Griffin 1989). The response of the SAM axis to a stressor is a sensitive indicator of stress in livestock and is activated immediately upon application of the stress stimuli (Niezgoda *et al.* 1993).

This immediate response results in the production of catecholamines by two discrete pathways: (1) directly, by release of norepinephrine from sympathetic nerve endings and (2) indirectly, by release of epinephrine and small amounts of norepinephrine from the innervated adrenal medulla (Griffin 1989). The body's response to catecholamines causes dramatic increases in cardiovascular function, vasoconstriction, splenic contraction and metabolism to effect an increased physical potential of the host which is manifest by the 'flight or fight' response (Griffin 1989; Guyton and Hall 2000).

Activation on the adrenal medulla is an effective response only to short-term problems and its activity is seldom of much use as an indicator of long-term problems. However, some long-term problems involve repeated brief stressful stimuli, so assessment of the consequences of these may be useful (Broom and Johnson 1993). Because different types of psychological and physical stressors produce varying levels of norepinephrine and epinephrine, there is a continuing need to understand the interaction between these factors and their impact on the host animal.

2.4 Hypothalamo-pituitary-adrenocortical (HPA) axis

Failure of the SAM axis to resolve stress on an animal leads to activation of the HPA axis, which is manifest behaviorally by the 'conservation-withdrawal' reaction. Persistence of the HPA axis activation causes biological changes, which may produce a multitude of pre-pathological states (Griffin 1989).

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The stress stimuli act upon the magnocellular region of the paraventricular and supraoptic nuclei in the hypothalamus to produce corticotrophin releasing hormone (CRH), which in turn activates the pars nervosa of the pituitary gland to secrete pro-opiomelanocortin (POMC) (Irvine *et al.* 1988). This POMC produces endorphins, adrenocorticotrophic hormone (ACTH) and melanocyte stimulating hormone (MSH). ACTH is the principle regulator of glucocorticoid synthesis and secretion from the adrenal gland. Of the glucocorticoids in cattle, cortisol is the predominant hormone with small amounts of corticosterone also produced. There is a negative feedback mechanism for cortisol that acts on both the pituitary and hypothalamus (Guyton and Hall 2000).

ACTH in turn is regulated by a variety of peptides but principally CRH (Minton 1994), arginine vasopressin (AVP) and catecholamines (Broom and Johnson 1993). In cattle, Minton (1994) states that ACTH has greater secretory responses to CRH. The role of AVP in mediating the HPA axis response to various stressors is unclear. However, it has been concluded that the nature of the stressor determines the hypothalamic response (Alexander *et al.* 1988).

Bolus injections of AVP have produced pituitary adrenocortical effects in the sheep. Redekopp *et al.* (1985) demonstrated that AVP strongly potentiates the action of CRH on ACTH release. Using exogenous infusions of AVP (0.02 IU/kg) and CRH (50 µg), Redekopp *et al.* (1985) demonstrated that when injected separately, maximal responses to ACTH were achieved with CRH inducing a more prolonged effect. However, the combined administration of AVP and CRH induced an ACTH response that was seven fold greater (47.7 ± 6.60 µg/min/L) than the administration of CRH alone (6.27 ± 2.56 µg/min/L). During hypertonic saline infusions in sheep, Redekopp *et al.* (1986) further demonstrated rises in osmolality and endogenous AVP concentration concurrently with an amplified ACTH response (12.91 ± 3.66 µg/min/L) to exogenous CRH compared to the response from exogenous CRH administered with isotonic saline (4.00 ± 0.93 µg/min/L).

In contrast, Matthews and Parrott (1991) suggested that a sustained high level of AVP in the circulation probably does not release ACTH from the ovine pituitary under physiological conditions. Further to this they stated that under normal

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conditions dehydrated animals show only minimal increases in cortisol and presumably ACTH release because CRH concentration in portal vessels is low. However, during stress, when greater amounts of CRH are released from the median eminence, the increased pituitary sensitivity of the dehydrated animal results in a greater stimulation of the HPA axis.

In considering the neuro-endocrine responses to differing stress stimulus Pacak and Palkovits (2001) suggest that each stressor has its own chemical 'signature', with quantitatively if not qualitatively distinct central mechanisms. This may imply that measurements in differing compensating systems, for example, epinephrine, nor-epinephrine, ACTH, AVP and the renin-angiotensin-aldosterone axis would yield clearly distinct patterns. This perhaps explains the contrasting information available on stress responses to differing stress stimulus, physiological status and environmental conditions of reported experiments.

2.5 Body fluid compartments

Total body water (TBW) is divided into two main compartments, the extra-cellular and the intra-cellular spaces. The TBW content has been approximated at 55 to 70% of the total body weight (Yousef and Johnson 1985). Extra-cellular water consists of 20% and the intra-cellular water makes up the remaining 50% of total body weight. The extra-cellular space consists of the extra-vascular space (15%) and the vascular space (5%) of body weight. The intra-cellular space is made up of the total fluids inside cells in the body (Seif 1973).

Extra-cellular fluid (ECF), composed of plasma, interstitial fluid, lymph, cerebrospinal fluid, and intra-ocular fluid, is vital because it is the immediate environment of the cells. It is also vulnerable because through it the exchanges with the external environment, the cells of the body and the GIT take place. Its regulation is of the utmost importance (Fitzsimmons 1979).

2.5.1 Measurement of body fluid compartments

As the volume of water in each compartment cannot be measured directly, indirect measurements are used, such as the dilution technique, in which a substance is placed into a compartment and the extent to which the substance becomes diluted is measured. The dilution principle technique has been utilized to measure the major body fluid compartments in a number of species with varying degrees of accuracy. To calculate fluid space in this way, the total concentration of the substance injected and the concentration in that fluid space after complete and uniform distribution of the substance need to be known. Both stable and radioactive substances have been advocated as markers for measuring body fluid compartments, the substance used being dependant on the diffusion characteristics of the marker (Seif 1973; Guyton and Hall 2000).

2.5.2 Total Body Water (TBW)

To measure TBW, the marker must be able to diffuse rapidly and homogenously through all water, into the cells, as well as the different spaces of the extra-cellular compartment. In addition, the substance must not be metabolized or excreted before uniformity in distribution, and preferably it should not be toxic or foreign to the body (De Campeneere *et al.* 2000). The substances that have been used in the past include tritiated water (Springell 1968; Little and Morris 1972; Kock and Preston 1979), deuterium (Arnold and Trenkle 1986), antipyrine, N-acetyl-1-4-aminoantipyrine (Guyton and Hall 2000), and urea (Preston and Kock 1973; Hammond *et al.* 1984; De Campeneere *et al.* 2000). All these substances yielded values that correlated well with those obtained from desiccation and specific gravity measurements. Of all the substances used, tritiated water is considered to be the 'gold standard' in dilution technique assays and consistently yields a high degree of accuracy (Springell 1968; Kamal and Seif 1969; Little and Morris 1972; Seif 1973; Kock and Preston 1979).

2.5.3 Extra-cellular Fluid Volume (ECF)

When injected into the blood stream, substances that diffuse readily throughout the ECF space must pass through the capillary membranes without penetrating the lipid membrane of cells to any significant extent. The radioactive substances used are sodium, chloride and bromide, while thiosulfate ions, thiocyanate ions, inulin and sucrose are among the non-radioactive markers used. None of these substances gives the exact volume of ECF. Sucrose and inulin give lower values than expected since they do not penetrate the bound-fluid of the collagen fibers. Others, such as thiocyanate and radioactive chloride, sodium and bromide penetrate cells to a lesser extent and are more commonly used (Seif 1973; Guyton and Hall 2000).

2.5.4 Intra-cellular Fluid Volume (ICF)

There is no known substance that will measure intra cellular fluid volume using the dilution technique. The ICF is calculated by subtracting the extra cellular fluid volume from the TBW volume.

2.5.5 Blood volume

There is no substance that will measure total blood volume. However, plasma volume is frequently measured utilizing Evan's blue dye, as this substance adheres to the proteins in plasma. Red cell volume can be calculated using the hematocrit percent. From these two measures total blood volume can be calculated (Guyton and Hall 2000).

2.6 Body water regulation

With the exception of disease states such as oedema or severe water dehydration, the intra-cellular compartment of the body water stays relatively constant. Therefore, body water regulation depends primarily upon the regulation of the ECF. The balance between intake and output of water and salt determines ECF volume. In

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most cases, salt and fluid intakes are dictated by an animal's habits or management protocols, rather than by physiologic control mechanisms. The burden of ECF volume regulation is usually placed upon the kidneys (Guyton and Hall 2000).

2.6.1 Regulation of ECF osmolarity

For the cells in the body to function properly, they must be bathed in ECF with a relatively constant concentration of electrolytes and other solutes. The total concentration of the solutes in the ECF, and therefore the osmolarity, is determined by the amount of solute divided by the volume of the ECF. Osmolarity and sodium concentration are regulated by the amount of extra-cellular water. Body water in turn is regulated by intake of fluids, which is controlled by factors influencing thirst, and renal excretion of water (Guyton and Hall 2000). Two primary mechanisms are involved in regulating the concentration of sodium and the osmolarity of ECF: The osmoreceptor anti-diuretic hormone (ADH) feedback system and the thirst mechanism.

2.6.2 Osmoreceptor-ADH feedback system

Figure 2.2 shows the basic components of the osmoreceptor-ADH feedback mechanism for the control of ECF sodium concentration and osmolarity. When osmolarity increases due to water deficit, for example, this feedback system operates as follows:

1. Osmoreceptor cells, located in the anterior hypothalamus sense an increase in osmolarity and subsequently shrink.
2. Shrinkage of the osmoreceptor cells causes them to fire, sending a nerve signal to other nerve cells in the supra-optic nuclei which in turn transport these signals to the posterior pituitary.
3. The secretory vesicles in the posterior pituitary are the storage center for ADH. Upon stimulation by nerve cells these vesicles release ADH into the blood stream.

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4. ADH enters the blood stream and is transported to the kidneys, where it increases the permeability of the late distal tubules, cortical collecting tubules and inner medullary collecting ducts.

The increased water permeability in the distal nephron segments causes increased water absorption and excretion of a small volume of concentrated urine (Guyton and Hall 2000).

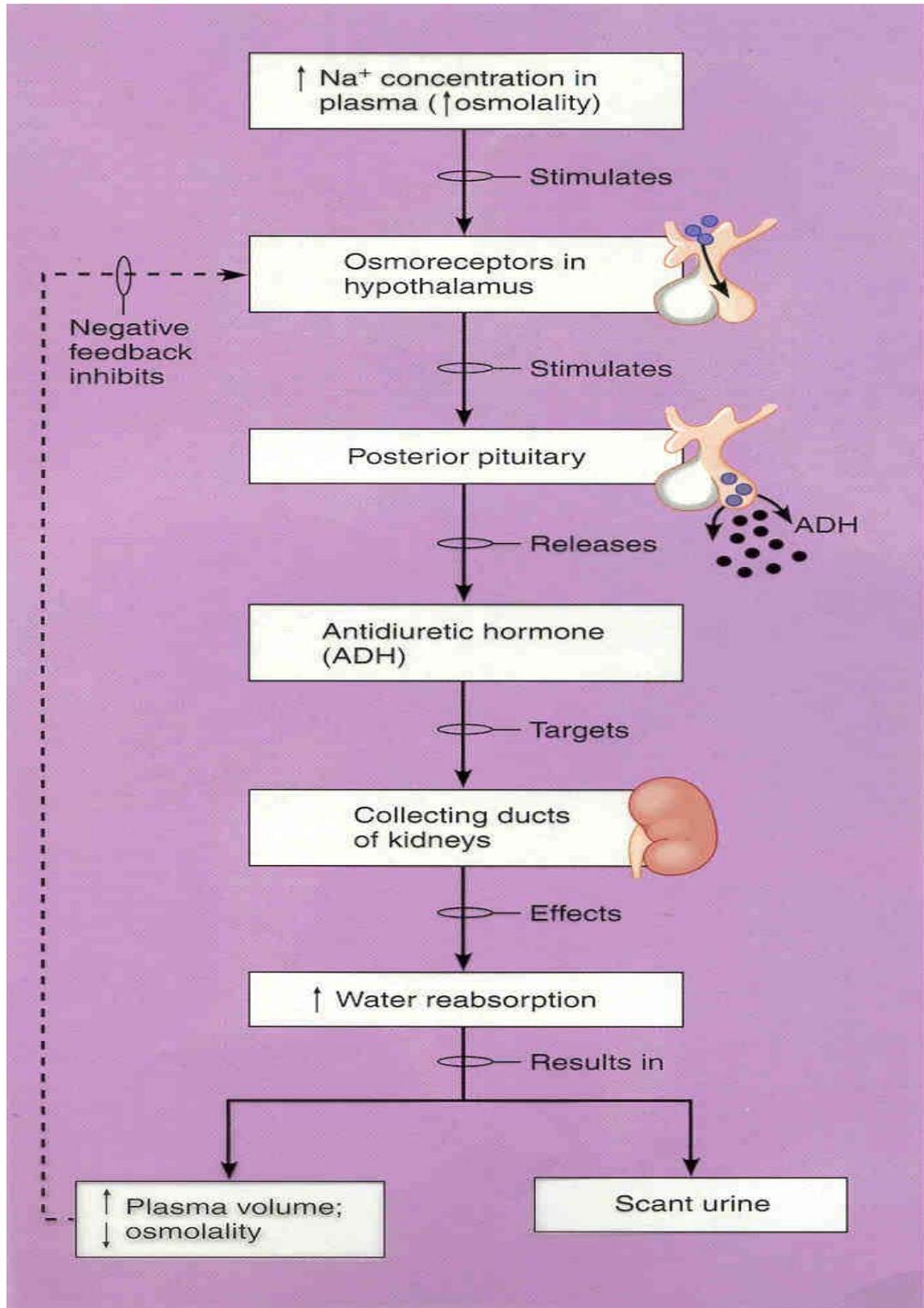


Figure 2.2. Mechanism and consequences of Anti-diuretic hormone release (Taken from; Marieb, E. 1999).

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2.6.3 Role of thirst mechanism and sodium appetite

Although the kidneys minimize fluid loss during water deficits through the osmoreceptor–ADH feedback system, adequate fluid intake is necessary to counter balance whatever fluid loss does occur through sweating, breathing and through the gastrointestinal tract. Fluid intake is regulated by the thirst mechanism, which, together with the osmoreceptor-ADH mechanism, maintains precise control of ECF osmolarity and sodium concentration. In the absence of the ADH-thirst mechanism, no other feed back mechanism is capable of adequately regulating plasma sodium concentration and osmolarity (Guyton and Hall 2000).

There are two main stimuli for thirst: cellular dehydration and extra-cellular dehydration. Cellular dehydration refers to a decrease in the water within the cells, or cell shrinkage. If the effective osmotic pressure of the plasma is increased, water will move out of the body cells. The anterior hypothalamus contains osmoreceptor cells; when these neurons shrink, they signal the cerebral cortex to give rise to the thirst sensation. Not all solutes are effective stimuli for the osmoreceptor cells. Urea and ethanol are ineffective because they readily penetrate the osmoreceptor cells and therefore do not cause them to shrink (Guyton and Hall 2000).

2.7 Regulation of cellular volume

The membranes of animal cells are highly permeable to water. Animal cell membranes cannot tolerate substantial hydrostatic pressure gradients, and water movement across those membranes is dictated by osmotic pressure gradients (Guyton and Hall 2000). Therefore, any imbalance of intra-cellular and extra-cellular osmolarity is paralleled by a respective water movement across cell membranes resulting in changes in cell volume.

To avoid excessive alterations in cell volume, cells utilize a number of volume regulatory mechanisms including transport across the cell membrane and metabolism. The following mechanisms are triggered by minute alterations in cell volume, and serve to readjust cell volume and modify cellular functions (Lang *et al.* 1998).

2.7.1 Ion Transport systems

Ion transport systems are the most efficient and rapid means of altering cellular osmolarity (Lang *et al.* 1998). During cell swelling, cells extrude ions, thus accomplishing regulatory volume decrease, whereas during cell shrinkage, cells accumulate ions to achieve regulatory volume increase. The major ion transport systems accomplishing electrolyte accumulation in shrunken cells are the Na⁺-K⁺-2Cl⁻ co-transporter and the Na⁺/H⁺ exchangers. The later alkalinizes the cell leading to a parallel activation of the acid exchanger. In some cells, e.g. muscle cells, electrolytes are accumulated during residual volume increase by activation of Na⁺ channels and/or non-selective cation channels. The depolarization induced by Na⁺ entry favors Cl⁻ entry into the cell (Lang *et al.* 1998).

2.7.2 Osmolytes

The cellular accumulation of electrolytes after cell shrinkage is limited because high ion concentrations interfere with the structure and function of macromolecules, including proteins. Furthermore, alterations of ion gradients across cell membranes would affect the respective transporters (Lang *et al.* 1998). To circumvent the effects of disturbed ionic composition, cells produce or accumulate osmolytes, molecules specifically designed to create osmolarity without compromising other cell functions. Unlike ions, osmolytes, are not inhibitory to most cellular processes even at molar concentrations and have been found to stabilize the native state of proteins and lipids (Burg 2001). Beyond their function in cell volume, Burg (2001) reported that osmolytes are protective against the destructive effects of excessive temperatures and desiccation. Three groups of osmolytes are used by mammalian cells: 1. polyalcohols, such as sorbitol and inositol; 2. methylamines such as glycerol, glycerophosphorycholine and betaines; and 3. amino acids and amino acid derivatives, such as glycine, glutamine, glutamate, aspartate, proline and taurine (Burg 2001; Lang *et al.* 1998; Robert *et al.* 2000).

Glycerol

Human sports science has inadvertently promoted the use of one particular osmolyte, Glycerol, a natural metabolite that is rapidly absorbed into the body, and touted as having osmotic properties such that it enables a greater fluid retention than ingestion of water alone (Wagner 1999). Recent studies in man (Riedesel *et al.* 1987; Hitchins *et al.* 1999) and equine (Schott *et al.* 1999) have focused on the use of glycerol solutions to achieve a state of hyper-hydration. They found that subjects drinking glycerol solutions achieved greater hyper-hydration compared to subjects drinking water while resting in temperate conditions.

Riedesel *et al.* (1987), demonstrated that glycerol with excess fluid could produce a state of hyper-hydration for four h in humans. Freund *et al.* (1995), reported that glycerol increased fluid retention by reducing free water clearance. Exercise and heat stress, however, decreased renal blood flow and free water clearance in monogastrics and therefore may reduce the effectiveness of glycerol as a hyper-hydrating agent in these species (Latzka *et al.* 1997). El-Nouty *et al.* (1980) have demonstrated an increase in urine output under conditions of heat stress in cattle and therefore an increase in the free water clearance. The administration of glycerol may reduce the water deficit incurred with stressors in the bovine.

One advantage of hyper-hydration is that it delays the development of a body water deficit when sweat losses are not replaced. Latzka *et al.* (1997) demonstrated that pre-exercise hyper-hydration delayed the development of a body water deficit until approximately 60 min of exercise. As expected, when hypo-hydration was present physiological strain was increased (temperature and heart rate). Latzka *et al.* (1997) postulated that pre-exercise hyper-hydration could be beneficial when fluid intake is restricted during compensatable exercise in humans. Similarly, the use of a glycerol containing solution on cattle prior to the onset of stressors involved in handling and transport may minimize the loss of body water in transit.

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Betaine

Betaine is accumulated in cells by a Na^+ -coupled transporter. Increased cellular ionic strength, but not urea, stimulates the transcription rate of the transporter and subsequently the accumulation of betaine. After cell swelling betaine is rapidly released (Lang *et al.* 1998). Betaine normally present in mammalian plasma, provides a source of betaine for uptake by cells (Burg 2001). Betaine is a by-product of the sugar beet industry and is readily available in Australia, although its use as an osmo- and thermo-protectant in cattle has yet to be fully investigated.

2.7.3 Acid-Base balance

The Henderson-Hasselbalch Equation

The Henderson-Hasselbalch equation is considered the traditional approach to describe acid-base balance. This equation focuses on how carbon dioxide tension (P_{CO_2}), bicarbonate concentration ($[\text{HCO}_3^-]$), the negative logarithm of the equilibrium constant (pK'_1), and the solubility of CO_2 in plasma (S) interact to determine plasma pH. This relationship is expressed as the Henderson-Hasselbalch equation:

$$(1) \text{ pH} = \text{pK}'_1 + \log \frac{[\text{HCO}_3^-]}{S P_{\text{CO}_2}}$$

The Henderson-Hasselbalch equation is well regarded for its robustness in determining acid-base physiology and is routinely employed to understand and treat acid-base disorders in mammals. It uses pH as the ultimate measure of acid-base status, P_{CO_2} as an independent measure of the respiratory component of acid base balance, and extra cellular HCO_3^- as an independent measure of the metabolic component (Constable 1999). The equation allows for four primary disturbances to be defined: respiratory acidosis (increased P_{CO_2}), respiratory alkalosis (decreased P_{CO_2}), metabolic acidosis (decreased extra-cellular HCO_3^-), metabolic alkalosis (increased extra-cellular HCO_3^-).

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Constable (1999) highlights the disadvantages of the Henderson-Hasselbalch equation as unsatisfactorily explaining the temperature dependence of plasma pH, why the value of pK_1' depends on pH, protein concentration, and sodium concentration and why a non-linear relationship exists between pH and $\log P_{CO_2}$ *in vitro* over a wide range of pH and *in vivo* during CO_2 equilibration studies. The Henderson-Hasselbalch equation can only be applied to ruminant plasma at approximately normal temperature, pH, protein concentration, and sodium concentration. Thus, this minimizes the utility of the Henderson-Hasselbalch approach for describing acid-base balance in ruminants, as large changes in blood temperature, pH, plasma protein, and sodium concentrations occur during specific disease states, particularly dehydration and heat stress.

Strong Ion Model

Stewart's (1983) strong ion model states that plasma pH is dependant upon three independent variables: The partial pressure of carbon dioxide in plasma, (P_{CO_2}); the difference between the charge of plasma strong cations and strong anions, termed the strong ion difference (SID); and the total concentration of non volatile plasma weak acids ($[A_{total}]$), consisting of albumin, globulin and phosphate. These physicochemical interactions between the independent variables contributing to acid-base control recognize the constraints imposed by the laws of electroneutrality, dissociation equilibrium of weak acids and water and the conservation of mass (Kowalchuk and Scheuermann 1994).

The strong ion model reduces the chemical reactions in plasma to those of simple ions in solution. This assumption is made on the basis that the major plasma cations (Na^+ , K^+ , Ca^{++} , Mg^{++}) and anions (Cl^- , HCO_3^- , protein $^-$, lactate $^-$, sulfate $^-$, ketoacids $^-$) bind each other in a salt-like manner. Ions that enter into oxidation-reduction reactions, complex ion interactions, and precipitation reactions, such as Cu^{++} , Fe^{++} , Fe^{+++} , Zn^{++} , Co^{++} , and Mn^{++} , are assumed to be quantitatively unimportant in determining plasma pH, due to their low concentrations in plasma (Constable 1999).

Conceptually, the ions in plasma can be differentiated into two types, non-buffer ions (strong electrolytes) and buffer ions. Stewart (1983) considered the strong

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electrolytes in plasma to be fully dissociated at physiologic pH and, as such, exerted no buffering capacity in plasma. However, strong electrolytes are held to exert an electrical effect because the sum of the dissociated strong cations does not equal the sum of the dissociated strong anions (Constable 1999). Stewart (1983) termed this difference in electrical charge as the SID.

Buffer ions are derived from plasma weak acids and bases that are not dissociated fully at physiologic pH. Buffer ions can be separated into two types, volatile buffer ion (bicarbonate) and non-volatile buffer ions (non-bicarbonate). In arterial plasma, the bicarbonate buffer system is an open system and is considered separately. Altered respiratory activity can change the tension of CO₂ in plasma and hence bicarbonate concentration. In contrast, the non-bicarbonate system is a closed system containing a fixed quantity of buffer (Constable 1999). Stewart (1983) stated that most membranes within the body are impermeable to protein, so that A_{total} interactions are not normally significant. However, A_{total} changes may occur in some pathological states, and when they do, they have consequences for [H] in the fluids in which they occur.

Quantitatively, three independent variables, pCO₂, SID, and total protein, and their physiological regulation by the lungs, kidneys, gut and liver offer an insight into the pathophysiology of mixed acid-base disturbances using the strong ion model. This approach enables our ability to understand, predict and control hydrogen ions in biological fluids (Constable 1999).

Stewart's (1983) strong ion approach is not universally accepted. The major limitation on its application is the difficulty in obtaining an accurate value for SID concentration. Determination of SID in plasma requires identification and measurement of all strong ions in plasma, which is an impossible task as unidentified strong ions such as lactate, β -OH butyrate, acetoacetate, sulfate and calcium and magnesium may be present in meq/L concentration in animals with a pathology (Constable 1999). Despite this shortcoming an estimate of SID concentration can be obtained in ruminants by determining the plasma concentrations of the four strong ions (Na⁺, K⁺, Cl⁻ and lactate⁻) (Stewart 1983).

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Cameron (1989) criticizes Stewart's strong ion model due to its mathematical complexity compared to the Henderson-Hasselbalch equation in determining pH. Aguilera-Tejero *et al.* (2000) stated that such a quantitative approach was not intended to merely measure pH of a biological fluid but to identify the inciting cause of the acid-base disturbance. The identification of the effects of hypoproteinaemia or hyperproteinaemia or a change in the SID of plasma would not be implicated using the Henderson-Hasselbalch equation.

2.8 Effects of HPA axis on Water and Electrolyte Balance

Dehydration has been touted as a stressor in the transportation chain for ruminants (Atkinson 1992). *Prima facie*, this appears to be an anthropomorphic observation as Thornton *et al.* (1987) demonstrated that dehydration for 24 h in wethers did not result in an elevation in plasma cortisol concentration. Similarly, Finberg *et al.* (1978) demonstrated that water deprivation alone in the camel for 9 d was not a prototypical stressor that would activate the HPA axis and elevate plasma cortisol. It should be noted that the above-mentioned experiments were with well-handled animals placed in stalls where the perception of novel stressors by the animals were minimal. Further to this, the sampling intervals for cortisol concentration analysis were at 24 h intervals, which may not have allowed for the pulsatile nature of cortisol secretions.

Matthews and Parrott (1991), provided evidence for a physiological interaction between stress, dehydration, and HPA axis function in the sheep. They suggested that endogenous AVP amplifies the responsiveness of the pituitary to CRH. Thus, under normal grazing conditions, dehydrated animals show only minimal increases in cortisol and ACTH because the CRH content of the portal vessels is low. However during stress, when greater amounts of CRH are released at the median eminence, the increased sensitivity of the pituitary in a dehydrated animal results in a greater stimulation of the HPA axis. This would imply that cortisol will be more readily released if sheep are stressed when deprived of water. The biological significance of an increased cortisol response in dehydrated ruminants and whether or not it confers an adaptive value remains to be established.

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Guerrini and Bertchinger (1982) demonstrated in sheep that increased plasma cortisol concentrations were associated with a decreased water intake ($P < 0.005$) and decreased urine output ($P < 0.005$). Parrott *et al.* (1987) observed that isolation stress in sheep resulted in a reduced, and in some animals caused a complete absence, of water intake. However the associated changes with dehydration did not occur. Parrott *et al.* (1987) concluded that acute stress may activate a mechanism that enables the volume, tonicity and ionic composition of extra cellular fluid in sheep to be maintained in the face of a severe reduction in water intake.

The effects of stressors on water balance lie with the secretion of hormones from the HPA axis. Glucocorticoids, especially cortisol, have been known to suppress the antidiuretic response of nicotine and ADH in human subjects. Studies of impaired water excretion in human patients indicate that glucocorticoids are necessary to suppress the inappropriately elevated levels of ADH (Forman and Mulrow 1975). Cortisol is recognized as a calorogenic agent in many mammalian species, including cattle (Yousef and Johnson 1985). Yousef and Johnson (1985) have stated that the increase in cortisol levels in cattle as a result of short-term exposure to heat is the result of a stress reaction, which is not specific to heat stress. The subsequent reduced cortisol levels, which occur with heat acclimation, have been said to be a regulatory mechanism for reducing the animal's heat production. Other studies indicate that the suppression of cortisol with chronic heat stress in cattle may be a water balance mechanism.

Dehydration has been reported to induce a sodium diuresis in sheep and other species, as well as cattle (Bianca *et al.* 1965; McKinley *et al.* 1983; Metzler *et al.* 1986). Blair-West *et al.* (1989) found that one day of dehydration failed to show a statistically significant loss of sodium in dehydrated cows, compared with hydrated control cows. However, the findings of Blair-West *et al.* (1989) may have been limited by the statistical power of their test. A population size of six animals and the lack of other stressors may have been the cause of a lack of significant results.

The natriuretic nature of cortisol associated with stress has been demonstrated in the bovine and other species (El Nouty *et al.* 1980; Baas *et al.* 1984; Wintour *et al.* 1985; Beede and Collier 1986; Marya *et al.* 1987). El Nouty *et al.* (1977) found in heat

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stressed Friesian cows that an increase in ADH was not associated with significant decreases in urine output (18.43 ± 1.51 and 19.55 ± 1.55 liters) for thermo-neutral and heat stress treatments, respectively. Although ADH levels increased in the heat stressed cow, plasma osmolality decreased [due in part to the significant decrease in serum sodium concentration ($P < 0.01$)].

Cortisol excess has been shown to cause polyuria in the dog by inhibition of the action of ADH (Baas *et al.* 1984). Marya *et al.* (1987), when subjecting rats to restraint stress, found an elevation of glucocorticoids and a corresponding statistically significant increase in the urinary excretion of water, sodium and calcium but not potassium or magnesium. Wintour *et al.* (1985), after infusing cortisol into cannulated ovine fetuses, observed four-fold increases in sodium and chloride excretion and a doubling in potassium and free water clearance.

2.9 Effects of Feeding Electrolytes and Water on Transport Stress

Liveweight losses incurred during transit were reduced when animals were offered water on arrival, resulting in greater carcass hydration (Wythes *et al.* 1980). The application of electrolyte solutions to minimize transport stress in cattle has been extensively investigated (Hutcheson *et al.* 1984; Gortel *et al.* 1992; Schaefer *et al.* 1992; Phillips, 1997; Schaefer, Jones and Stanley 1997). There is a trend in the literature for statistically significant increases in the ECF of cattle when electrolyte solutions are fed compared to no fluids offered post-transport. The effects of the electrolyte solutions fed in these studies were to replenish lost TBW in the animals involved (Schaefer *et al.* 1990; Schaefer *et al.* 1992; Gortel *et al.* 1992). However, the study by Gortel *et al.* (1992) offered a third group of bulls water only. There were no statistically significant differences in carcass and rumen weights, extra cellular fluid volume, plasma volume, hematocrit, serum sodium, potassium, glucose or β -hydroxybutyric acid between the water and electrolyte fed groups. Lower statistically significant values for plasma osmolality (281.2 ± 2.8 , and 291.8 ± 2.6), serum chloride (97.2 ± 1.4 and 101.3 ± 1.3) and serum lactate (3.42 ± 0.67 and 4.25 ± 0.60) were found between the water and electrolyte groups, respectively. This was a

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reflection of the amount of fluid consumed post-transport between the treatments (water, 29.7 liters \pm 3.9 and electrolyte 17 liters \pm 3.4 per animal).

Apple *et al.* (1993) found no significant differences in a similar experiment in sheep offered either distilled water or an electrolyte solution, and subjected to restraint and isolation stress. They concluded that electrolyte administration had no appreciable effect on pituitary – adrenal secretions or any other measured component of blood. Ruppanner *et al.* (1978), looked at the electrolyte profile of shipped calves in feedlots found it difficult to associate any difference in the mean values for sodium, potassium and chloride between electrolyte treated and control calves. In Australia, Phillips (1997) offered an electrolyte supplement to mixed cattle being transported over 1500 kilometers to slaughter. In all three trials there were no significant differences in liveweight loss or carcass weight between the supplemented groups and cattle that drank normal water supplies.

The experimental design of these trials relied on feeding an electrolyte solution post-transit / stress, with the exception of the experiments of Apple *et al.* (1993) and Phillips (1997). If stress and dehydration induce a sodium diuresis in ruminants, conducting further experiments utilizing electrolyte mixes alone pre-transport may exacerbate the level and onset of dehydration.

Ross *et al.* (1994), observed a significant linear increase in feed intake and subsequent average daily gain with increasing dietary electrolyte balance [mEq (Na +K) – mEq (Cl)]. This was achieved with increasing sodium content in the diet. The intake of water was not reported. Church (1971) citing Riggs *et al.* (1953) stated the quantitative effects of NaCl on the intake of water indicates that added salt may increase water consumption from 22-100% over no salt addition. For a given feed ration and ambient temperature, water intake is related to feed consumed (Church 1971). Data from other workers suggests that the time and stage of recovery is associated with the level of fluid consumed (Whythes *et al.* 1980; Gortel *et al.* 1992). The interrelationships between salt (NaCl) intake, water intake and feed intake in live export cattle need to be investigated post-transit/stress during the rest and recovery stage prior to boarding ships for transporting.

2.10 Impact of stress on animal metabolism

All living organisms require a continuous supply of energy to cover energy expenditure. The supply of energy to the tissues of the body is derived from anaerobic glycolysis and aerobic breakdown of the main energy substrates glucose and free fatty acids (FFA). Circulating glucose and FFA are derived from the fairly large stores of glycogen in the liver and muscle, and from the large amounts of triglycerides in fat tissues, respectively.

Blood glucose levels must be accurately defended as both hyperglycemia and hypoglycemia lead to pathological states. When blood glucose falls below 65 mg/dl nervous tissue does not function properly because glucose uptake by the tissue is hampered. Hyperglycemia (blood glucose levels above 140 mg/dl), causes glycosylation of cell membrane proteins leading to dysfunction of numerous membrane processes (Steffens and De Boer 1999).

Deviations in FFA do not have such serious consequences and are therefore not defended so accurately. However, a chronically elevated level of FFA can lead to cell membrane damage. In the stressed state, peripheral glucose uptake is increased even if plasma glucose is low (Steffens and De Boer 1999).

2.10.1 Regulation of Glucose and Free Fatty Acids

In principle, glucose and FFA turnover are closely linked because an increase in blood glucose and plasma insulin leads to increased lipogenesis and glycogenesis, whereas a decrease in glucose and insulin results in lipolysis and glycogenolysis. During times of food deprivation and exercise, three lines of defence challenge hypoglycemia.

The first of these consists of suppressing insulin release from the B-cell and enhancing glucagon release from the A-cell of the islets of Langerhans. Insulin and glucagon released from the pancreas act on the liver as their first target. Suppression of insulin inhibits glucose uptake via the insulin dependant glucose transporters. In addition, glycogenesis in the liver is inhibited by the inactivation of liver glycogen

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synthase which is converted from the active D form to the inactive I form. Glucagon converts inactive phosphorylase-b to active phosphorylase-a, causing glycogenolysis. The liver contains the enzyme glucose-6-phosphatase, which converts glucose-6-phosphate to glucose unlike muscle tissue and enables the release of glucose into the blood stream. Glucagon is the major stimulus for gluconeogenesis (Steffens and De Boer 1999; Guyton and Hall 2000).

The sympathetic nervous system can be considered to act as the second line of defence. The sympathetic nervous system consists of two branches; (1) the neural branch and the cardiovascular system and, (2) the adrenal medullary branch (SAM). Measuring plasma noradrenaline and adrenaline concentrations can assess activation of both branches of the sympathetic nervous system. Norepinephrine and epinephrine stimulate liver glycogenolysis by direct stimulation of α_1 -adrenoceptors present in the parenchymal cells and indirectly by the prostaglandin released by the non-parenchymal cells after stimulation by the α_1 -adrenoceptors. Lipolysis in fat cells is stimulated by activation of β_3 -adrenoceptors in the adipocyte membrane. Noradrenaline has a higher affinity for β_3 -adrenoceptors than adrenaline.

The third line of defence consists of activation of the HPA axis leading to increased corticosteroid release. Growth hormone also plays a role in glucose counter regulation (Steffens and De Boer 1999).

2.10.2 Pathology of gastrointestinal system affected by stress

The loss of appetite associated with chronic stress results in pathological changes to the gastrointestinal tract, namely the intra-cellular storage of secretory products in the exocrine and endocrine cells of the system and cellular involution and loss of epithelial cell types. These changes lead to a decrease in the absorptive and secretory capacity of these cell types.

Although these changes are typical of low feed intake or starvation in general, stress induces further pathological changes in the intestinal and gastric mucosa, in particular, mucosal erosion and atrophy, hyperkeratosis, and ulceration. These

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pathological changes have been known to occur in humans, chickens, cattle, pigs and horses (Selye 1976; Pfeiffer 1992; Mitchell and Carlisle 1992; Rebhun 1995; Bonga and Balm 1999).

Bonga and Balm (1999), quoting Hessing *et al.* (1992), stated that in domestic pigs the incidence of gastric ulceration increased from 8% in controls to 40% in animals after transport and mixing. Rebhun (1995) stated abomasal ulcers are a common clinical problem in dairy cattle and calves but pathogenesis is more related to intensive management and highly acidic diets with a majority of cases presenting themselves at 4 – 6 weeks post calving, which coincides with the highest degree of stress on the animal. Mitchell and Carlisle (1992) demonstrated in chickens that heat stress decreased feed intake and growth rate with an associated decrease in villi height and wet and dry mass per unit length of jejunum.

2.10.3 The effect of stress on immunocompetence

Transportation, as well as some environmental and management situations, may be stressful to ruminants, and it is generally agreed that such impositions can result in a compromised function of the immune system (Lan *et al.* 1995). There is evidence in the literature of increased morbidity and mortality due to infectious diseases up to 4 weeks following transportation of feeder calves (Knowles 1995). The increased incidence of morbidity has frequently been attributed to stress-induced suppression of the host's immune system making the animal more susceptible to pathogens (Mackenzie *et al.* 1997).

Transportation stress studies have demonstrated significant associations between elevated plasma glucocorticoid concentrations and a suppressed immune system. This has resulted in the belief that cortisol is the primary agent in mediating the negative effects of stress on the immune system (Crookshank *et al.* 1979; Roth, Kaeberle and Hsu 1982; Murata and Hirose 1990). Although glucocorticoids have been demonstrated to have immunosuppressive effects on ruminants (Roth and Kaeberle 1982; Roth, Kaeberle and Hsu 1982), other factors may also play a role in immuno-suppression following the application of a stressor. These have been implicated as an interdependence between the central nervous system and its

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activation of the SAM and HPA axes, (Minton *et al.* 1995), level of nutrition (Pollock *et al.* 1993) and, neutrophil numbers in the peripheral blood (Murata and Hirose 1990).

Total and differential white blood cell counts have been used to evaluate immunosuppression in transported ruminants (Blecha *et al.* 1984; Murata *et al.* 1987; Murata and Hirose 1990). The classic white blood cell profile of a stressed animal results in the 'stress triad' leukogram consisting of a leukocytosis, neutrophilia and lymphopenia (Lan *et al.* 1995). The calf responds to transportation stress by increasing the total population of leukocytes. Kegley *et al.* (1997) and Richardson *et al.* (2002) reported increases in total white cell population for 263 kg and 490 kg steers, respectively, immediately following transportation as compared with non-transported controls.

The neutrophil: lymphocyte ratio is often reported as indicative of an animal suffering from stress. Typical ratios for unstressed cattle are 1: 2, while in a stressed animal, increased neutrophil counts and decreased lymphocyte counts may result in ratios of 2: 1 (Richardson *et al.* 2002).

There are marked species differences in susceptibility to glucocorticoid hormones. Roth and Kaberle (1982) suggest that cattle are relatively resistant to glucocorticoids on the basis that glucocorticoid-induced lymphopenia in cattle is not as profound or as easily produced.

2.10.4 The effects of stress on liveweight

Chronic stress is typically associated with increased energy demand as well as reduced appetite. This leads to a depletion of energy stores, namely liver glycogens and body fat (Hutcheson and Cole 1986; Bonga and Balm 1999). Adrenaline is the major glycogenolytic factor during stress, with glucocorticoids in a supportive role.

Glucocorticoids stimulate glyconeogenesis at the expense of body proteins, and together with CRH, they are central switches in reallocating energy sources away from productive processes such as reproduction and growth toward functions

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promoting immediate survival. This leads to a reduction in body mass, in particular a decrease in the mass of the liver, fat tissue, striated musculature and gonads (Bonga and Balm 1999).

Liveweight loss has been shown to increase with fasting time, and although this relationship is not linear, maximum liveweight loss occurs in cattle within 24 h (Whythes *et al.* 1980; Smith *et al.* 1982; Jones *et al.* 1988). Tennessen *et al.* (1984), quoting Monin and Royant (1980), stated that the incidence of dark cutting in beef bulls increased with the time elapsed between departure from the feedlot and arrival at the abattoir.

Knowles *et al.* (1999) illustrate that the physiological changes taking place in cattle being transported by road indicates that the animals coped with a journey of up to 31 h. However, it highlighted the fact that as the journey continued past 14 h, plasma osmolality and urea concentrations progressively increased, suggesting that the animals were becoming dehydrated and suffering increasing protein breakdown. It was of significance in this study, and that of Tarrant *et al.* (1992), that animals chose to lie down after 20 h, presumably because the animals were becoming fatigued. This is supported by the elevated cortisol, urea and creatinine kinase activity. Sinclair *et al.* (1992) stated that long distance haulage does not appear to stress cattle any more than short distance haulage. Sinclair *et al.* (1992) must have been referring to psychological stress as they reported liveweight losses, dehydration and perhaps exhaustion to be greater at the end of the journey, compared to the control group.

There is a trend in the literature for slower rates of liveweight loss to be reported when water is available during fasting than when animals were feed and water deprived (Figure 2.3). Truscott and Gilbert (1978) found liveweight loss to be higher in cattle fasted for five days without water than in those supplied with water. Smith *et al.* (1982), found that transport with fasting resulted in a carcass weight loss relative to fasting alone. They suggested that transport stresses may cause a reduction in carcass weight via loss of moisture or tissue catabolism which may not be detectable in terms of liveweight, or was perhaps negated by a reduced weight loss of non-carcass components (either gut fill or offal) in the transported steers.

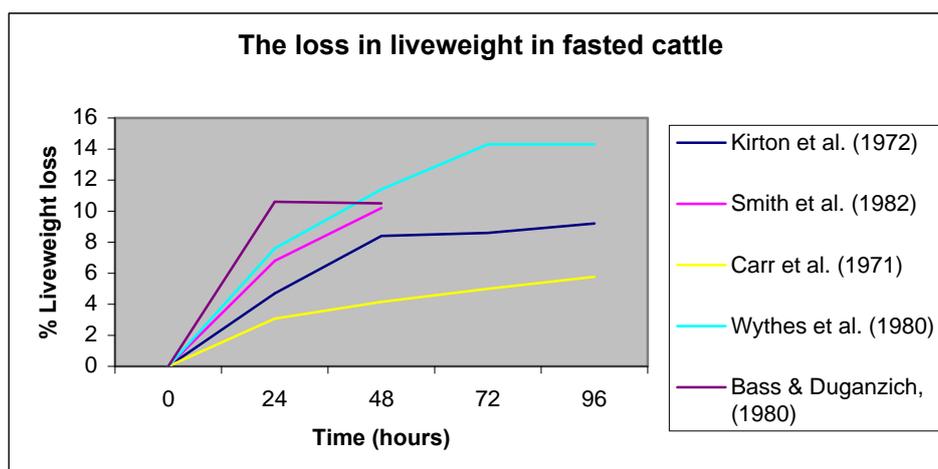


Figure 2.3. The loss in liveweight in fasted cattle. Adapted from: Warriss (1990).

2.10.5 Changes in Body composition

Large losses in liveweight within 24 h of fasting and transportation have been attributable to urination and defecation, as well as some carcass loss (Whythes *et al.* 1980; Jones *et al.* 1988). During transportation by rail and fasting, Heever *et al.* (1967) found loss of liveweight due to elimination of ingesta did not exceed 5%, in any of their treatment groups. Total liveweight loss in this trial ranged from 11.6% to 13.1% throughout the groups suggesting that the remaining 6 – 8% of loss was due to dehydration.

Schaefer *et al.* (1990) suggests that carcass tissues, rather than red blood cells, were being dehydrated in response to increasing time off feed and water. They argued that although plasma volume decreased, dehydration of the red blood cells *per se* was not occurring, as mean cell volume and mean cell hemoglobin content did not change significantly with time off feed and water. This agrees with Haussinger *et al.* (1993), who state that in humans there is evidence to suggest that cellular hydration state, particularly in liver and skeletal muscles, is an important factor in controlling cellular protein turnover. Protein synthesis and degradation are affected in opposite directions by cell swelling and shrinking. An increase in cellular hydration acts as an anabolic proliferative signal, whereas cell shrinkage is catabolic.

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Starvation has been shown to reduce the weight of edible meat and excess fat in cattle, as well as offal weights. Kirton *et al.* (1972) demonstrated that reductions in liveweight could not be accounted for by tissue dehydration alone. Statistically significant losses occurred in empty stomach weight, liver weight, omental and perinephric fat depot weights with increasing time fasted – up to eight days. Fat was reduced more quickly than carcass weight and edible meat percent was unchanged in the carcass of starved cattle indicating that muscle decreased in weight at the same rate as the reduction in carcass weight. Truscott and Gilbert (1978), when measuring fat depth at the 12th/13th rib site with ultrasound during fasting derived a regression equation (rate of change of rib fat depth = 0.33 – 0.065 * initial fat depth (mm)) which indicated that below 5 mm of rib fat there was a positive rate of change of fat depth, i.e., fat was deposited.

2.10.6 Rest and recovery periods

In Northern Australia, cattle must travel large distances from property of origin to export depot, sale yard or abattoir. Under these conditions cattle are sometimes unloaded during their journey to rest, eat, drink and exercise. The resting period allows time for animals to recover from the stressors associated with transportation, to adapt to their new environment and to replenish metabolites needed for homeostasis (Wythes *et al.* 1988).

Some studies show little or no detrimental effects on the metabolism or health of animals during transportation (Todd *et al.* 2000). However, relatively high mortality and morbidity rates during the weeks that follow the transport event suggest that significant detrimental effects related to the transport stress can subsequently develop and delay the recovery period (Cole *et al.* 1988; Knowles 1995).

Knowles *et al.* (1999) demonstrated that a rest period of 24 h in lairage provided an adequate, although not complete period of recovery for animals transported from 14 to 31 h. Level of hydration, total protein, and osmolality returned to pre transport levels after 36 h of recovery. However, the animal's liveweight had not returned to pre-transport levels after 72 h of rest. In other reports transported feeder calves did

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compensate for liveweight loss by day 56 in a feedlot, with no difference in liveweight or daily gain after this time (Crookshank *et al.* 1979; Cole *et al.* 1988).

2.10.7 Injuries, Death and Disease from Transport

Reactions of susceptible animals to handling and transportation encountered during the marketing process can result in defects in the ultimate meat quality, such as dark cutting beef and pale soft exudative pork (Pearson 1977). More severe physiological responses include a susceptibility to transport tetany (Martens and Schweigel 2000) and the development of the shipping fever disease complex (Hutcheson and Cole 1986).

The death rate in cattle being transported by road or rail varies between different geographical regions. Deaths among cattle transported by rail and road in Queensland have been estimated to be less than 0.25% and 0.001%, respectively (Lapworth 2001). This figure referred to cattle loaded in good condition and at satisfactory densities. Higher losses resulted from loading too loosely or densely, if stock were in poor condition (as in drought), or from inexperienced or careless drivers and handlers.

The death rates for sea voyages are relatively low with overall death rates ranging from 0.05% to 0.16% in short voyages to South East Asia (3 - 7 d), and 0.35% to 0.69% in longer voyages to the Middle East (21 d) (Norris *et al.* 2003). Norris *et al.* (2003) observed that *Bos indicus* animals had lower death rates overall and a greater tolerance of the high humidity and heat experienced on voyages than *Bos taurus* cattle. Heat stroke was the most frequently recorded cause of death with all of these deaths in *Bos taurus* genotypes. They further noted that there were no deaths from pneumonic pasteurellosis (Shipping Fever) in the first few days after embarkation, and argued that this reflected pre-embarkation stress being kept to a minimum during collection on property, transport to the wharf and loading onto the ship (Norris *et al.* 2003).

It is important to note that most cattle do not die from transport *per se*, but they are weakened by the process and usually die from secondary causes several days to

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weeks after the event (Knowles 1995). Calves transported for 12 h had higher morbidity, and mortality, became ill earlier and became ill or died during a longer period than did the control calves (Cole *et al.* 1988). Staples and Haugse (1974) related five factors to be closely related with death and sickness in calves that had been transported. They were: age when purchased; origin of purchase; time of year; type of feed used to start calves; and genotype. Staples and Haugse (1974) also observed that pneumonia was reported to be the most frequent cause of death followed by scours.

Less severe, but widespread and costly are the weight losses, and in some circumstances, the bruising attributable to ante-mortem stress (Pearson 1977). Yeh *et al.* (1978) demonstrated that a positive regression relationship ($y = 0.3 + 0.16x$; $r = 0.53$; $P < 0.01$) existed between time of journey and bruise trim weight in cows but not for bullocks. They suggested that fatigue may have resulted in a reluctance of these animals to move out of the way of other animals or objects and thus increased the likelihood of bruising.

Wythes *et al.* (1988), suggested that the cumulative fatigue of a long rail journey (965 km) was more detrimental in terms of bruising than the handling associated with unloading and re-loading *enroute* at a pre-determined resting point. However, for cows, two resting periods were as harmful in terms of bruising as a non-stop journey, reflecting extra handling at unfamiliar yards

There is a belief among pastoralists that fasting prior to transport contributes to bruising. Poorly constructed and unfamiliar yards, re-grouping of animals in confined spaces, mixing bulls and steers, and mixing horned and hornless cattle have been advocated as inciting causes for an increase in bruising (Blockey and Lade 1974; Dodt *et al.* 1979; Wythes *et al.* 1988). Dodt *et al.* (1979), substantiated this claim demonstrating the weight of bruise trim from carcasses of unfasted bullocks to be significantly less ($P < 0.01$) than from carcasses of bullocks fasted for 24 or 48 h prior to transport for slaughter. The lower level of bruising in the unfasted animals was believed due to insufficient time to establish a social order before transporting.

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Barnett *et al.* (1984) hypothesized that the susceptibility to bruising was associated with the physiological state (stress responsiveness) of the animal. They suggested that chronic stress suppressed the adrenocortical response to the short term stressors of handling and transport, which in turn increased the susceptibility to bruising. The data produced in this manuscript for bruising amount and corticosteroid response for the groups failed to support this hypothesis. The author's discussion was based on statistically significant ($P < 0.05$) values for the basal (1.6, 1.2 and 1.1 ng/ml) and peak (1.7, 1.5 and 1.5 ng/ml) levels of corticosteroid between the chronic, acute and control groups respectively to restraint stress. However, the response between basal and peak values for all groups (0.1, 0.3 and 0.4 ng/ml) may suggest an adaptive response by the chronic stressed group and not inhibition as the authors argue.

2.10.8 Sex differences in coping ability

Tenessen *et al.* (1984) compared the response of 15 to 17 month old bulls and steers to trucking for two h and found neither sex was particularly disturbed by the experience. However, in this trial animals were shipped in the company of pen mates. If re-grouping of unfamiliar cattle were carried out then considerable differences would have been expected due to the stress in establishing new social relationships.

Kenny and Tarrant (1987b) showed little response to cortisol level in steers when confined on a stationary truck, compared with a previous study with bulls which showed a substantial increase in cortisol levels (Kenny and Tarrant 1987a). These authors suggested that this was due to a lower resting cortisol level of bulls compared to steers (2.8 vs. 9.0 ng/ml). Tennessen *et al.* (1984), quoting Moberg (1983) stated that castration probably leads to loss of feedback effects between the pituitary adrenal and pituitary gonadal axes. The deviations in normal steroid synthesis would alter the steroid balance of castrated animals and perhaps explain the higher serum cortisol levels seen here.

2.11 Conclusion

The physiological changes that occur within cattle during the transport and handling phase of their export journey may predispose the animals to a higher level of stress on board ship, resulting in a loss of appetite and immuno-suppression (Hutcheson and Cole 1986). The metabolic changes that occur in the animal due to stress may induce a catabolic state and contribute to a lack of growth of tissues (Haussinger *et al.* 1993). Haussinger *et al.* (1993) and Lang *et al.* (1998) provide evidence that cell volume is a regulator of catabolism of tissues, especially muscle and liver cells. It is hypothesized that maintaining eu-hydration in cattle will alleviate the catabolic processes acting on tissues from stressors and dehydration. Stress and dehydration in cattle and other species induces a sodium diuresis that places a greater strain on the animal's homeostatic mechanisms (Bianca *et al.* 1965; El Nouty *et al.* 1980; McKinley *et al.* 1983; Baas *et al.* 1984; Wintour *et al.* 1985; Beede and Collier 1986; Metzler *et al.* 1986; Marya *et al.* 1987).

This literature review has addressed the hypothesis that stress in cattle may manifest itself in a relationship between the principal stress hormone – cortisol, and the level of hydration of the animal. Studies have recognized the need to minimize stress caused by transport and handling by hydrating the animals involved with water, (Whythes *et al.* 1980) and with electrolyte solutions (Hutcheson *et al.* 1984; Gortel *et al.* 1992; Schaefer *et al.* 1992; Phillips, 1997; Schaefer, Jones and Stanley 1997). It has been recognized by Phillips (1997) that the electrolyte solutions fed by Schaefer *et al.* (1992) and Gortel *et al.* (1992) contained amino acids and that this may have contributed to Phillips' lack of significant data. Some amino acids are used by cells as osmolytes in cell volume regulation and as cell protectants, but amino acid uptake into cells is stimulated by cell shrinkage.

Glycerol and betaine may be taken up by cells prior to the onset of shrinkage and thus may impose a protective effect on the hydration and thermal status of the animal. To date, no studies have employed the use of osmolytes alone to minimize dehydration and the effects of stress in cattle. This thesis will investigate the effects of these osmolytes on transport and handling stress in cattle. Finally it is worth noting that there is a lack of data which quantifies the physiological response of

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cattle to transportation stressors, in particular transport stress and the associated dehydration imposed upon the animal.

CHAPTER THREE

DEHYDRATION IN STRESSED RUMINANTS MAY BE THE RESULT OF A
CORTISOL INDUCED DIURESIS

ABSTRACT: The effect on water and electrolyte balance of stress, simulated by intravenous infusion of cortisol, was studied using 24, 18 mo Merino wethers (37.0 ± 0.94 kg mean body weight) over 72 h. The sheep were allocated to one of four groups: 1) no water/no cortisol ($n = 6$); 2) water/no cortisol ($n = 4$); 3) no water/cortisol ($n = 6$); and 4) water/cortisol ($n = 4$). Animals allocated to the two cortisol groups were given $0.1 \text{ mg} \cdot \text{kg BW}^{-1} \cdot \text{hr}^{-1}$ of hydrocortisone suspended in isotonic saline, to simulate stress for the duration of the experiment. TBW, plasma cortisol, osmolality and electrolytes and urine electrolytes were determined at 3 hly intervals for 72 h. In the presence of cortisol, TBW was maintained in the face of a water deprivation insult for 72 h. Water deprivation alone did not induce elevated plasma concentrations of cortisol, in spite of a 13% loss of TBW between 48 and 72 h. Infusion of cortisol was found to increase urine output ($P = 0.003$) and decrease total urinary sodium output ($P = 0.032$), but had no effect on plasma electrolyte levels or water intake. Water deprivation was found to increase plasma sodium concentrations ($P = 0.037$). These results indicate that sheep given cortisol to stimulate stress suffer from a loss of body water in excess of that associated with a loss of electrolytes and support the hypothesis that elevated physiological concentrations of cortisol induce a diuresis in ruminants that contributes to dehydration.

3.1 Introduction

Researchers have endeavored to discover the physiological changes that occur when animals are exposed to stressors by utilizing models that mimic the effects of the HPA axis. The HPA axis, when activated by stressors such as transport and handling, responds with the release of glucocorticoids and other hormones which have physiological effects. Cortisol is the principal stress hormone associated with the activation of the HPA axis, and has been shown to induce pathophysiological changes to the immune (Roth and Kaeberle 1982), metabolic (Sapolsky Romero and

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Munck 2000) and reproductive (Macfarlane *et al.* 2000) systems of animals. Water deprivation has been touted as a significant stress stimulus in the transportation and handling of ruminants (Atkinson 1992) and that this contributes to an elevated stress response by the animal (Matthews and Parrott 1991).

Swanson and Morrow-Tesch (2001) highlighted the need for a valid model system for evaluating the physiological effects of transport stress in ruminants. Lay *et al.* (1996) proposed a stress response model based on an ACTH challenge test but failed to accurately predict physiological disturbances seen in cattle subjected to transport and handling stress. Other authors have utilized glucocorticoids; Anderson *et al.* (1999), used dexamethasone to quantify the effects of potential stressors on immune competence in ruminants and Macfarlane *et al.* (2000) utilized stress-like infusions of cortisol to model reproductive responses to stressors in Merino sheep.

The application of the Macfarlane *et al.* (2000) model to test the effects of stress-like infusions of cortisol on water and electrolyte balance has yet to be investigated.

Thus, this study was conducted to test the hypothesis that elevated plasma concentrations of cortisol induce a diuresis that contributes to water loss in excess of electrolyte loss in Merino sheep.

3.2 Materials and Methods

3.2.1 Animals and management

Twenty four, 18 mo Merino wethers (37.0 ± 0.94 kg mean body weight) were sorted in ascending order of body weight, allocated to metabolism crates at random and fed oaten chaff *ad libitum* for 10 d prior to the commencement of the experiment. Upon entry to the crates, all animals were dosed with Ivermectin (Ivomec-RV, 1 mL/10 kg BW) (Merial Australia Pty Ltd, Parramatta, NSW, Australia) and their necks and pizels were shaved. A temperature and humidity index (THI) was calculated by the formula presented by Gaughan *et al.* (1999). The mean daily (THI) during the experimental period for days 0, 1, 2, 3 were 76, 76, 77 and 77, respectively. There was no significant difference between THI for the acclimatization period or the experimental period.

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All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (Approval No. A664-01).

3.2.2 Treatments

Crate numbers were assigned at random, in a 2 x 2 factorial arrangement, to one of four groups: 1) no water/no cortisol (n = 6); 2) water/no cortisol (n = 6); 3) no water/cortisol (n = 6); and 4) water/cortisol (n = 6). On day 0, all animals were catheterized with a poly-vinyl chloride tube (OD 2.0mm x ID 1.0mm; Critchley Electrical Products Pty Ltd, Silverwater, NSW, Australia) inserted into the jugular vein under local anaesthetic. Urine collectors were also fitted to the animals. All animals allocated to the two cortisol groups were given $0.1\text{mg}\cdot\text{kg BW}^{-1}\cdot\text{hr}^{-1}$ of hydrocortisone (Solu-Cortef[®], Upjohn Pty Ltd, Rydalmere, NSW, Australia) suspended in isotonic saline administered at a rate of $0.1\text{mL}\cdot\text{kg BW}^{-1}\text{hr}^{-1}$, to simulate stress for the duration of the experiment as per Macfarlane *et al.* (2000). The non-cortisol groups were given an equivalent placebo infusion of isotonic saline. Animals that were in water deprived groups had their water withdrawn for 72 h.

3.2.3 Sample collection

On day 0, 10 mL of blood was manually collected from all treatment groups into lithium heparin containing tubes (Disposable Products Pty Ltd, Adelaide, SA, Australia) and continued at 3 h intervals for 72 h. Intakes of water were measured daily by weighing the animals drinking container pre and post-daily intake. Feed intake was measured by weighing all feed into the animals feed container and weighing total feed rejected daily. Total urine excreted was collected, measured and sub-sampled (200 mL) daily for three days during the study. Urine samples were stored at -20°C until they were analyzed. Blood samples were immediately placed into an ice water slurry then centrifuged at $200 \times g$ for 15 min and plasma poured off within two h and frozen (-20°C) for analysis at a later date. Plasma cortisol concentration was measured using a radioimmunoassay kit (Spectria Cortisol ¹²⁵I coated tube kit, Orion Corporation, Espoo, Finland).

3.2.4 Urea space measurements

Urea space was used as a measure of total body water in all animals. Urea space was determined on days 0, 1, 2, 3 for each animal using the technique described by Preston and Kock (1973). In brief, following catheterization of the jugular vein, a solution containing 20% (wt/vol) urea dissolved in 0.9% (wt/vol) saline was administered through the catheter over a 2 min period. The volume injected was calculated to provide 130 mg urea/kg liveweight. The catheter was flushed with 10 mL of isotonic saline followed immediately by 10 mL heparinized saline solution (35,000 I.U./mL 0.9% saline) to prevent clotting between samplings. Blood samples were collected through the catheter prior to infusion and at 15 min post-infusion. The following formula was used to calculate urea space as a percentage of liveweight (Kock and Preston 1979):

$$\text{Urea space \%} = \frac{[\text{Volume infused (mL)} * \text{concentration of solution (mg urea-N/dL)}]}{[\text{Plasma Urea Nitrogen / liveweight in kg}]}$$

TBW was recorded as the pool available to the urea molecule.

3.2.5 Urea and Electrolyte measurement

Plasma urea nitrogen was analyzed with a Technicon Auto-analyzer 2 (Bran + Leubbe Pty Ltd, Homebush, NSW, Australia) according to the Technicon auto-analyzer method SE40001FD4. Analysis of Na, K and Mg in sheep plasma and urine samples were conducted using a Liberty Series 2 inductively coupled plasma atomic emission spectrometer, (Varian Australia Pty Ltd, Melbourne, VIC, Australia).

3.2.6 Statistical analysis

A 2 x 2 factorial arrangement with the main effects for water (*ad libitum* water and no water) and cortisol (cortisol infusion and no cortisol), and the interaction effects of water x cortisol with time taken into account were analyzed statistically with a repeated measures ANOVA using Statistical Package for the Social Sciences (SPSS 10[®]) software package (SPSS 2001). Quantitative variables (plasma and urinary electrolytes, plasma cortisol, TBW and urine output) were independently sampled.

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Tests for sphericity and homogeneity were conducted to test assumptions for the repeated measures ANOVA, and in all cases these tests were satisfied. Arithmetic means and standard errors have been presented and multiple comparison tests within the factors were not performed because there were fewer than three groups and therefore any difference would be clearly perceived. Differences were considered significant for $P < 0.05$. Four animals had to be withdrawn from the experiment, one for scours and three for blocked catheter lines.

3.3 Results and Discussion

3.3.1 Plasma cortisol concentration

Macfarlane *et al.* (2000) maintained plasma cortisol concentration at 72.0 ± 2.5 ng/mL to simulate stress in sheep. The infusion rate chosen in this study appears to offer a physiological dose rate, when mean plasma cortisol concentrations (Figure 3.1) are compared to those found in sheep exposed to the stress of isolation and restraint (70 ng/mL) (Apple *et al.* 1993), shearing and shearing noise (78.8 ng/mL and 58.1 ng/mL) (Hargreaves and Hutson 1990), and handling stress prior to slaughter (22.0 to 77.8 ng/mL) (Pearson *et al.* 1977). Of note is the fact that water deprivation alone for 72 h in the no water/no cortisol group did not increase plasma cortisol concentration to the same levels as reported by other authors (Pearson *et al.* 1977; Hargreaves and Hutson 1990; Apple *et al.* 1993). Others have touted water deprivation as being a significant stressor in the marketing process for ruminants (Atkinson 1992). The lack of cortisol response between the water/no cortisol and no water/no cortisol group may be due to the animals having been derived from a population in the seasonally dry tropics in which water deprivation for 72 h, to a well hydrated animal, with ample water in the gastro-intestinal tract, may not be a significant stressor. Similarly, Finberg *et al.* (1978), found no significant change in plasma cortisol concentration throughout eight days of water deprivation in the camel. It would appear that water deprivation alone for 72 h in merino sheep is not a prototypical stressor that will activate the HPA axis. However, a HPA axis response may be invoked at an increased time of water deprivation. Blair-West *et al.* (1972) demonstrated a significant rise in plasma cortisol concentrations in sheep after nine days of water restriction.

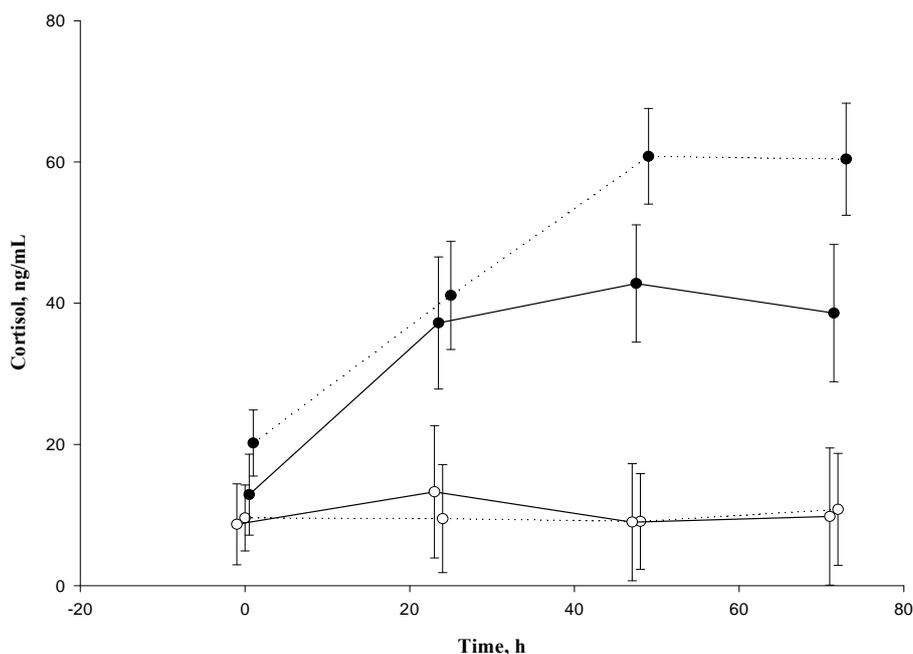


Figure 3.1. Plasma cortisol concentrations (mean \pm SEM) at 0, 24, 48 and 72 h for four groups of sheep in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

3.3.2 Body Water

There was no change in body water (Figure 3.2) within any group at 0, 24 or 48 h. The no-water/no cortisol group sustained body water at 24 and 48 h ($52.2\% \pm 4.2\%$ and $53.2\% \pm 5.1\%$, respectively) before losing 13% by 72 h ($40.2\% \pm 5.7\%$). A time \times water interaction demonstrated body water loss for the no water groups between 24 and 48 h ($P = 0.034$) and 48 and 72 h ($P = 0.052$), compared to the groups on *ad libitum* water. Preston and Kock (1973) concluded that urea space in the ruminant was a measure of empty body water (TBW less the water in the gastrointestinal tract). The lack of reduction in body water for the water/cortisol group in spite of the presence of a diuretic effect may be due to the replacement of water in the urea space of the animal with water from the gastrointestinal tract, in a bid to maintain homeostasis in the face of a net water deficit. Alternatively, TBW may not be a good

estimate of total body water.

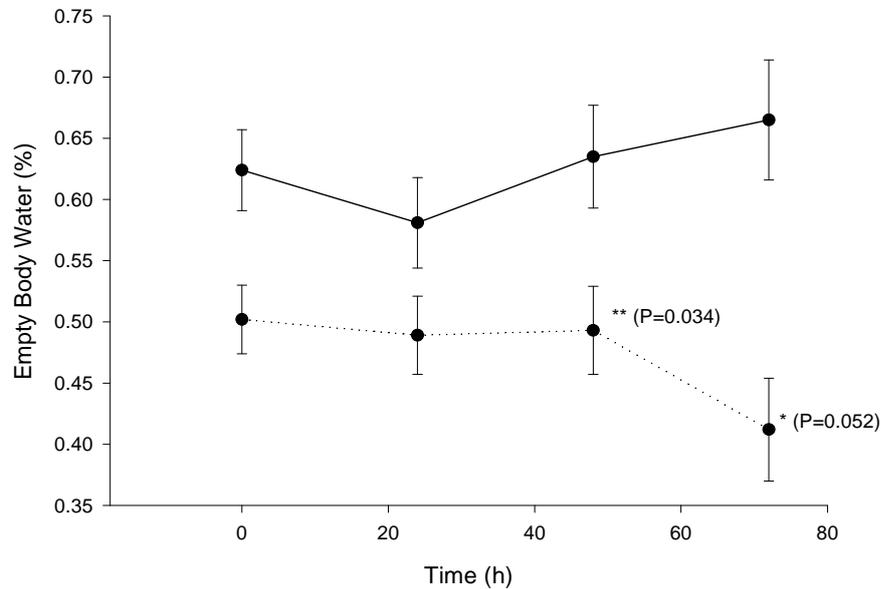


Figure 3.2. Empty body water (mean \pm SEM) at 24, 48 and 72 h for two groups of sheep which were either water deprived (dotted line) or given ad libitum access to water (solid line).

3.3.3 Urine output

There were cortisol x water x time ($P = 0.037$) and cortisol x time ($P = 0.003$) interactions, between 24 and 48 h, demonstrating an increase in urine output for the water/cortisol group over the other groups during the same period (Figure 3.3). This interaction was not significant at the 48 to 72 h interval for the water/cortisol group, although a trend ($P = 0.07$) toward increased urine output continued for this group. One of the proposed avenues of weight loss in domestic animals placed under stress is an increase in urination (El Nouty *et al.* 1977; Hutcheson and Cole, 1986; Kenny and Tarrant 1987a; Phillips *et al.* 1991; Knowles 1999), and it is believed that this increase in urination contributes to dehydration in the animal when water is unavailable (Phillips *et al.* 1991; Atkinson 1992).

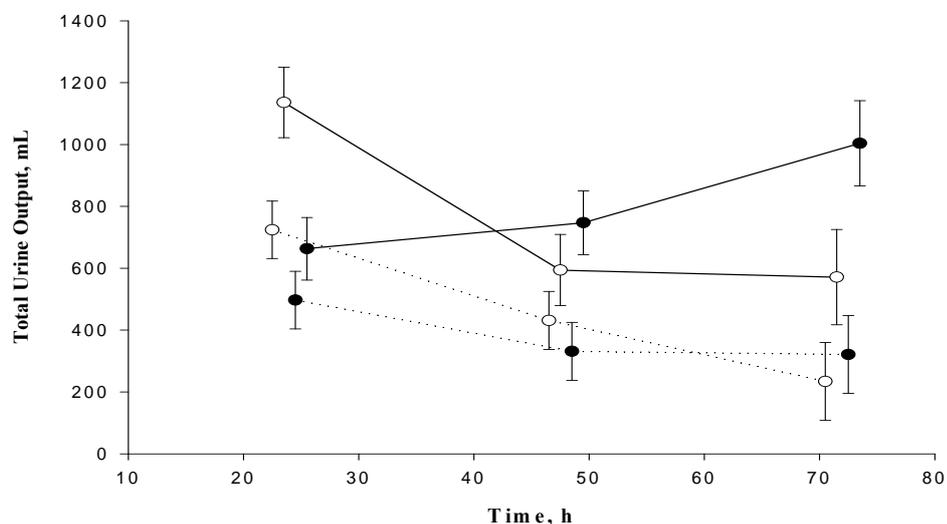


Figure 3.3. Total urine output (mean \pm SEM) at 24, 48 and 72 h for four groups of sheep in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given ad libitum access to water (solid line).

The mechanism by which cortisol induces a diuresis is still under debate. Pharmacological doses of cortisol in the dog (Baas *et al.* 1984) have been reported to induce a polyuria via inhibition in the action of the ADH, AVP. El-Nouty *et al.* (1977), demonstrated in cattle, a significant increase in AVP concentrations during heat stress over thermo-neutral conditions. The increase in AVP in heat stressed cows was not associated with significant changes in urine output or glucocorticoid concentrations. The failure of El-Nouty *et al.* (1977) to detect changes in glucocorticoids to heat stress may lie in their sampling regimen. Cattle subjected to heat stress have displayed rapid increases in plasma corticoid concentration followed by a decline (Lee *et al.* 1974). El-Nouty *et al.* (1977) sampled the animals after 2 d of heat stress where the cows may have adapted to the stressor. Short term isolation stress in sheep by Parrott *et al.* (1987) invoked a similar trend toward a negative

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relationship between cortisol and AVP. High plasma cortisol concentrations were associated with low plasma AVP concentrations.

Glucocorticoids inhibit the vasoconstrictive and water retentive effects of AVP by increasing the glomerular filtration rate (Wintour *et al.* 1985) and increasing the secretion and efficacy of atrial natriuretic peptide, both of which enhance water excretion. This mechanism has been suggested to prevent an overshoot by the vasoconstrictive effects of AVP (Sapolsky *et al.* 2000). This response may explain why the greatest contributing factor to the two-way and three-way interaction involving cortisol seen in the present study was the water/cortisol group which showed the greatest increase in urine output at 24, 48 and 72 h, while the no water/cortisol group appeared to stabilize urinary output at 24, 48 and 72 h. This suggests that stress-like concentrations of cortisol will induce a diuresis if water is available in a bid to prevent hypervolemia and in the absence of water will protect water balance by decreasing urine output. The diuresis could not be explained by polydipsia as both watered groups increased their water intake from 24 to 48 h. However it is likely in this case, i.e. in the presence of *ad libitum* water, that glucocorticoids promoted a diuresis by increasing the glomerular filtration rate (Rang and Dale 1991).

El-Nouty *et al.* (1980), demonstrated a significant decrease in aldosterone concentrations during heat stress in cattle and considered this to be the main factor resulting in the polyuria associated with heat stress. It has been known for some time that repeated treatment with ACTH or glucocorticoids results in a diminished response of the glomerulosa zone of the adrenal gland in a number of species (Coghlan *et al.* 1979). Coghlan *et al.* (1979) demonstrated that prolonged ACTH treatment in the sheep significantly reduced the aldosterone response to known stimulating vectors including angiotensin II (AII) and salt depletion. Sustained stimulation of the HPA axis, as may occur in acute stress, has quite different effects on mineralocorticoids and glucocorticoids.

Stressor stimulation results in the aldosterone response decreasing to normal or even low concentrations within 24 h, whereas cortisol and other glucocorticoid secretions are well maintained. In contrast to the suppressive effects of excessive stimulation of

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the HPA axis on aldosterone secretion, other aldosterone secretagogues (AII and plasma potassium) have specific actions on the adrenal glomerulosa alone, and do not stimulate glucocorticoids. This perhaps explains the sustained and high levels of aldosterone associated with hypovolemic stress, where the renin-angiotensin system is the driving force (Espiner 1987). Although in well hydrated animals elevated concentrations of cortisol induces a diuresis, it would appear from the results of the present study that the principal effect of cortisol on the ruminant body is to protect and maintain water balance in times of stress.

3.3.4 Water and feed intake

High cortisol concentrations associated with stress have been noted to reduce and, in some sheep, cause complete abstinence from drinking (Guerrini and Bertchinger 1982, Parrott *et al.* 1987). The cortisol/water group failed to repeat the responses observed by Guerrini and Bertchinger (1982) and Parrott *et al.* (1987), and demonstrated a time effect, increasing water intake between 24 and 48 h ($P = 0.001$) along with the no cortisol/water group (Table 3.1). There was also a time effect for decreasing feed intake between 48 and 72 h for all groups ($P < 0.001$).

Although mean daily THI increased by 1 unit at 48 h it is doubtful that this would have had a significant effect itself on water or feed intake. In support of a lack of effect of THI on water and feed intake, the THI dropped below 74 for nine h per d allowing nighttime relief and wind speed remained relatively constant at 9 km/h throughout the adaptation and experimental periods. In addition, throughout the adaptation period of 10 d, the animals appeared to settle into their environment and were calm in the presence of the experimenters.

3.3.5 Urinary electrolytes

A cortisol x time interaction for total sodium output ($P = 0.032$) between 24 and 48 h indicated that cortisol treatment resulted in less total daily sodium output in the urine of treated sheep than in non-treated animals (Figure 3.4). There were no differences between groups at 72 h for total urinary sodium output.

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Dehydration has been reported to induce a natriuresis in sheep and other species including cattle (Bianca *et al.* 1965; McKinley *et al.* 1983; Metzler *et al.* 1986).

This increase in sodium content in the urine of dehydrated animals is a homeostatic mechanism that allows sodium balance in the body to be maintained. This study demonstrates that although a natriuresis does occur with water deprivation (no water/no cortisol group), total urinary sodium content excreted per day actually decreases with total urinary volume as dehydration ensues. Studies that have evacuated the bladder of animals post-transport (Schaefer *et al.* 1992), merely illustrate the animal's natriuretic mechanism due to water deprivation at that point in time. To extrapolate these results to promote the use of electrolyte solutions containing sodium in minimizing stressors is physiologically unsound.

Table 3.1. Mean \pm SEM for water and feed intake by the four treatment groups of sheep at 24, 48 and 72 h after stress was simulated by injection of cortisol.

Treatment				
Time, h	No water/no cortisol ^a	Water/no cortisol ^b	No water/cortisol ^a	Water/cortisol ^b
<i>Water Intake, kg/d</i>				
24		2.08 \pm 0.49		2.45 \pm 0.44
48		2.84 \pm 0.31		3.01 \pm 0.28
72		2.58 \pm 0.28		2.49 \pm 0.25
<i>Feed Intake, kg/d as-fed</i>				
24	0.69 \pm 0.13	0.86 \pm 0.14	0.67 \pm 0.14	0.86 \pm 0.14
48	0.49 \pm 0.05	0.73 \pm 0.59	0.48 \pm 0.59	0.83 \pm 0.59
72	0.23 \pm 0.82	0.45 \pm 0.09	0.28 \pm 0.90	0.59 \pm 0.09

^an=6
^bn=4

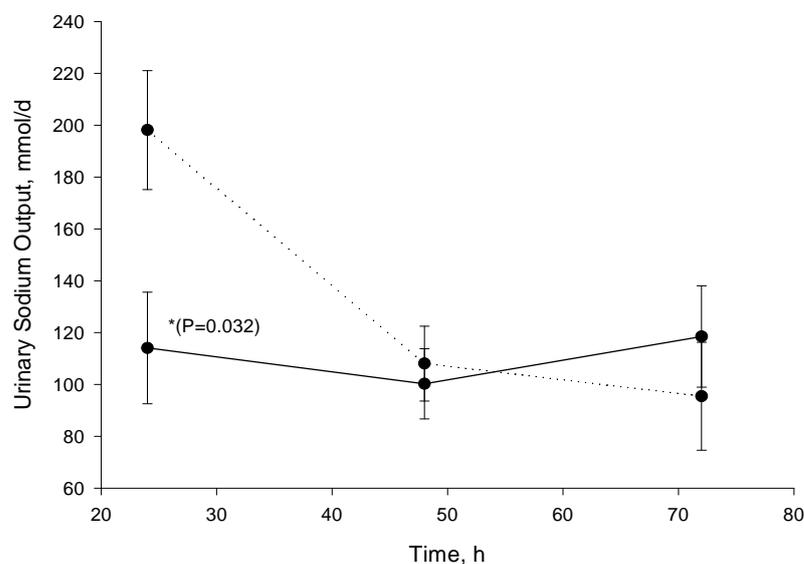


Figure 3 4. Total urine sodium output (mean \pm SEM) at 24, 48 and 72 h for two groups of sheep in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

There was a significant water \times time interaction ($P = 0.044$) for total daily potassium output between 48 and 72 h demonstrating an increase in potassium output with animals given access to water. Urinary potassium output tended to follow a similar trend to daily urine volume output. Water deprivation decreases the glomerular filtration rate of the kidney and, as such, less potassium would be excreted in urine compared to an animal offered *ad libitum* water. A time effect was significant between 24 and 48 h for the water/cortisol group ($P = 0.041$) (Figure 3.5) suggesting an increase in daily potassium output over the other groups. The time effect may be a reflection of the decreased feed intake experienced by the other groups. However, cortisol does cause a degree of potassium loss through two pathways; 1) high physiological concentrations of cortisol can occupy mineralocorticoid receptors and induce mineralocorticoid activity (Rang and Dale 1991), and 2) cortisol has been reported to increase the glomerular filtration rate promoting diuresis (Wintour *et al.* 1985; Rang and Dale 1991).

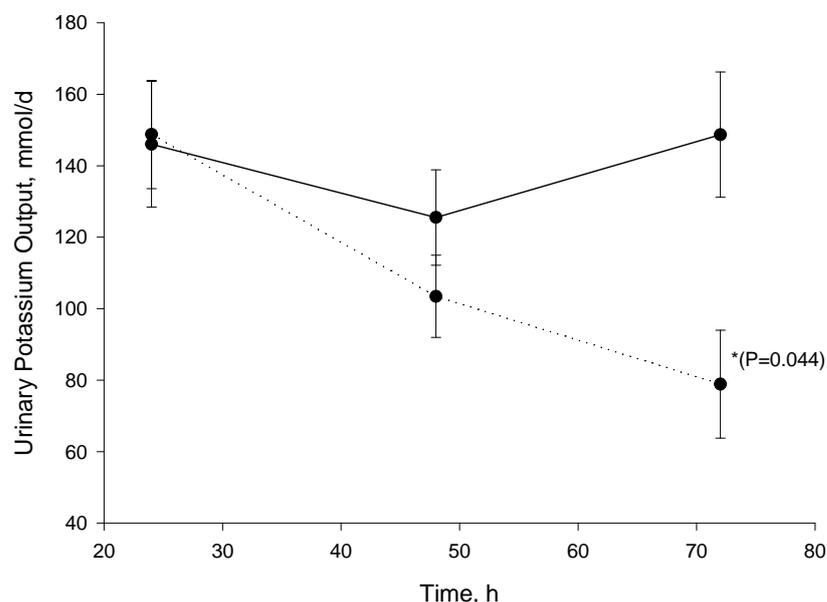


Figure 3.5. Total urine potassium output (mean \pm SEM) at 24, 48 and 72 h for two groups of sheep which were either water deprived (dotted line) or given ad libitum access to water (solid line).

There was a water \times time interaction between 48 and 72 h ($P = 0.016$) for magnesium indicating that animals that were water deprived excreted less magnesium in their daily urine output than did animals which had *ad libitum* access to water (Figure 3.6). A time effect was significant ($P = 0.042$) at 48 h for the water/cortisol group, which had a higher level of daily magnesium excreted in urine over the other groups. The actions of calciotropic hormones are similar for calcium and magnesium and are said to influence magnesium in the kidney, affecting reabsorption (Saris *et al.* 2000). The action of cortisol is said to induce a negative calcium balance by decreasing calcium absorption in the GIT and increasing its excretion by the kidney (Rang and Dale 1991). Although a trend continued for urinary magnesium loss in the water/cortisol group, plasma magnesium was unaffected. Hypomagnesaemia in newly arrived feedlot sheep occurs within 10 d of arrival and is often associated with an increase in water consumption and loss of appetite (Franklin and Macgregor 1944; Lucas 1983). The increased water load must be excreted and if outflow of magnesium exceeds inflow, hypomagnesaemia occurs (Martens and Schweigel

2000). Simulated stress via cortisol infusion failed to have any influence on plasma magnesium concentrations over 72 h.

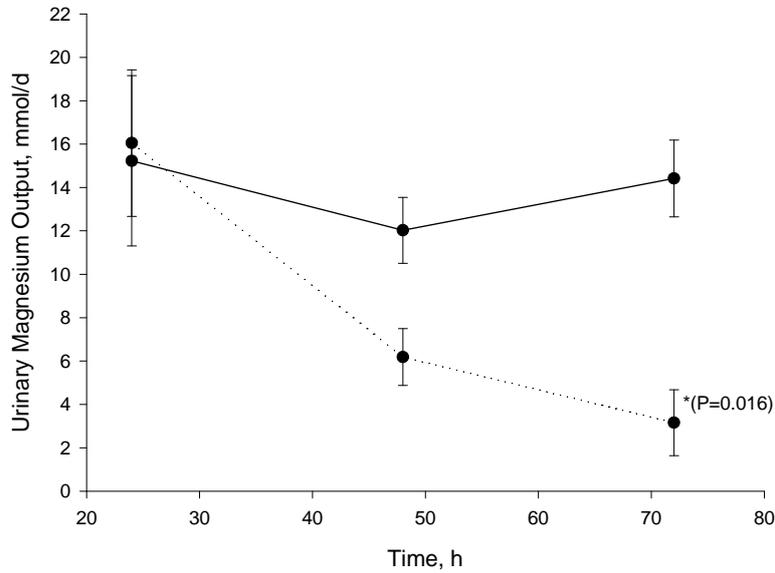


Figure 3.6. Total urine magnesium output (mean \pm SEM) at 0, 24, 48 and 72 h for two groups of sheep which were either water deprived (dotted line) or given ad libitum access to water (solid line).

3.3.6 Plasma electrolytes

Plasma sodium concentrations had a significant water x time interaction between 24 and 48 h ($P = 0.037$), indicating that water deprived animals had a higher plasma sodium concentration than animals that had access to water (Figure 3.7). This trend was maintained throughout the rest of the study. Despite any mineralocorticoid effect cortisol may have had on sodium retention, water deprivation caused a greater increase in plasma sodium.

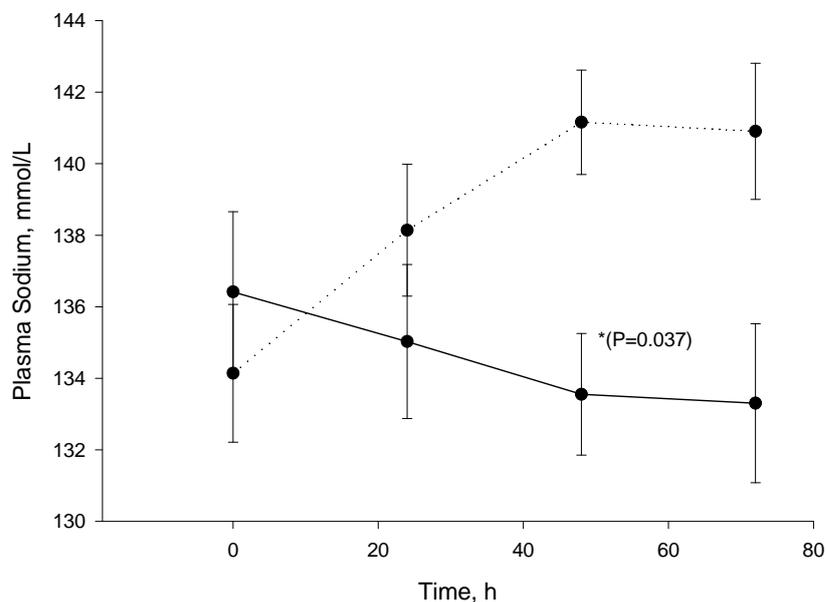


Figure 3.7. Plasma sodium concentration (mean \pm SEM) at 0, 24, 48 and 72 h for two groups of sheep which were either water deprived (dotted line) or given ad libitum access to water (solid line).

There was a trend toward a time x cortisol interaction on plasma potassium concentrations from 0 to 24 h ($P = 0.078$) (Figure 3.8), indicating a lower plasma potassium concentration in cortisol treated animals than animals that received no cortisol. Plasma potassium concentrations for all groups were less than the reported normal values for blood chemistry in sheep (4.8 to 5.9 mmol/L) (Blood and Radostits 1989). Although no clinical signs of potassium deficiency were detected in the experimental sheep or their flock mates.

Plasma magnesium concentrations were not affected by water deprivation or cortisol treatment. Cortisol treatment had no significant effect on plasma sodium, potassium or magnesium concentrations. Infusion of cortisol by Fan *et al.* (1975) into sheep resulted in a similar outcome to that seen in the present study. Furthermore, these results are supported by other authors, who have demonstrated that isolation and restraint stress in sheep had no effect on plasma sodium or potassium concentrations (Parrott *et al.* 1987; Apple *et al.* 1993).

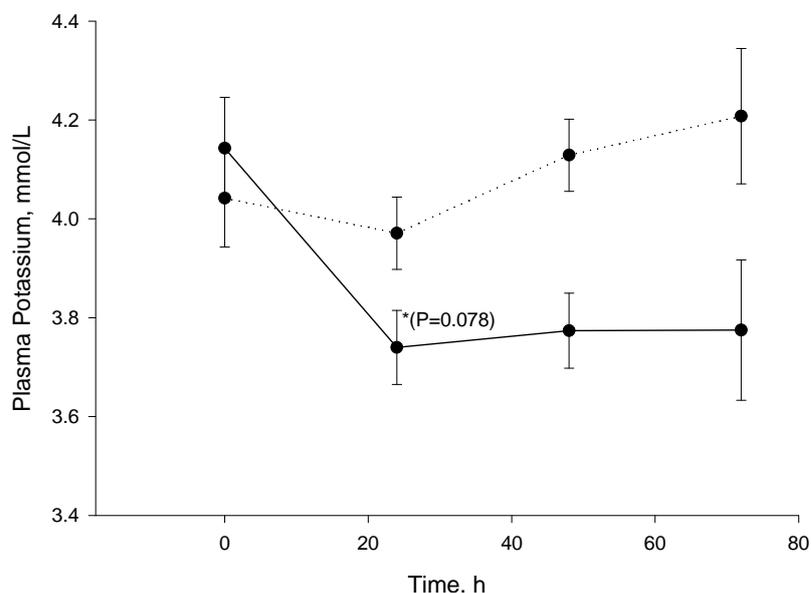


Figure 3.8. Plasma potassium concentration (mean \pm SEM) at 0, 24, 48 and 72 h for two groups of sheep in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

Cole (2000) also demonstrated that feed and water deprivation for 72 h had no effect on plasma or whole blood sodium, potassium or magnesium concentrations compared with hydrated, fed control sheep. Similarly in other ruminants, Gaylean *et al.* (1981) demonstrated no difference in plasma sodium concentration compared with unstressed controls in steers subjected to fasting or transportation and fasting stress. Throughout their study, Gaylean *et al.* (1981) did, however, demonstrate a difference ($P = 0.05$) between plasma potassium concentrations at one sample point only (18 h) between the fasted and transported animals and control animals.

In stress related research, the measurement of single variables (i.e. cortisol) are of little value when not considered in the context in which the substance is released and in not knowing the consequences a particular level of the variable has for an animals well being (Von Borell 2001). We concur with Parrott *et al.* (1987) that acute stress may activate a mechanism that enables the volume, tonicity and ionic composition of the extracellular fluid in the sheep to be maintained in the face of a severe reduction in water intake.

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Cortisol appears to play a major role in activating this protective mechanism for the animal.

3.4 Implications

We would conclude from this model based on cortisol infusion that well-hydrated ruminants placed under stressful conditions will respond with a diuresis. However, because the animal may draw upon water reserves within its gastrointestinal tract, 72 h of cortisol infusion was not sufficient to see a significant decrease in body water, in the cortisol treated animals. As animals subjected to intravenous infusions of cortisol to simulate stress appear to suffer from a loss of water in excess of that associated with a loss of electrolytes, administration of water alone is likely to be the most effective treatment for these animals.

CHAPTER FOUR

EXCESS CORTISOL INTERFERES WITH A PRINCIPLE MECHANISM OF RESISTANCE TO DEHYDRATION IN *BOS INDICUS* STEERS

ABSTRACT: This study investigated the effects of excess cortisol on physiological mechanisms that resist dehydration in *Bos indicus* steers (n = 31, 2 yr-old, 193 ± 21.47 kg mean BW) during a 90 h period. The steers were assigned randomly to one of four groups: 1) no water/no cortisol, (n = 8); 2) water/no cortisol (n = 8); 3) no water/cortisol, (n = 8); 4) water/cortisol, (n = 7). Animals allocated to cortisol treatment groups were given 0.1 mg•kg BW⁻¹•h⁻¹ of hydrocortisone suspended in isotonic saline for the duration of the study. TBW, osmolality, heamatocrit, urine output, feed and water intake, and plasma concentrations of AVP, AII, electrolytes, total protein, and albumin were determined at 24 h intervals for 90 h. In the presence of excess plasma cortisol, TBW body water was maintained in the presence of a water deprivation insult for 90 h. Hydration indices such as total plasma protein and albumin did not change throughout the study, supporting the body water data. However, plasma osmolality increased for the water-deprived groups from 24 h (P = 0.008). Heamatocrit did not reflect dehydration in any group. Water deprivation induced an increase in endogenous plasma cortisol concentrations post 60 h of the study (P = 0.023). Plasma concentrations of AVP increased with water deprivation (P = 0.006). Excess cortisol decreased the plasma concentration of AVP at 72 h only (P = 0.027). Plasma concentrations of AII were suppressed by excess plasma cortisol at 24 and 72 h (P = 0.000; P = 0.036), respectively. Animals treated with excess cortisol maintained urinary output for 48 h before decreasing at 72 h (P = 0.057), but cortisol had no effect on water or feed intake. Water deprivation increased plasma sodium concentrations (P < 0.05) until 72 h, while potassium decreased under the influence of excess plasma cortisol (P = 0.001) at 24 h. Water deprivation increased plasma chloride concentration at 72 and 90 h (P = 0.051; P = 0.026), respectively. Plasma phosphorous decreased under the influence of excess plasma cortisol at 24 h (P = 0.001) and remained at these lower concentrations for the duration of the study (P = 0.05). These results highlight the complexity of endocrine interactions associated with water balance in *Bos indicus* steers. We are able to accept our

hypothesis that, the renin-angiotensin-aldosterone (RAA) axis is suppressed in the presence of excess cortisol however, homeostasis is achieved through other physiological systems.

4.1 Introduction

Ruminants exposed to the stressors of transport and handling respond with an activation of the SAM axis and the HPA axis (Schaefer *et al.* 2001). Because activation of the SAM provides for a short-term response, models that mimic the effects of the HPA axis have been favoured to investigate the longer-term effects of a stressor upon the physiology of an animal. The HPA axis when activated by a stressor such as transport and handling results in the release of glucocorticoids and other hormones which have pathophysiological effects on an animal's body.

We have previously adapted a stress model based upon cortisol infusions from Macfarlane *et al.* (2000) to investigate the effects of excess cortisol infusions upon water balance in the Merino sheep (Parker *et al.* 2003a). As a consequence of this previous research, it appeared that cortisol had the capacity to interfere with a principal mechanism of resistance to dehydration. Cortisol has been implicated in inhibiting the effects of AVP in dogs (Baas *et al.* 1984) and there is evidence to suggest elevated ACTH and glucocorticoids also inhibit the RAA axis (Coghlan *et al.* 1979). Our working hypothesis was that excess plasma concentrations of cortisol would interfere with the principle mechanisms of resistance to dehydration in *Bos indicus* genotypes, in particular the arginine vasopressin-thirst mechanism and the RAA mechanism.

4.2 Materials and Methods

4.2.1 Animals and management

Bos indicus steers of high grade Brahman genotype (n = 32, 2 yr of age, 193 ± 21.47 kg mean BW) were halter broken and taught to lead, and were fitted with canvas urine collectors for training 21 d prior to initiation

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of experimentation to minimize stress during sample collection. The steers were ranked on BW, allocated to individual stalls at random and fed a commercial forage cube (ME 8.5 MJ/kg DM, crude protein 12.5% and crude fibre 31.1%; Cane Fibre Products, Brandon, QLD, Australia) *ad libitum* for 14 d prior to the commencement of the experiment. Urine collectors were fitted to animals at the start of the study (1: no water/no cortisol, n = 3; 2: water/no cortisol, n = 3; 3: no water/cortisol, n = 4; 4: water/cortisol, n = 6). All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (Approval No. A664-01).

4.2.2 Treatments

Stall numbers were assigned at random, in a 2 x 2 factorial arrangement, to one of four groups (1: no water/no cortisol, n = 8; 2: water/no cortisol, n = 8; 3: no water/cortisol, n = 8; 4: water/cortisol, n = 7). On day -1, all animals were catheterized with a poly-vinyl chloride tube (OD 2.0mm x ID 1.0mm; Critchley Electrical Products Pty Ltd, Silverwater, NSW, Australia) inserted into the jugular vein under local anesthetic. All animals allocated to the two cortisol groups were given $0.1 \text{ mg} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ of hydrocortisone (Solucortef, Upjohn Pty Ltd, Rydalmere, NSW, Australia) suspended in isotonic saline administered at a rate of $0.1 \text{ mL} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$, for the duration of the experiment. The non-cortisol groups were given an equivalent placebo infusion of isotonic saline. Animals that were in water-deprived groups had their water withdrawn at the commencement of the experiment for 86 h.

4.2.3 Sample collection

On day 0, 20 mL of blood was collected from all treatment groups and decanted into 2 x 10 mL tubes containing lithium heparin (Disposable Products Pty Ltd, Adelaide, SA, Australia). The catheters were then flushed with 10 mL of heparinized saline ($15000 \text{ IU heparin} \cdot \text{L}^{-1}$ 0.9% saline) to prevent clotting between sampling periods. The sampling regimen continued at 6 h intervals for 90 h. Blood samples were immediately placed into an ice water slurry and then centrifuged at $200 \times g$ for 15

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min and plasma poured off within 2 h and frozen (-20° C) for analysis. Total urine excreted was collected, measured and sub sampled (200 mL) every 24 h.

4.2.4 Urea, electrolyte and metabolite measurement

Analysis of Na and K in steer plasma was conducted using ion selective electrodes (Lablyte 830 electrolyte analyzer, Beckman Instruments, Inc. Brea, California, USA). Plasma Ca, P, Cl, total protein, and albumin were analyzed spectrophotometrically using a Cobas-Mira auto analyzer (Roche diagnostics, Brisbane, QLD, Australia) with Trace scientific reagents (Trace Calcium, TR29248; Inorganic Phosphorous TR30025; Chloride, TR38025; Total Protein, TR34025; Albumin TR36025). Plasma urea nitrogen was analyzed with a Technicon auto-analyzer 2 (Bran + Leubbe Pty Ltd, Homebush, NSW, Australia) according to the technicon auto analyzer method SE40001FD4. Heamatocrit was measured using a micro-centrifuge (Quantum Scientific, Milton, QLD, Australia). Plasma osmolality was measured using an automatic osmometer (Knauer Osmometer, Berlin, Germany).

Plasma cortisol concentration was measured using a radioimmunoassay kit (Spectria Cortisol ¹²⁵I-coated tube kit, Orion Corporation, Espoo, Finland). Plasma AVP and AII concentrations were assayed using a radioimmunoassay method by Austin Biomedical services (Prosearch International Australia Pty Ltd, Melbourne, VIC, Australia). Intra- and inter- assay coefficient of variation were 8 and 12%, respectively, for both AVP and AII hormone assays. Urea space measurements were determined on 0, 24, 48, 72 and 90 h for each animal using the technique described by Preston and Kock (1973), and validated in our laboratory (see Appendix 1). TBW was recorded as the pool available to the urea molecule.

4.2.5 Statistical analysis

A 2 x 2 factorial arrangement with the main effects for water (*ad libitum* water and no water) and cortisol (cortisol infusion and no cortisol), and the interaction effects of water x cortisol with time taken into account were analyzed statistically with a repeated measures ANOVA using SPSS 10 (SPSS Inc. Chicago, IL). Quantitative

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variables (plasma electrolytes, plasma cortisol, AVP, AII, total protein, glucose, TBW, hematocrit, water intake and urine output) were independently sampled. Tests for sphericity and homogeneity were conducted to test assumptions for the repeated measures ANOVA, and in all cases these assumptions were satisfied. Least squares means and standard errors have been presented and multiple comparison tests within factors were not performed because there were fewer than three groups and therefore any difference would be clearly perceived. Differences were considered significant for $P < 0.05$. Due to the pattern of secretion of the cortisol concentrations in the no-water/no-cortisol group (Figure 4.1) a one-way ANOVA was performed based on the areas under the time curve, integrated using the multiple-application trapezoidal rule for the 30 - 60 h and the 60 - 90 h periods.

4.3 Results and Discussion

4.3.1 Plasma cortisol concentration

Plasma cortisol concentrations from *Bos indicus* genotypes exposed to transport stress of 24.2 km, have ranged from 25 to 35 ng/mL taken 1 h after transport (Lay *et al.* 1996). Other authors have reported physiological cortisol concentrations in cattle to range from a baseline of 0.5 - 9.0 ng/mL (Grandin 1997) to extreme stress of 120 ng/mL (Locatelli *et al.* 1989). The cortisol concentrations of the cortisol-infused groups could arguably reflect a pharmacological rather than a physiological dose rate with a range of 276 - 442 ng/mL of plasma in the cortisol infused groups throughout the experimental period.

Finberg *et al.* (1978) and Parker *et al.* (2003a) demonstrated that water deprivation alone was not a prototypical stressor that will activate the HPA axis and elevate plasma cortisol in the camel and sheep, respectively. However, the concentrations of cortisol in the no-water/ no-cortisol group began to increase at 60 h of the experimental period (Figure 4.1). The peak plasma cortisol concentrations for the no-water/no-cortisol and the water/no-cortisol groups (51.65 ± 17.88 ng/mL and 16.80 ± 4.42 ng/mL) were recorded at 60 h and 66 h respectively. The area under the plasma cortisol concentration curve during the 60 - 90 h period was higher ($P = 0.028$) for the no-water/no-cortisol group than the water/cortisol group. There were

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no differences among groups for area under the cortisol curve during the 30 – 60 h period.

Matthews and Parrott (1991) suggested a physiological interaction between stress, dehydration and HPA function, in that HPA axis activity becomes sensitized to stressors as dehydration ensues. Their claim is supported by others who have indicated that endogenous AVP is of physiological importance in amplifying the ACTH response to stress (Redekopp *et al.* 1985). This evidence has significant animal welfare implications, in that the dehydration associated with long distance transportation becomes a circumstance of aggravation to transport and handling stressors, resulting in higher HPA axis responses than if the animals were well hydrated.

While it is likely that the hypothesis of Matthews and Parrott (1991) may explain the increased cortisol concentrations from a novel stimuli, after 60 h in the present study, the observed changes in the no-water/no-cortisol group may not necessarily be indicative of a HPA axis response to a stressor per se but rather to the very high levels of AVP and AII expressed in these animals. As AVP and AII are potent vasoconstrictor agents, it may be possible that endogenous cortisol levels increased in these animals to prevent excessive vasoconstrictive effects of these water retentive hormones (Sapolsky *et al.* 2000).

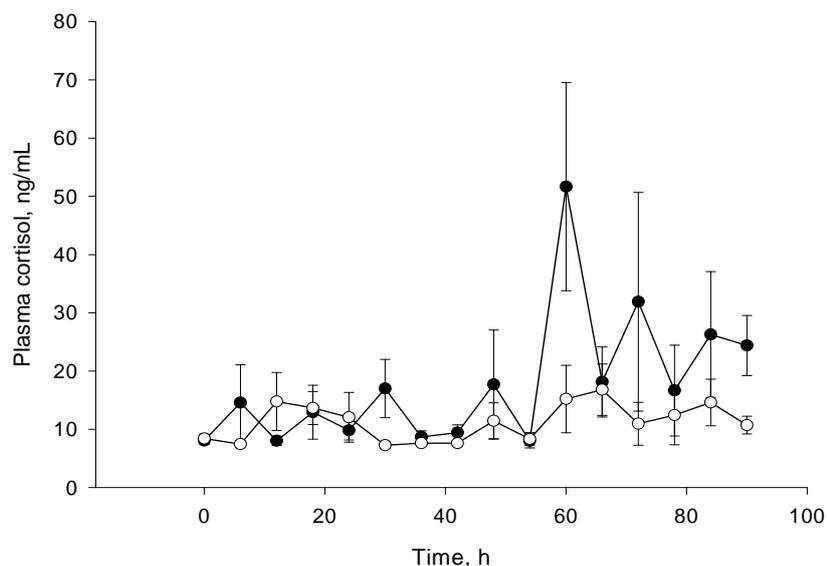


Figure 4.1. Plasma cortisol concentration (mean \pm SEM) at 6 h intervals for 90 h in the no water/no-cortisol (●) and water/no-cortisol (○) steer groups. The no-water/no-cortisol group demonstrated a group-x-time interaction ($P = 0.028$) toward increasing the area under the plasma cortisol concentration curve from 60 – 90 h compared to the water/no-cortisol group.

4.3.2 Arginine vasopressin

There were water x time interactions for AVP concentrations ($P < 0.006$) at 0 and 24 h, 24 and 48 h, and 48 and 72 h (Figure 4.2), demonstrating the effects of water deprivation on these animal groups. A cortisol x water x time interaction ($P = 0.027$) occurred between 48 and 72 h indicating that the no-water / no-cortisol group demonstrated a greater AVP concentration as compared with the no-water/cortisol group. Aubury (1965) reported that cortisol increased the osmotic threshold for AVP release in humans. This was demonstrated in the present study at 72 h only. At all other times, there were no differences between AVP concentrations in the water deprived groups.

The water deprived steers produced maximum AVP concentrations at 90 h of 31.88 ± 4.60 pg/mL and 30.38 ± 4.60 pg/mL for the no-water/no-cortisol and no-water/cortisol groups respectively. In contrast, Ben Goumi *et al.* (1993) demonstrated in camels an increase within 24 h of 5.3 ± 2.2 pg/mL that did not change for the rest of the water deprivation period of 14 d (5.7 ± 2.2 pg/mL).

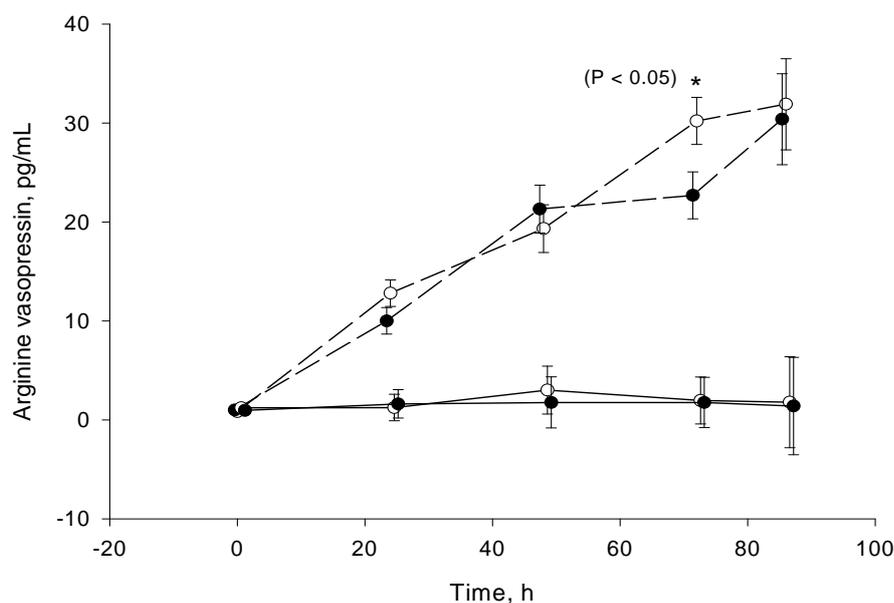


Figure 4.2. Plasma concentrations of arginine vasopressin (AVP) in *Bos indicus* steers (mean \pm SEM) at 0, 24, 48, 72 and 90 h for hour groups of steers in which stress was simulated by injection of cortisol (\bullet) or not (\circ), and which were either water deprived (dashed line) or given ad libitum access to water (solid line). * Denotes a water-x-time interaction ($P < 0.05$).

AVP may stimulate ACTH secretion and potentiate the response to corticotrophin releasing factor (Redekopp *et al.* 1985; Redekopp *et al.* 1986; Rittmaster *et al.* 1987). This effect has caused some authors to label AVP as a stress hormone. However, AVP may not mediate ACTH responses to all stressors (Irvine *et al.* 1989) and, in some cases, stressors have reduced plasma AVP levels (Keil and Severs 1977). Parrott *et al.* (1987) reported that short-term isolation stress in sheep resulted in a negative relationship between cortisol and AVP. Greater cortisol concentrations were associated with lesser AVP concentrations, however, this relationship was not significant. Similarly, El-Nouty *et al.* (1977) demonstrated an increase in AVP with heat stress in cows but did not detect changes in glucocorticoids. This differs from other authors who demonstrated that increased plasma cortisol was associated with a decreased urine output with longer term environmental stressors, suggestive of cortisol increasing AVP concentrations or alternatively a mineralocorticoid effect of cortisol (Guerrini and Bertchinger 1982). Exogenous cortisol had little effect on the concentration of AVP in the plasma of *Bos indicus* steers in the present study. Water deprivation, however, had a consistent effect in increasing AVP concentrations.

4.3.3 Angiotensin II

There was a cortisol-x-water-x-time interaction occurring between 0 and 24 h ($P < 0.001$) and 48 and 72 h ($P = 0.083$) of the experimental period demonstrating an increase in AII concentrations for the no-water/no-cortisol group as compared with the other groups. Water x time interactions between 0 and 24 h ($P = 0.000$), 24 and 48 h ($P = 0.053$), and 48 and 72 h ($P = 0.053$) demonstrated increases in AII concentrations for the water deprived groups, compared with the groups offered water *ad libitum*. However, the no-water/no-cortisol group largely influenced this effect. Cortisol x time interactions between 0 and 24 h ($P < 0.001$) and 48 and 72 h ($P = 0.036$) demonstrated a decrease in AII concentrations with the infusion of exogenous cortisol. The no-water/cortisol group maintained the same AII concentrations as the water/no-cortisol group, and the water/cortisol group's AII concentration was below that of the water/no-cortisol group from 24 h (Figure 4.3).

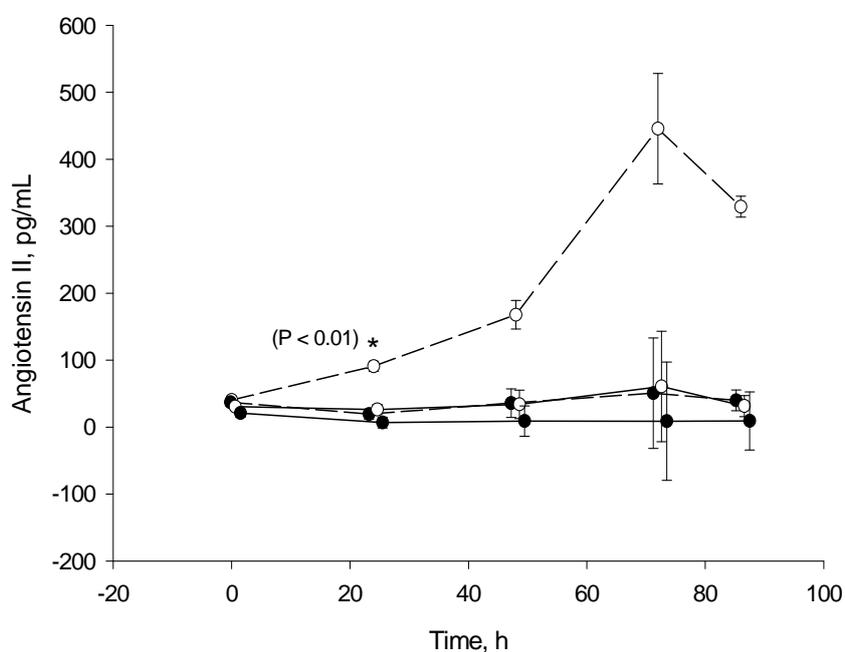


Figure 4.3. Plasma concentrations of angiotensin II (AII) in *Bos indicus* steers (mean ± SEM) at 0, 24, 48, 72 and 90 h for four groups of steers in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dashed line) or given ad libitum access to water (solid line). * Denotes a cortisol-x-water-x-time interaction ($P < 0.01$).

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The RAA axis remains a principle mechanism in the resistance to dehydration (Guyton and Hall 2000). While acute hypovolaemic stress consistently activates the HPA and RAA axes along with AVP and catecholamine secretion, lesser degrees of fluid loss result in inconsistent hormonal secretory patterns (Espiner 1987). AII has a tropic action on the kidney to retain Na and water as well as stimulating the production of aldosterone from the adrenals. The repeated treatment with ACTH or glucocorticoids results in a diminished response of the adrenal glomerulosa and in the suppression of rennin (Coghlan *et al.* 1979). Changes in Na status appear to be the predominant factor in the suppression of AII associated with excess cortisol infusion in the present study and in that of Coghlan *et al.* (1979). The hypernatremia that accompanies dehydration has also been implicated for the disruption of the nexus between the renin-angiotensin system and aldosterone in the sheep and camel (Blair-West *et al.* 1972; Ben Goumi *et al.* 1993). In the presence of a concurrent water deprivation, the complexity of endocrine interactions associated with water balance results in homeostasis occurring regardless of a deficit in one of the physiological system employed. Cortisol has a suppressive effect on the RAA axis, however, our results are consistent with previous reports that in the presence of water deprivation it serves to protect and maintain water balance in times of stress (Parrott *et al.* 1987; Parker *et al.* 2003a).

4.3.4 Urine output

There was a cortisol-x-water-x-time ($P = 0.057$) interaction, between 48 and 72 h of the treatment period, demonstrating that the water/cortisol group maintained urine output for 48 h then decreased their urine output at 72 h of the treatment period (Figure 4.4).

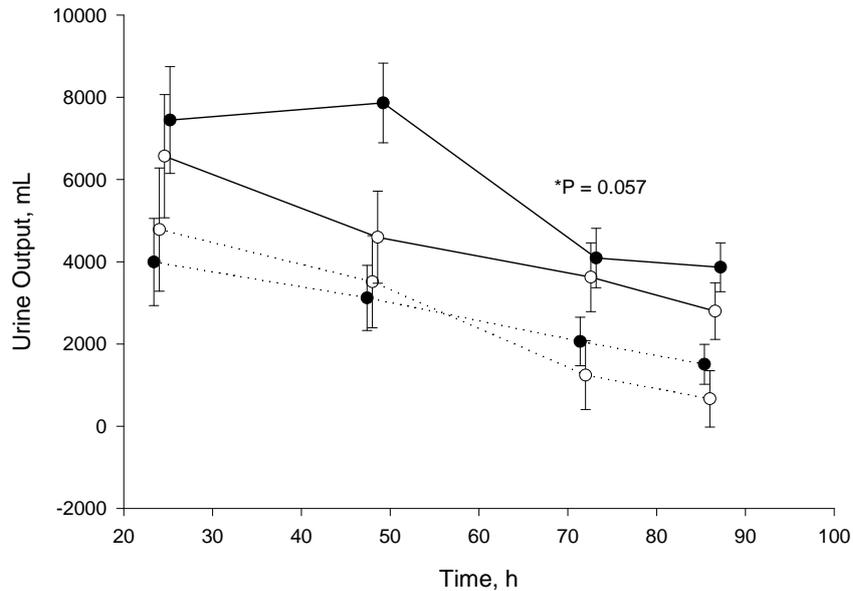


Figure 4.4. Total urine output (mean \pm SEM) at 24, 48, 72 and 90 h for four groups of steers which were given an injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given ad libitum access to water (solid line).

Glucocorticoids have been shown to antagonize the effects of AVP by increasing the glomerular filtration rate (De Matteo and May 1999) and the secretion of atrial natriuretic peptide. Baas *et al.* (1984) indicated that the mechanism in which pharmacological doses of cortisol induced a polyuria in the dog was due to an inhibition of the action of AVP, causing a decreased water and urea reabsorption by the kidney. Their findings were associated with polydipsia in well-hydrated animals. Similarly, a diuresis was also found in well-hydrated sheep offered *ad libitum* water, and given stress-like infusions of cortisol. However, when sheep were water deprived and infused with cortisol, the diuretic effect ceased (Parker *et al.* 2003a). Concentrations of AVP and AII were not elevated in the water/cortisol group, which would allow a diuresis to occur via an increase in the glomerular filtration rate. Post 48 h of the treatment period, the decrease seen in the urine production of this group may have been associated with the mineralocorticoid effect of cortisol on the steers causing Na and water retention.

4.3.5 Hydration effects

A water-x-time interaction occurred between 0 and 24 h ($P = 0.008$) of the experimental period indicating that animals that were water deprived had a greater plasma osmolality as compared with animals that had *ad libitum* access to water (Figure 4.5). The decrease in osmolality at 72 h for all groups may have been due to fluctuations in water compartments within the animal.

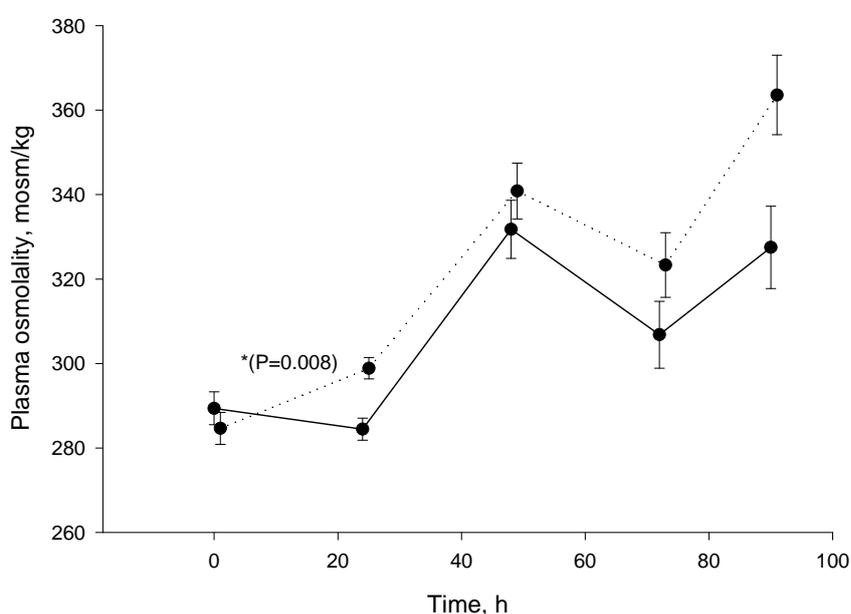


Figure 4.5. Plasma osmolality (mean \pm SEM) at 0, 24, 48, 72 and 90 h for two groups of steers that were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

An elevation of total protein and albumin is indicative of hemoconcentration due to dehydration. The elevation of plasma proteins was not found in the present study and changes across time were similar for all groups for total protein and albumin.

Although hematocrit data remained within normal physiological limits for all groups there was a water x cortisol x time interaction at 90 h ($P = 0.028$) of the treatment period, demonstrating that the water/cortisol group had lower hematocrit as compared with the water deprived groups at 90 h (Figure 4.6). Hematocrit data did not indicate dehydration in any group.

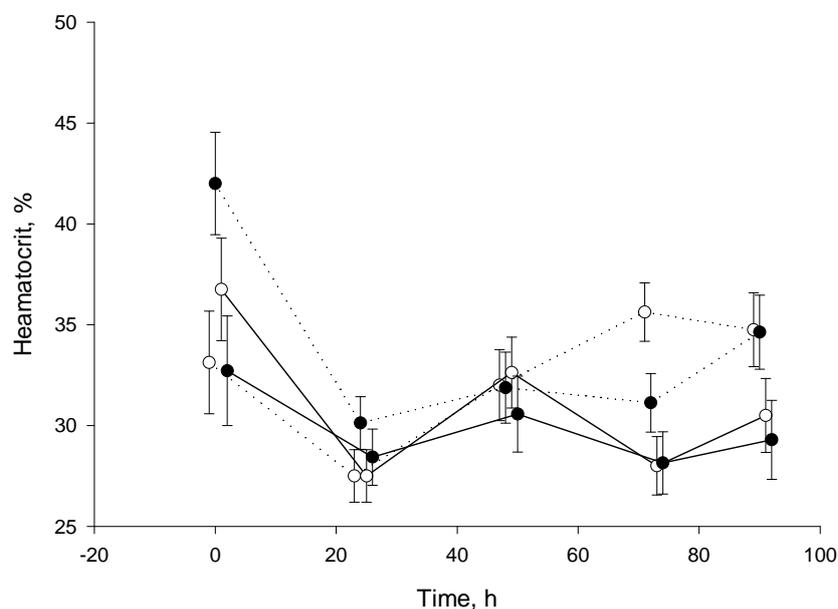


Figure 4.6. Haematocrit (mean \pm SEM) at 24, 48, 72 and 90 h for four groups of steers which were given an injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given ad libitum access to water.

The body water content of the four groups of steers did not differ at 0, 24, 48, 72 or 90 h of the experimental period. Preston and Kock (1973) concluded that urea space was proportional to empty body water (TBW less the water in the GIT). We previously reported that the replacement of water from the GIT may have been responsible for a maintenance of body water in Merino sheep in the presence of a cortisol-induced diuresis (Parker *et al.* 2003a). Data supporting the body water assay appears equivocal. The AVP and AII hormonal data suggests a loss of water from the vascular space in the water deprived groups especially the no-water/no-cortisol group. This interpretation is supported by a time \times water effect on plasma osmolality in the water-deprived groups. In contrast, the hematocrit, total protein, and albumin data indicated no difference among groups in the present study. The water pools in the ruminant body are dynamic, moving from the lumen of the GIT to the extra-cellular fluid freely. This flux of water pools has resulted in considerable variation in the determination of body water loss from stressors (Cole 1995). Other procedural considerations in undertaking the urea space assay have been implicated by Bartle *et al.* (1988) as significant sources of error. However, we are confident that sampling

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and analytical errors were minimized in the present study. It would appear that during 90 h of water deprivation, *Bos indicus* steers were able to utilize sufficient water from the GIT to prevent a decrease in empty body water content.

4.3.6 Plasma Electrolytes

Plasma Na concentrations had significant water x time interactions between 0 and 24 h ($P = 0.014$), 48 and 72 h ($P = 0.022$) and 72 and 90 h ($P = 0.076$) of the treatment period (Figure 4.7), indicating that water deprived animals had greater plasma Na concentrations as compared with animals that had access to water. The no-water/cortisol group had the greatest plasma sodium concentrations of all the groups from 24 h of the treatment period until the completion of the experiment. Despite the pharmacological dose rate given to the cortisol infused animals in the present study, water deprivation alone had a greater effect on plasma Na concentration.

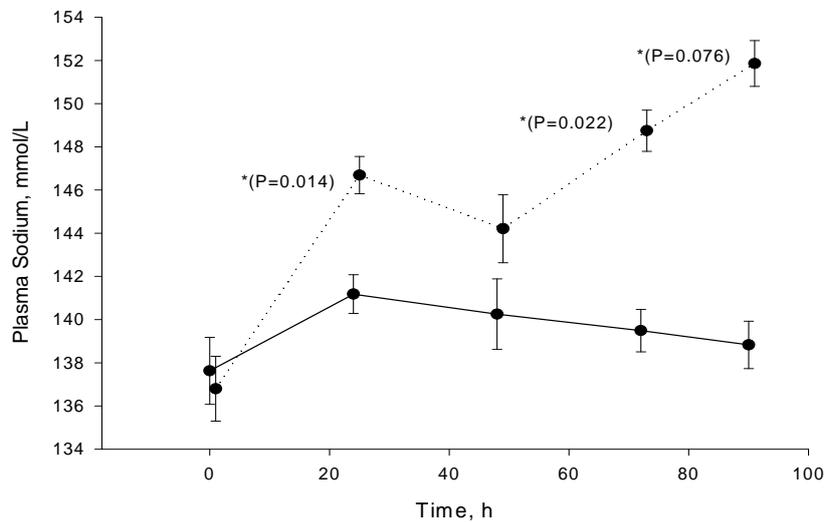


Figure 4.7. Plasma sodium concentration (mean \pm SEM) at 0, 24, 48, 72 and 90 h for two groups of steers that were either water deprived (dotted line) or given ad libitum access to water (solid line).

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A cortisol x time interaction ($P = 0.001$) was detected between 0 and 24 h of the treatment period demonstrating that cortisol infusion induced a decrease in plasma K at 24 h of treatment (Figure 4.8). The difference between the cortisol and no-cortisol groups was maintained until 90 h of the treatment period when the no water/no-cortisol group had decreased its plasma K to the same extent as the cortisol groups. The pre/post ANOVA performed on data collected at 60 h of the treatment period for cortisol concentration in the no-water/no-cortisol group demonstrated an increase in the concentration of endogenous cortisol after 60 h of the experimental period. This increase may have been sufficient to induce the decrease in plasma K. Alternatively, Bianca *et al.* (1965) reported a similar effect and proposed the reduced feed intake to have decreased plasma K concentrations.

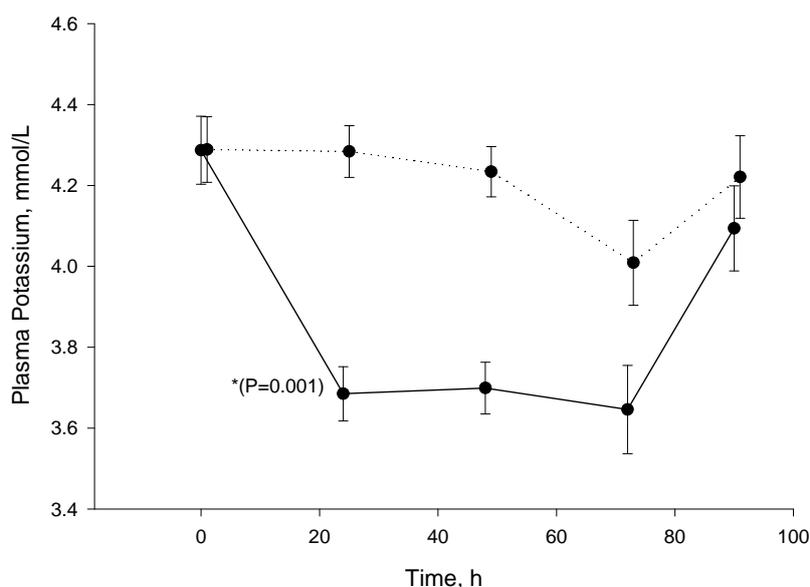


Figure 4.8. Plasma potassium concentration (mean \pm SEM) at 0, 24, 48, 72 and 90 h for two groups of steers in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

There were water x time interactions for Plasma Ca concentrations between 24 and 48 h ($P = 0.045$), and 72 and 90 h of the experimental period ($P = 0.015$) indicating an increase in plasma Ca for the groups receiving water as compared with the water deprived animals (Figure 4.9). The reduced feed intake by water deprivation and the subsequent effects on gastrointestinal motility and absorption in the water-deprived

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groups did not have a significant effect until 90 h. The plasma Ca concentration of the no-water/no-cortisol group were $1.75 \text{ mmol/L} \pm 0.12 \text{ mmol/L}$ at 90 h of the experimental period which falls below the range for calcium in cattle ($2.00 - 2.62 \text{ mmol/L}$) (Blood and Radostits 1989), although all other groups were within the normal range.

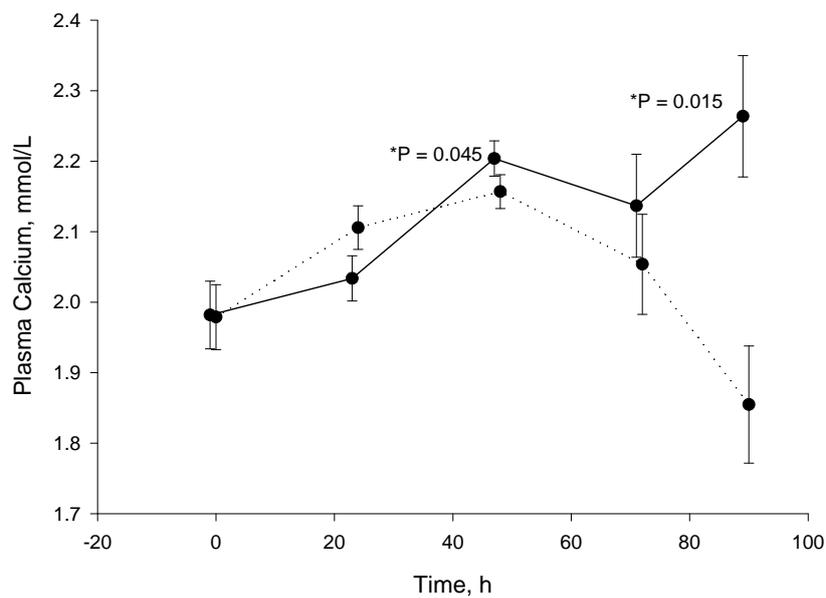


Figure 4.9. Plasma calcium (mean \pm SEM) at 0, 24, 48, 72 and 90 h for two groups of steers that were either water deprived (dotted line) or given ad libitum access to water (solid line).

Water \times time interaction occurred for plasma Cl concentrations between 48 and 72 h ($P = 0.051$) and 72 and 90 h of the experimental period ($P = 0.026$), demonstrating that water deprived groups had greater concentrations of chloride in their plasma (Figure 4.10). Plasma concentrations and interactions of Cl followed Na concentrations in plasma. All groups were within normal ranges for plasma Cl in cattle ($95 - 110 \text{ mmol/L}$) (Blood and Radostits 1989).

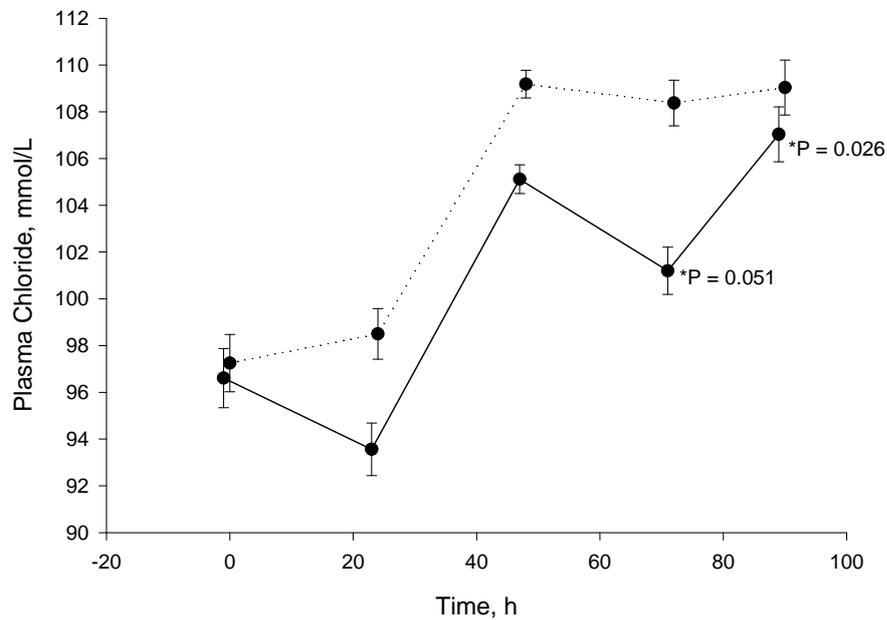


Figure 4.10. Plasma chloride concentration (mean \pm SEM) at 0, 24, 48, 72 and 90 h for two groups of steers that were either water deprived (dotted line) or given ad libitum access to water (solid line).

Cortisol x time interaction occurred between 0 and 24 h ($P = 0.01$) for plasma P demonstrating a decrease in P concentration for those animals infused with cortisol. This trend continued to be significantly different from the no-cortisol groups ($P = 0.05$) until the conclusion of the experiment (Figure 4.11). The plasma P concentration of the cortisol groups remained within normal limits for cattle (1.30 – 2.25 mmol/L) (Blood and Radostits 1989).

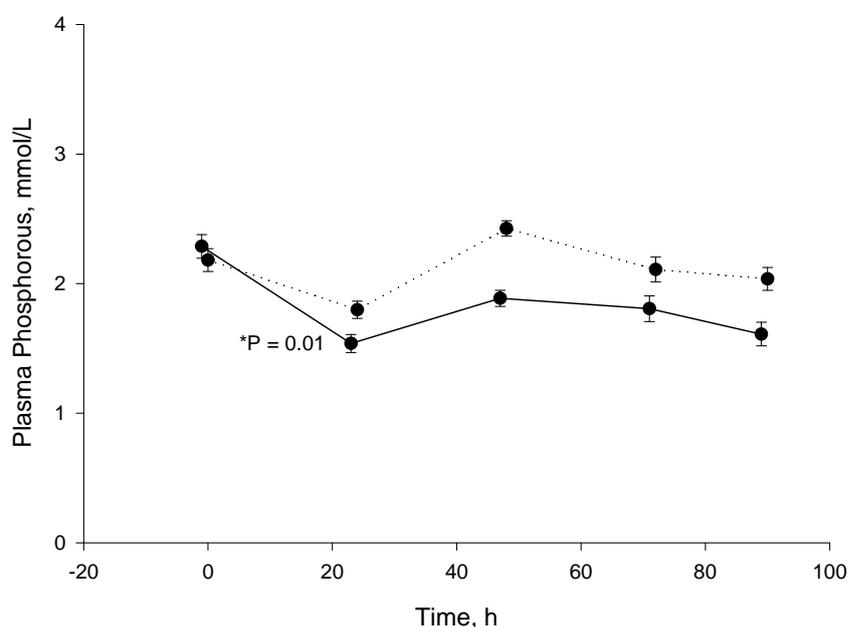


Figure 4.11. Plasma phosphorous concentration (mean \pm SEM) at 0, 24, 48, 72 and 90 h for two groups of steers in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

4.3.7 Water and Feed Intake

The animals offered water demonstrated a time effect, with water intake decreasing between 24 and 48 h ($P = 0.016$) before increasing their intake from 48 and 72 h ($P=0.001$) (Table 4.1). However, feed intake of the watered groups did not vary throughout the study. High cortisol concentrations in sheep may reduce water intake or cause complete abstinence from drinking (Guerrini and Bertchinger 1982; Parrott *et al.* 1987). The water/cortisol group failed to repeat the behaviors reported by Guerrini and Bertchinger (1982) and Parrott *et al.* (1987). The isolation and or restraint stress in previous experiments may have activated the SAM and HPA axes resulting in a number of neuroendocrine products that collectively may have altered drinking behavior. This would suggest that excess cortisol alone is not responsible for the fluctuations in water intake in the present study. Data for feed intake indicated a time x water interaction between 0 and 24 h ($P = 0.005$), 24 and 48 h ($P = 0.032$), and 72 and 90 h ($P = 0.013$) of the treatment period, demonstrating a

decreased feed intake from the water deprived groups as compared with the groups offered *ad libitum* water.

4.4 Implications

In the presence of water deprivation, the complexity of endocrine interactions associated with water balance results in homeostasis regardless of a deficit in one of the physiological system employed. Excess cortisol has a suppressive effect on the RAA axis, but does not affect the circulating concentrations of arginine vasopressin. Plasma electrolytes in the present study exhibited small but significant changes over time. However, electrolytes and metabolite concentrations remained within physiologically normal range limits. In the presence of water deprivation cortisol may serve to protect and maintain water balance in times of stress.

Table 4.1 Mean \pm SEM for water and feed intake at 0, 24, 48, 72, and 90 h for four groups of steers after stress was simulated by infusion of cortisol or not and which were either water deprived or given *ad libitum* access to water.

Treatment				
Time, h	No water/no cortisol^a	Water/no cortisol^a	No water/cortisol^a	Water/cortisol^b
Water intake, kg/d				
0		19.23 \pm 1.95		18.09 \pm 2.08
24		16.44 \pm 1.36		14.36 \pm 1.46
48		10.36 \pm 1.58		11.86 \pm 1.69
72		21.86 \pm 1.28		22.14 \pm 1.37
90		9.94 \pm 1.25		12.64 \pm 1.33
Feed intake, kg/d, as fed				
0	3.96 \pm 0.28	4.63 \pm 0.28	4.13 \pm 0.28	4.74 \pm 0.30
24	2.83 \pm 0.45	4.80 \pm 0.45	2.48 \pm 0.45	4.91 \pm 0.48
48	2.14 \pm 0.57	5.25 \pm 0.57	2.35 \pm 0.57	6.43 \pm 0.61
72	2.04 \pm 0.52	5.50 \pm 0.52	2.13 \pm 0.52	6.05 \pm 0.56
90	2.26 \pm 0.49	3.96 \pm 0.49	2.61 \pm 0.49	4.46 \pm 0.53

^an = 8

^bn = 7

CHAPTER FIVE

QUANTITATIVE ANALYSIS OF ACID BASE BALANCE IN *BOS INDICUS*
STEERS SUBJECTED TO TRANSPORTATION OF LONG DURATION

ABSTRACT: There is a lack of information pertaining to the effects of transport stress on the acid-base physiology of ruminants. The effect of transportation and/or feed and water deprivation on acid-base balance was studied using 24, 2 yr old, *Bos indicus* steers. The steers were allocated to one of three groups: 1) Control, offered feed and water, *ad libitum* (n = 8); 2) Water and feed deprived, offered no feed or water for 60 h (n = 8); and 3) Transported, offered no feed or water for 12 h and then transported for 48 h (n = 8). Blood gases, electrolytes, lactate, total protein, albumin, anion gap (AG), SID and total weak acids were determined at the conclusion of transportation. Arterial blood pH did not differ among experimental groups. Partial pressure of carbon dioxide (pCO₂) was lower for the water and feed deprived (P = 0.023) group than the control group. Plasma total protein, albumin and total weak acid concentrations were higher for the transported (P = 0.001, P = 0.03, P = 0.01) and water and feed deprived (P = 0.000, P = 0.003, P = 0.001) groups respectively, compared to the control group. Transported animals had a lower plasma concentration of potassium compared to the control animals (P = 0.026). This study demonstrates that although blood pH remains within normal values in transported and fasted steers, the primary challenge to a transported or feed and water deprived animal is a mild metabolic acidosis induced by elevated plasma proteins which may be the result of a loss of body water. The loss of electrolytes has little effect on the acid-base balance of the animals.

5.1 Introduction

The standard management practices of transport, assembly, mixing, handling, and the associated deprivation of feed and water are significant contributors to a transport stress syndrome characterized by loss of appetite and body mass (Hutcheson and Cole 1986), and compromised immune function (Atkinson 1992; Murata 1989). Transport stress has led to liveweight loss *en route*, (Phillips *et al.* 1991) and greater carcass shrink (Schaefer *et al.* 1992), while it is also accepted that animals dehydrate

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with increasing transit time (Sinclair *et al.* 1992; Tarrant *et al.* 1992; Knowles *et al.* 1999). Management strategies for dealing with the problems caused by transport stress have included pre-conditioning regimens (Pritchard and Mendez 1990), rest periods during and after transport (Wythes *et al.* 1988), the use of supplemental potassium (Hutcheson *et al.* 1984), and the use of electrolyte solutions (Schaefer *et al.* 1992; Gortel *et al.* 1992; Phillips 1997; Schaefer *et al.* 1997).

The use of electrolyte solutions for minimizing the effects of stressors on animals in the marketing process has been advocated in the sheep and beef industries, without a full understanding of the effects of transport stress on the acid-base physiology of ruminants (Schaefer 1997). Reported studies have shown transportation stress to have no effect on the pH of the bovine animal's blood (Schaefer *et al.* 1988; Schaefer *et al.* 1992), but there have been small but significant changes in some electrolytes that make up the strong ion group. As the strong ion group plays an important role in regulating plasma pH, the changes seen in the plasma electrolyte status of transported animals must be minimal or are compensated by another system to maintain pH within normal values.

This study was undertaken to assess the compensatory mechanisms involved in the maintenance of acid-base balance in *Bos indicus* steers subjected to transportation of long duration.

5.2 Materials and Methods

5.2.1 Animals and management

Twenty four, 2 yr old *Bos indicus* steers of high grade Brahman genotype (276 ± 14.65 kg mean BW) were sorted in an ascending order of liveweight and allocated to one of three treatment groups: 1) Control, offered *ad libitum* feed and water (n = 8); 2) Water and feed deprived, offered no feed or water for 60 h (n = 8); and 3) Transported, offered no feed or water for 12 h and then transported for 48 h (n = 8). Animals in the control group were offered a commercial dietary cube: ME 8.5 MJ, CP 12%, CF 31.1% per kg/DM (Cane Fibre Products, Brandon, QLD, Australia). Animals in the feed and water deprived and trucked groups had their water and feed

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withdrawn 12 h prior to departure of the transported group. The transported group were trucked for 48 h (3600 km), before being unloaded, and sampled. The truck stopped for 30 min every 5 h due to re-fueling and regulatory control of driver rest stops. The animals did not have access to feed and water in the yards while waiting to be sampled and were immediately processed upon exiting the unloading ramp. The transported animals were conveyed in a body truck with an eight tonne tare. The truck was equipped with an adjustable gate separating the holding compartment into two areas. The transported animals were loaded into the forward compartment at a density of 0.86m²/animal. A preliminary study had shown plasma concentrations of cortisol to be significantly elevated during periods of transportation (see Appendix 2).

At sampling, all animals were forced into a race where the animals were captured in a cattle head bail and restrained. A halter was placed on the individual animals and their heads were then restrained to the side with an attendant holding the head whilst samples were taken. When cattle are captured and restrained, abnormal physiological reactions to the restraint may be expressed as an increase or decrease in respiration rates. Subsequently, the blood gas parameters of the animal become affected. While the possibility exists for an abnormal measurement in blood gas parameters to have taken place in an animal from this study, it is unlikely when the current results are compared to that of other authors (Fisher *et al.* 1980; Mirakhur *et al.* 1985). Further to this, the animals used in the present study were accustomed to being handled, and behaved in a quiet and amicable manner when sampled.

A temperature and humidity index (THI) was calculated by the formula presented by Gaughan *et al.* (1999). The mean daily THI during the experimental period for days 0, 1 and 2 were 74, 74 and 73 respectively. The THI in the rear of the truck was not measured. The control and water and feed deprived groups were housed in outdoor pens with minimal shade.

All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (Approval No. A730-02).

5.2.2 Sample collection

After 48 h of transportation, 22.5 mL of blood was manually collected by jugular venepuncture from all groups; 20 mL into 2 x 10 mL tubes containing lithium heparin (Disposable Products Pty Ltd, Adelaide, SA, Australia); and 1 x 2.5 mL tube containing fluoride oxalate (Sarstedt Australia, Technology Park, SA, Australia). The samples containing fluoride oxalate were used for the analysis of plasma lactate and the tubes containing lithium heparin were used for all other analyses. Blood samples were immediately placed into an ice water slurry, centrifuged at 200 x g for 15 min, and the plasma poured off within 2 h and frozen (-20° C) for analysis at a later date.

A 22 G (0.9 x 25 mm) intra-arterial catheter (Optiva, Johnson and Johnson Int. Belgium) was utilized with a 2 mL blood gas syringe containing lithium heparin (Sarstedt Australia, Technology Park, SA, Australia) to sample arterial blood gases. Arterial blood samples for blood gas analysis were obtained from the caudal auricular artery (Riley and Thompson 1978). Blood gas syringes were capped and placed into an ice water slurry for immediate analysis of blood gases. All blood gas assays were performed within 0.5 h of collection.

5.2.3 Measurement

Arterial blood pH, partial pressure of carbon dioxide (pCO₂) and bicarbonate (HCO₃⁻) were measured using a blood gas analyzer (Ciba Corning Model 278, Bayer Diagnostics, Brisbane, QLD, Australia). Plasma concentrations of Na and K were measured using ion selective electrodes (Lablyte System 830, Beckman Instruments Inc, California, USA). Sodium and K samples were completed on singular samples and quality control samples (Liquichek controls; 16171 and 16172, Bio-Rad Laboratories, Regents Park, NSW, Australia) were performed every 10 samples. Lactate, P, albumin, total protein, Ca and Cl concentrations in plasma were measured using a Mira Autoanalyzer (Roche Diagnostics, Brisbane, Australia) with standard enzymatic and spectrophotometric kits (Lactate, Roche Diagnostics, Australia; and P, TR30025; Albumin, TR36025; Total Protein, TR34025; Ca, TR29248; and Cl, TR38025, Trace Scientific Ltd. Noble Park, Australia).

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AG (Polancic 2000) was obtained from the equation: $AG \text{ (mEq/L)} = [(Na^+ + K^+) - (Cl^- - HCO_3^-)]$, while SID (Stewart 1983) was obtained from the equation: $SID \text{ (mEq/L)} = [(Na^+ + K^+) - (Cl^- - Lactate)]$. Total weak acids (A_{total}) were calculated from the equation by Figge *et al.* (1992): $A_{total} \text{ (mEq/L)} = [Albumin * (1.23 * pH - 6.31) + [(Phosphorous (0.309 * pH - 0.469) * 10)]/30.97]$.

5.2.4 Statistical analysis

Least squares means and standard errors are presented. Data were analyzed by one-way ANOVA with treatment as the sole source of variation in the model. The ANOVA was conducted using SPSS 10 software package (SPSS, Chicago, IL). Multiple comparison tests were undertaken using Tukey's honestly significant difference test, where the level of significance was set at $P < 0.05$. Five animals had to be withdrawn from the analysis, water and feed deprived group ($n = 2$); and the transported group ($n = 3$) for difficulty in sampling arterial blood gases from these animals.

5.3 Results and Discussion

Stewart (1983) proposed a comprehensive quantitative method of acid-base analysis that required the distinction between independent and dependant variables involved in acid-base balance. That author demonstrated that acid-base homeostasis in plasma is regulated by changes in three independent variables: pCO_2 , SID and A_{total} , which can be changed independently of each other (Stewart 1983). While pCO_2 is regulated by the respiratory system, SID is mainly regulated by trans-membrane ionic exchanges, and A_{total} , although it has a significant influence on acid-base status, is not primarily regulated to maintain acid base homeostasis (Aguilera-Tejero *et al.* 2000).

This approach to acid-base analysis has offered an excellent qualitative framework for clinical interpretation of acid base disorders in a number of species (Weinstein *et al.* 1991; Pieschl *et al.* 1992; Frischmeyer and Moon 1994; Aguilera-Tejero *et al.* 2000). It offers researchers and clinicians the ability to identify the mechanisms

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involved in changing acid-base status and thereby focusing an appropriate treatment on the inciting cause (Constable 2002).

Blood gases, plasma electrolytes and metabolites are presented in Table 5.1. There was no difference in the pH of arterial blood in the treatment groups, confirming other data, albeit on venous blood, that transportation stress causes no difference in the acid-base status of transported compared to non-transported ruminants (Schaefer *et al.* 1988, 1992). Arterial pH values recorded in all treatment groups in the present study were similar to those reported by Mirakhur *et al.* (1985) in normal *Bos indicus* cattle (7.47 ± 0.04) and also in *Bos taurus* cattle (7.43 ± 0.03) by Fisher *et al.* (1980). The water and feed deprived animals had a lower pCO₂ compared to the control animals ($P = 0.023$). However, the pCO₂ values for the transported animals did not differ from those of the control group ($P = 0.126$). A lowering of the pCO₂ concentration in the blood of the water and feed deprived animals and a trend toward the same in the transported group demonstrates a compensatory mechanism used to buffer against a mild metabolic acidosis caused by dehydration. The primary pathology for metabolic acidosis, a low [HCO₃⁻], results in a low pH that stimulates respiration. This produces a low pCO₂, which reverts the pH towards normal. However, this process reaches a compensatory limit at approximately 12 h (Walmsley *et al.* 1988). If the inciting cause of the acidosis persists, it is the renal system that reverts the pH back to normal via reabsorption and production of HCO₃⁻ and secretion of H⁺ ions (Guyton and Hall 2000). It is difficult to state which compensatory system contributed to the differences seen in [pCO₂] in the water and feed deprived and transported groups. However, the fact that the feed and water deprivation and transit was prolonged (48 h), leads to the speculation that it is a metabolic system that is influencing [pCO₂].

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Table 5.1. Least squares means \pm SEM for blood pH, blood gases, plasma lactate, electrolytes, albumin, total protein, anion gap (AG), strong ion difference (SID) and total weak acids (A_{total}) in *Bos indicus* steers subjected to 48 h of transportation and fasting (Transported), or fasting alone (Water and feed deprived), or offered *ad libitum* feed and water (Control).

Variables	Control (n = 8)	Water and feed deprived (n = 6)	Transported (n = 5)
pH	7.44 \pm 0.01 ^a	7.46 \pm 0.01 ^a	7.46 \pm 0.02 ^a
PCO ₂ mm Hg	42.63 \pm 1.45 ^a	36.08 \pm 1.66 ^b	37.82 \pm 1.82 ^{a,b}
HCO ₃ mmol/L	28.87 \pm 1.16 ^a	25.47 \pm 1.34 ^a	27.08 \pm 1.47 ^a
Lactate mmol/L	0.59 \pm 0.11 ^a	0.75 \pm 0.12 ^a	0.62 \pm 0.13 ^a
Na ⁺ mmol/L	139.48 \pm 1.83 ^a	141.13 \pm 2.11 ^a	140.72 \pm 2.32 ^a
K ⁺ mmol/L	4.41 \pm 0.12 ^a	4.10 \pm 0.14 ^{a,b}	3.86 \pm 0.15 ^b
Ca ⁺⁺ mmol/L	2.30 \pm 0.06 ^a	2.48 \pm 0.07 ^a	2.34 \pm 0.07 ^a
Cl ⁻ mmol/L	97.75 \pm 1.82 ^a	102.67 \pm 2.10 ^a	98.80 \pm 2.30 ^a
PO ₄ ⁻ mmol/L	2.17 \pm 0.18 ^a	2.80 \pm 0.20 ^a	2.69 \pm 0.22 ^a
AG mEq/L	17.26 \pm 3.33 ^a	17.10 \pm 3.85 ^a	18.70 \pm 4.21 ^a
SID mEq/L	49.02 \pm 3.00 ^a	45.80 \pm 3.47 ^a	48.73 \pm 3.80 ^a
Total Protein g/L	64.13 \pm 1.92 ^d	81.33 \pm 2.43 ^e	78.60 \pm 2.22 ^e
Albumin g/L	35.12 \pm 1.00 ^{a,d}	41.17 \pm 1.15 ^{a,c}	39.60 \pm 1.26 ^{b,d}
A ^{total} mEq/L	11.07 \pm 0.34 ^d	13.46 \pm 0.40 ^c	12.95 \pm 0.44 ^c

a, b, c with a row means that do not have a common superscript letter differ, P < 0.05.
d, e, f within a row means that do not have a common superscript letter differ, P < 0.01.

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There were no differences in HCO_3^- concentrations in the treatment groups and all groups remained within normal limits for cattle (20 – 30 mmol/L) (Blood and Radostits 1989). Schaefer *et al.* (1988, 1990) demonstrated a decrease in pCO_2 and subsequently HCO_3^- concentrations in the venous blood of cattle exposed to the marketing process and feed and water deprivation, respectively. Total protein or albumin concentrations were not reported in these studies, however, animals were withheld from water and feed for up to 72 h, and Schaefer *et al.* (1990) recorded increases in hematocrit, hemoglobin and red blood cells indicating a state of dehydration. Thus, the changes seen post-treatment by Schaefer *et al.* (1988, 1990) are more likely the result of a respiratory and/or metabolic compensation for a mild metabolic acidosis secondary to water loss.

As a consequence of dehydration, the biosynthesis of the L-isomer of lactic acid from anaerobic glycolytic metabolism by skeletal muscle may be increased (Nappert and Johnson 2001). There was no difference between treatment groups for plasma lactate concentrations. Transportation or water and feed deprivation, in the present study, failed to elevate lactate concentrations to values as high as those demonstrated by Mitchell *et al.* (1988) or Schaefer *et al.* (1988). Mitchell *et al.* (1988), working with *Bos indicus* x *Bos taurus* steers and heifers, demonstrated a difference for lactate values between handling (3.1 ± 1.8 mmol/L), transport for 2 h (4.0 ± 2.2 mmol/L) and animals which had not been handled or transported (0.3 ± 0.2 mmol/L). Schaefer *et al.* (1988), who transported *Bos taurus* steers and heifers for 6 h obtained plasma lactate levels of 5.53 mmol/L prior to the stress of transportation and 6.50 mmol/L at slaughter.

The lactate data reported by Schaefer *et al.* (1988) may be artefactually elevated due to a pre-analytical error, especially to the pre-transport values, because there is no indication that an anti-glycolytic agent was used in collection tubes. If the analysis of plasma lactate by Schaefer *et al.* (1988) were collected in tubes containing lithium heparin, anaerobic glycolysis may have occurred within the samples (Polancic 2000).

The difference seen between studies for lactate concentrations may be due to time in transit. Tarrant (1990) indicated that 24 h of transportation fatigued steers enough to induce resting behaviors in transit. Resting in transit during the current study may

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have been sufficient to decrease lactate concentration in the transported steers compared to that expressed in the other groups housed in pens. Tarrant (1990) further stated that, during short transit times, cattle tend not to lie down in trucks while they are moving. The constant standing and bracing during short haul transit may elevate lactate concentrations in cattle during these journeys. This is consistent with other reports that indicate that the major effects of transport stress take place during the early portions of transport (Cole *et al.* 1988).

Mitchell *et al.* (1988) noted that the animals used in their study were unaccustomed to being handled. In contrast, the animals used in the present study had been extensively handled by experienced stockmen. The lactate concentrations reported here may also reflect the beneficial effects of a sound management program in minimizing lactate accumulation in cattle subjected to transportation of long duration.

In agreement with the work of Galyean *et al.* (1981), plasma Na was not influenced by treatment, and remained within normal values for cattle (Blood and Radostits 1989). Transported animals had lower concentrations of plasma K compared to the control animals ($P = 0.026$). It is well recognized that stressor-induced activation of the HPA axis stimulates the secretion of cortisol, resulting in the excretion of K (Parker *et al.* 2003a). The hypokalemia associated with the transported group may also be the result of a lack of feed intake, although, this observation was not replicated by the water and feed deprived group.

Plasma concentrations of Ca, Cl and P did not differ between groups. However, there was a trend ($P = 0.07$) for plasma concentrations of P to be higher in the water and feed deprived group than in the control group. Galyean *et al.* (1981) reported plasma P concentrations to be higher in fasted than in transported animals at 32 h, although, as in the present study, changes were small and within physiological limits.

The AG is a diagnostic concept that demonstrates the difference between unmeasured anions and unmeasured cations (Guyton and Hall 2001). Usually, the unmeasured anions exceed the unmeasured cations, with the AG for cattle ranging between 14 to 26 mEq/L (Blood and Radostits 1989). Strong ions move between

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body fluids through membranes, and the resulting changes in SID values provide the major mechanism for acid-base interactions between fluids (Stewart 1983). Despite the small but significant changes in the K concentration of plasma in the transported animals, there were no differences between groups for the AG or SID calculations. This would suggest that electrolyte solutions fed to these steers post-transport would provide little benefit in correcting their acid-base balance compared to water alone. In support of this suggestion, AG did not differ significantly between low and moderate stress groups of cattle offered water when compared to moderately stressed cattle offered only an electrolyte solution (Schaefer *et al.* 1994).

The application of electrolyte solutions to minimize transport stress in cattle has been extensively investigated (Schaefer *et al.* 1988; Gortel *et al.* 1992; Schaefer *et al.* 1992; Phillips 1997; Schaefer *et al.* 1997). There is a trend in the literature for increases in the extra-cellular fluid, carcass weight and body weight of cattle when electrolyte solutions are fed compared to no fluids offered post-transport. The effects of the electrolyte solutions fed in these studies were to replenish lost TBW in the animals involved (Schaefer *et al.* 1992; Gortel *et al.* 1992; Schaefer *et al.* 1997). Similarly, the same effects can be seen when cattle are offered water post-transport (Wythes *et al.* 1980; Wythes *et al.* 1983). A comparative study using an electrolyte solution and water as treatments by Gortel *et al.* (1992) demonstrated no difference ($P < 0.05$) in carcass yield as a proportion of farm weight, rumen weights, extra cellular fluid volume, plasma volume, hematocrit, serum Na, K, glucose or β -hydroxybutyric acid between the water and electrolyte fed groups. Lower values for plasma osmolality, serum Cl and serum lactate were found between the water and electrolyte groups, respectively. This was a reflection of the amount of fluid consumed post-transport between the treatments. There was a difference recorded for hot carcass weight as a proportion of the pre-slaughter weight between the electrolyte and water treated groups, this difference, however, could be attributed to the higher intake of fluid by the water group causing the animals to be heavier at slaughter than the electrolyte group. Subsequently, the carcass yield as a proportion of the pre-slaughter weight would be lower for the group offered water if carcass weights were similar. Unfortunately, carcass weights were not reported in that study.

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Plasma total protein, albumin and A_{total} concentrations were higher for the transported ($P = 0.001$, $P = 0.03$, $P = 0.01$) and water and feed deprived ($P = 0.001$, $P = 0.003$, $P = 0.001$) groups, respectively, than for the control group. Transport stress has been observed to cause dehydration and may manifest itself as a hyperproteinemia (Atkinson 1992; Schaefer *et al.* 1997). Serum proteins, especially albumin, act as weak acids in plasma. The role of proteins in acid-base balance has practical importance: hypoproteinemia and hyperproteinemia by themselves cause metabolic alkalosis and acidosis respectively (Figge *et al.* 1991, 1992). Hemoconcentration secondary to dehydration elevates total protein and is a contributing factor toward metabolic acidosis (Walmsley *et al.* 1988; Figge *et al.* 1991, 1992; Nappert and Johnson 2001).

Transportation and water and feed deprivation resulted in an increase in A_{total} compared to control animals due to an elevation in albumin concentration. The changes seen in plasma albumin and hence total protein concentrations in the water and feed deprived and transported groups are likely to be due to hemoconcentration secondary to water loss. The increase in albumin and hence total protein and A_{total} would contribute to a mild metabolic acidosis. The resulting hypovolemia and low tissue perfusion may also cause a limited supply of oxygen to tissues and a decrease in H excretion by the kidneys (Nappert and Johnson 2001).

It would appear, in the present study, that the water and feed deprived group incurred a greater deviation from the control group in Cl, total protein, albumin and total acids compared to the transported group, in spite of the winter THI for the region being mild for tropically adapted cattle. These parameters would indicate a greater level of dehydration in the feed and water deprived group although statistically this is not different to the transported group. This may imply that the tractable genotype used in this study found feed and water deprivation to be a greater challenge to acid-base homeostasis than transportation itself. Further to this, the increased airflow created by transportation may have had a cooling effect on the animals thereby decreasing the amount of water loss, and hence, the degree to which the animals compensatory acid-base mechanisms were employed.

5.4 Implications

In conclusion, the results of this study indicate that *Bos indicus* steers transported for 48 h are able to maintain their acid-base balance within normal values. The primary challenge to these animals appears to be the elevation of total weak acids via an increase in plasma albumin concentration due to dehydration. This was compensated for by the respiratory and renal systems decreasing the pCO₂ concentration in arterial blood. Plasma electrolytes were selectively altered, however, and the SID and AG did not differ between the control, water and feed deprived and transported groups. Offering electrolyte solutions to dehydrated, transported, nutrient deprived and stressed *Bos indicus* cattle is unlikely to resolve the physiological stressors any more efficiently than water alone.

CHAPTER SIX

PHYSIOLOGICAL AND METABOLIC EFFECTS OF PROPHYLACTIC TREATMENT WITH THE OSMOLYTES GLYCEROL AND BETAINE ON *BOS INDICUS* STEERS DURING LONG DURATION TRANSPORTATION

ABSTRACT: The physiological and metabolic effects of prophylactic treatment with osmolytes were investigated using twenty four, 2.5 yr old *Bos indicus* steers. Animals were allocated to one of four treatment groups: 1) Control, feed and water deprived (n = 6); 2) Transported, transported for 48 h (n = 6); 3) Glycerol, dosed with glycerol (2 g/kg BW) and transported for 48 h (n = 6) and; 4) Betaine, dosed with betaine (80 g/steer) and then transported for 48 h (n = 6). Body water, electrolytes, blood pH and gases, plasma lactate, glucose, albumin, total protein, AG, strong ion difference, total weak acids and liveweight were determined at the conclusion of 24 and 48 h of transportation. The Glycerol group had significantly higher body water concentrations than the Control (P = 0.05) and Transport (P = 0.02) groups. The Glycerol, Transported (P = 0.02) and Betaine (P = 0.02) groups had lower plasma concentrations of Mg than the Control group at 24 h, while the Glycerol group maintained lower plasma concentrations of Ca than the Control group (P = 0.04). The Betaine group had significantly lower hematocrits than the Control group (P = 0.04), at 24 and 48 h. Plasma bicarbonate and pCO₂ were 13 and 17% higher (P = 0.01) in the Glycerol group (P = 0.04) at 24 h, compared to Control and Transported animals. However, the ratio of [HCO₃]/[CO₂] in the Glycerol group was not significantly different from the other groups, and thereby maintained pH. The Betaine group demonstrated a similar acid-base profile to the Glycerol group. The Glycerol group maintained a 30% higher plasma concentration of glucose than the Control (P = 0.00), and 14% higher than the Transport and Betaine groups. In contrast, the osmolyte betaine had little effect on increasing blood glucose compared to glycerol. Glycerol-linked hyperhydration at 24 h may not only have aided in the conservation of water loss during long distance transportation, but the increased blood glucose may have had important protein-sparing effects due, in part, to higher insulin levels inhibiting the breakdown of muscle proteins, and to countering the amino-acid mobilising effect of cortisol after 24 h. Therefore the osmolyte glycerol shows promise in attenuating the effects of long distance transportation by

maintaining body water, decreasing the energy deficit, and preserving muscle quality.

6.1 Introduction

Prolonged periods of water deprivation, as occurs during long haul transportation of livestock, ultimately results in dehydration and a switch to a gluconeogenic state (Parker *et al.* 2003b, Schaefer *et al.* 1992; Tarrant *et al.* 1992). Hydration strategies currently employed rely on the replacement of lost body water and electrolytes at the completion of the journey after the welfare of the animals has been compromised (Schaefer *et al.* 1990, 1997; Wythes *et al.* 1980). However, expansion of plasma volume before exposure to a dehydrating environment such as the marketing process is problematic for most mammalian species. Hyper-hydration with water alone is transitory, as the kidney rapidly excretes any excess fluid. Prophylactic hyper-hydration with osmolytes has been used to prevent or delay the development of dehydration in human athletes and improve performance in the heat and high humidity (Hitchins *et al.* 1999; Freund *et al.* 1995). The administration of osmolytes prior to transportation of long duration may have merit in attenuating the deleterious effects of dehydration and promote an early and pronounced gluconeogenic state while sparing muscle protein degradation. Investigated here were the effects of two organic osmolytes, betaine and glycerol, on the TBW, electrolyte, glucose and acid-base balance of *Bos indicus* steers subjected to transportation of 48 h. The working hypothesis was that prophylactic treatment of steers with osmolytes would attenuate the loss of body water, enhance the gluconeogenic state, decrease the energy deficit and spare muscle protein degradation during transportation of long duration.

6.2 Materials and Methods

6.2.1 Animals and management

Twenty four, 2.5 yr old *Bos indicus* steers of high grade Brahman genotype (321.00 ± 4.60 kg mean BW) were sorted in an ascending order of liveweight and allocated to one of four treatment groups at random: 1) Control, feed and water deprived (n = 6); 2) Transported, deprived of feed and water and transported for 48 h (n = 6); 3)

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Glycerol, dosed with glycerol, (2 g/kg BW, Biotech Pharmaceuticals Pty Ltd, Carole Park, Qld, Australia) deprived of feed and water and transported for 48 h (n = 6) and; 4) Betaine, dosed with betaine (Betafin 80 g/steer, Danisco Animal Nutrition, Wiltshire, UK) deprived of feed and water then transported for 48 h (n = 6). All transported animals were dosed using a naso-gastric tube (7 mm O.D x 5 mm I.D) and all treatments had 500 mL of distilled water added to aid the flow of the product through the tube. The transported group received a placebo of 500 mL of distilled water. All groups had their water and feed withdrawn 12 h prior to departure of the transported groups.

The study was divided into 3 journeys of 48 h over a 2 wk period, with animals allocated to journeys at random. The transported groups were trucked for 24 h, unloaded, sampled and weighed and then transported for a further 24 h, before being unloaded, sampled, and weighed again. The sampling process, including the body water assays, took 2.5 h to complete. The animals did not have access to feed and water in the yards while waiting to be sampled and were immediately processed upon exiting the unloading ramp. The transported animals were conveyed in a rigid truck equipped with an adjustable gate separating the holding compartment into two areas. The transported animals were loaded into the forward and rear compartments at a density of 1.20 m²/animal. This stocking rate enabled balanced treatment groups per trip and is within normal stocking densities for cattle transported over long distances.

At sampling, all animals were forced into a race where the animals were captured in a cattle head bail and restrained. A halter was placed on the individual animals and their heads were then restrained to the side with an attendant holding the head while samples were taken. When cattle are captured and restrained, abnormal physiological reactions to the restraint may be expressed as an increase or decrease in respiration rates. Subsequently, the blood gas parameters of the animal become affected. While the possibility exists for an abnormal measurement of blood gas parameters to have taken place in an animal from this study, it is unlikely when the current results are compared to those of other authors (Mirakhur *et al.* 1985). Further to this, the animals used in the present study were accustomed to being handled, and behaved in a quiet and amicable manner when sampled.

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During journey 1, the blood gas machine developed a mechanical problem at the 24 h sampling. Subsequently, the samples for blood gas analysis could not be processed within the required time limit (<0.5 h) to yield meaningful data. The data in Table 6.1 reflect this incident where for the 24 h analysis for blood gas measurements; n = 4/group, except the control group where n = 5.

A temperature and humidity index (THI) was calculated by the formula presented by Gaughan et al. (1999). The mean daily THI during the experimental period for the transit period were 74 and 73, respectively. The Control group was housed in outdoor pens with minimal shade.

All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (Approval No. A730-02).

6.2.2 Sample collection

After 24 and 48 h of transportation, 24.5 mL of blood was manually collected by jugular venepuncture from all groups; 20 mL into 2 x 10 mL tubes containing lithium heparin (Disposable Products Pty Ltd, Adelaide, SA, Australia); 1 x 2.5 mL tube containing fluoride oxalate (Sarstedt Australia, Technology Park, SA, Australia), and a 2 mL blood gas syringe containing lithium heparin (Sarstedt Australia, Technology Park, SA, Australia) to sample venous blood gases. The samples containing fluoride oxalate were used for the analysis of plasma lactate and glucose. The tubes containing lithium heparin were used for all other analyses. Blood samples were immediately placed into an ice water slurry then centrifuged at 200 x g for 15 min and plasma poured off within 2 h and frozen (- 20° C) for analysis at a later date. Blood gas syringes were capped and placed into an ice water slurry for immediate analysis of blood gases. All blood gas assays were performed within 0.5 h of collection.

6.2.3 Measurement

Venous blood pH, partial pressure of carbon dioxide (pCO₂) and bicarbonate (HCO₃⁻) were measured using a blood gas analyzer (Ciba Corning Model 278, Bayer Diagnostics, Brisbane, Qld, Australia). Plasma concentrations of Na and K were measured using ion selective electrodes (Lablyte System 830, Beckman Instruments Inc, Brea, California, USA). Sodium and K samples were completed on singular samples and quality control samples (Liquichek controls; 16171 and 16172, Bio-Rad laboratories, Regents Park, NSW, Australia) were performed every 10 samples. Lactate, glucose, albumin, total protein, P, Ca, Mg, and Cl concentrations in plasma were measured using a Mira Autoanalyzer (Roche Diagnostics, Brisbane, Australia) with standard enzymatic and spectrophotometric kits (Lactate, Roche Diagnostics, Brisbane, Qld, Australia; and Glucose, TR 15004; P, TR30025; Albumin, TR36025; Total Protein, TR34025; Ca, TR29248; and Cl, TR38025, Trace Scientific Ltd. Noble Park, Australia). Plasma urea N was analysed with a Technicon Auto analyser 2 (Bran + Leubbe Pty Ltd., Homebush, NSW, Australia) according to the Auto-analyser method SE40001FD4.

AG (Polancic 2000) was obtained from the equation: $AG \text{ (mEq/L)} = [(Na^+ + K^+) - (Cl^- - HCO_3^-)]$, while SID(SID) (Stewart 1983) was obtained from the equation: $SID \text{ (mEq/L)} = [(Na^+ + K^+) - (Cl^- - Lactate)]$. Total weak acids (A_{total}) were calculated from the equation by Figge *et al.* (1992): $A_{total} \text{ (mEq/L)} = [Albumin * (1.23 * pH - 6.31) + [(Phosphorous (0.309 * pH - 0.469) * 10)]/30.97]$.

6.2.4 Urea Space Measurements

Urea space was determined at the conclusion of each 24 h transit period for each animal using the technique described by Preston and Kock (1973). In brief, following catheterisation of the jugular vein, a solution containing 20% (wt/vol) urea dissolved in 0.9% (wt/vol) saline was administered through the catheter over a 2-min period. The volume injected was calculated to provide 130 mg urea/kg liveweight. The catheter was immediately flushed with 15mL of heparinized saline solution (35,000 IU/L of 0.9% saline) to prevent clotting between samples. Blood samples were collected through the catheter prior to infusion and at 15 min post-infusion.

6.2.5 Statistical analysis

A two way ANOVA, with the main effects of time (24 h and 48 h) and group (Control, Transported, Glycerol or Betaine) and an interaction effect of group nested within time were analyzed statistically using SPSS 10 software package (SPSS, Chicago, IL). Quantitative variables (plasma electrolytes, albumin, total protein, glucose, hematocrit, osmolality, and blood gases) were independently sampled. Liveweight and accumulated BW loss (Table 6.5) were analyzed using a two way repeated measures ANOVA, with the main effects of time (24 and 48 h) and group (control, transported, glycerol or betaine) and the interaction effect of group x time using SPSS 10. Least squares means and standard errors are presented. Multiple comparison tests within the factors were performed using Tukeys honestly significant difference test. Differences were considered significant for $P < 0.05$.

6.3 Results and Discussion

6.3.1 Blood Acid-Base status

The acid-base balance of body fluids is closely regulated because all functional proteins within the body are influenced by hydrogen ion concentration (Guyton and Hall 2000) and, consequently, pH homeostasis of body fluids is guarded vigorously. There were no differences in the pH of venous blood between treatment groups at the specified sampling times (Table 6.1). This confirms other data that it is likely that transportation stress results in no difference in the acid-base status of transported, arterial or venous blood compared to non-transported ruminants (Parker *et al.* 2003b, Schaefer *et al.* 1988, 1992).

Plasma HCO_3 concentration was significantly higher in the Glycerol group compared to the Control group at 24 h ($P = 0.04$). No further difference was detected between the groups at 48 h. The end effect of the increased plasma HCO_3 concentration in the Glycerol-treated animals is complex it may involve respiratory and/or metabolic compensation, which in turn could lead to a higher HCO_3 and a higher pCO_2 for a constant $[\text{HCO}_3]/[\text{CO}_2]$, thereby maintaining blood pH. Indeed, this is evident in all

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groups withheld off feed and water in the present study, and regardless of the compensatory mechanisms or inciting cause involved this ratio remained constant.

A possible explanation for the elevation of HCO_3^- in the Glycerol group may be the down regulation of the urea cycle in these animals. Normally, in a cortisol-induced gluconeogenic state, skeletal muscle protein supplies most of the carbon for the liver needed for net glucose synthesis. As a result of the excess nitrogen arising from the metabolic breakdown of amino acids, the urea cycle is up-regulated to bring together two amino groups as NH_4 and one as HCO_3^- ion to form urea, which is excreted in the urine (Lehninger *et al.* 1993). If glycerol became a preferential fuel source and spared the catabolism of muscle protein, NH_4 would be low and HCO_3^- may remain the same or increase. Although no significant difference was found in plasma urea concentrations between groups at 24 or 48 h (Table 6.4), the urea concentration was lower in the glycerol-treated animals at 24 h. In addition, slowing the urea cycle would be beneficial because urea production is energetically expensive requiring 5 moles of ATP for each mole of urea formed (Lehninger *et al.* 1993), and this would be of particular importance during nitrogen- or energy-limiting situations such as starvation or during livestock transport.

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Table 6.1. Least squares means \pm SEM for acid base parameters from venous blood in *Bos indicus* steers treated with osmyolytes and subjected to road transportation for 48 h.

	Control	Transported	Glycerol	Betaine
24 h				
pH	7.40 \pm 0.02	7.38 \pm 0.02	7.38 \pm 0.02	7.35 \pm 0.02
pCO ₂ mm/Hg	42.72 \pm 1.70 ^{a,d}	47.18 \pm 1.90	51.08 \pm 1.90 ^d	51.70 \pm 1.90 ^a
HCO ₃ mmol/L	26.44 \pm 0.78 ^b	27.93 \pm 0.85	30.38 \pm 0.85 ^b	28.33 \pm 0.85
Anion Gap meq/L	11.73 \pm 2.82	18.08 \pm 3.15	13.84 \pm 3.15	20.98 \pm 3.15
SID meq/L	35.88 \pm 2.25	41.94 \pm 2.25	44.46 \pm 2.25	44.79 \pm 2.25
A _{total} meq/L	12.38 \pm 0.29	12.45 \pm 0.33	12.26 \pm 0.33	11.93 \pm 0.33
[HCO ₃]: [CO ₂]	20.66 \pm 0.73	19.75 \pm 0.82	19.82 \pm 0.82	18.50 \pm 0.82
48 h				
pH	7.41 \pm 0.02	7.42 \pm 0.02	7.38 \pm 0.02	7.37 \pm 0.02
pCO ₂ mm/Hg	42.92 \pm 1.55	42.50 \pm 1.55 ^a	47.12 \pm 1.55	48.18 \pm 1.55 ^a
HCO ₃ mmol/L	27.10 \pm 0.69	27.68 \pm 0.69	27.88 \pm 0.69	27.78 \pm 0.69
Anion Gap meq/L	15.47 \pm 2.58	18.00 \pm 2.58	18.42 \pm 2.58	16.78 \pm 2.58
SID meq/L	41.20 \pm 2.25	44.75 \pm 2.25	44.87 \pm 2.25	42.40 \pm 2.25
A _{total} meq/L	13.18 \pm 0.27	12.68 \pm 0.27	12.76 \pm 0.27	12.42 \pm 0.27
[HCO ₃]: [CO ₂]	21.05 \pm 0.67:1	21.74 \pm 0.67:1	19.91 \pm 0.67:1	19.36 \pm .67:1

Within a row, means with a common superscript letter differ. ^{a,b}P < 0.05, ^{c,d}P < 0.01

24 h n = 4 per group except control where n = 5; 48 h n = 6 per group

pCO₂ = partial pressure of carbon dioxide

HCO₃ = bicarbonate concentration

SID = Strong Ion Difference

A_{total} = Total weak acids

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There were no differences in AG between groups at 24 or 48 h (Table 6.1). The AG values demonstrated for all groups at 24 and 48 h remained within previously reported values for penned *Bos indicus* steers that had access to feed and water (17.26 ± 3.33 mEq/L) (Parker *et al.* 2003b). This is in contrast to the data presented by Schaefer *et al.* (1990) in which the AG of bulls transported for 6 h decreased by 23 mEq/L between pre- and post-transport values. Schaefer *et al.* (1990), state that the physiological implications of such a large shift in the AG by the animals in their study may not be as significant as the statistics imply, due to large variances within populations for AG values. The use of the AG suffers from poor diagnostic accuracy, and reliance on the AG can result in misinterpreting the animal's actual condition. Thus, the AG is no substitute for measurement of electrolytes, blood pH, blood gases, lactate and other data pertinent to the condition of the animal (Kleinman and Lorenz 1996). Indeed, if the entire acid-base clinical picture is portrayed there is little difference between the pre and post-transportation acid-base status of the bulls used by Schaefer *et al.* (1990).

Similarly, the SID also demonstrated no treatment group effects at either 24 or 48 h. Total weak acids showed no difference between groups or any effect due to time. The values reported were similar to previous results in *Bos indicus* steers subjected to long haul transportation of 48 h (Parker *et al.* 2003b).

6.3.2 Plasma Electrolytes

Plasma electrolytes are presented in Table 6.2. There was no difference between groups for plasma Na, and all groups remained within normal values for cattle. Potassium showed a trend ($P = 0.06$) for increasing plasma K as time increased. Potassium remained within normal limits for cattle (Blood and Radostits 1989); this result is in contrast with other authors who demonstrated a decrease in K with increasing time in transit ascribed to a lack of feed intake or the activation of the hypothalamo-pituitary-adrenal axis (Schaefer *et al.* 1990; 1994; Parker *et al.* 2003a,b).

Table 6.2. The effects of osmolyte treatment prior to transit on serum electrolytes after 24 h and 48 h of transit.

Group	Control	Transported	Glycerol	Betaine
24 h				
Sodium mmol/L	134.88 ± 2.30	145.31 ± 2.30	141.65 ± 2.30	137.98 ± 2.30
Potassium mmol/L	4.00 ± 0.20	3.75 ± 0.20	3.97 ± 0.20	4.00 ± 0.20
Chloride mmol/L	101.67 ± 1.46	97.67 ± 1.46	97.17 ± 1.46	98.17 ± 1.46
Calcium mmol/L	2.49 ± 0.05 ^a	2.45 ± 0.05	2.36 ± 0.05 ^a	2.42 ± 0.05
Magnesium mmol/L	0.81 ± 0.02 ^{a,b}	0.74 ± 0.02 ^a	0.75 ± 0.02	0.71 ± 0.02 ^b
Phosphorous mmol/L	2.15 ± 0.21	2.44 ± 0.21	2.25 ± 0.21	2.08 ± 0.21
48 h				
Sodium mmol/L	141.13 ± 2.30	141.33 ± 2.30	143.00 ± 2.30	142.73 ± 2.30
Potassium mmol/L	4.10 ± 0.20	3.85 ± 0.20	4.64 ± 0.20	4.16 ± 0.20
Chloride mmol/L	102.67 ± 1.46	99.50 ± 1.46	101.33 ± 1.46	102.33 ± 1.46
Calcium mmol/L	2.49 ± 0.05 ^a	2.37 ± 0.05	2.34 ± 0.05 ^a	2.45 ± 0.05
Magnesium mmol/L	0.77 ± 0.02 ^a	0.69 ± 0.02 ^a	0.72 ± 0.02	0.72 ± 0.02
Phosphorous mmol/L	2.80 ± 0.21	2.69 ± 0.21	2.72 ± 0.21	2.30 ± 0.21

Within a row, means with a common superscript letter differ. ^{a,b}P < 0.05, ^{c,d}P < 0.01

n = 6 per group

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The Glycerol, Transported ($P = 0.02$) and Betaine ($P = 0.02$) groups had lower plasma concentrations of Mg than the Control group at 24 h, and the Glycerol group maintained lower plasma Ca concentrations than the Control group ($P = 0.04$).

Fisher *et al.* (1999) demonstrated a decline in plasma Mg during long haul transportation in dairy cows despite treatment with supplemental Mg prior to transportation. The Mg concentration in cows from that study continued to decline after transportation ceased.

Calcium concentration of the Control group was higher than the Glycerol ($P = 0.04$) group. Stress and an inadequate intake of minerals have been associated with episodes of transport tetany. Treatment protocols that include electrolyte solutions that contain P, Mg, and Ca have been advocated in the sheep industry (Lucas 1982). Despite differences between groups for Mg and Ca, all groups remained within physiological limits at 24 and 48 h (Blood and Radostits 1989). A time effect was demonstrated for Cl ($P = 0.00$) and P ($P = 0.01$) increasing in concentration with time for all groups.

6.3.3 Metabolites

The Glycerol group maintained greater plasma glucose concentrations than the Control group ($P = 0.01$). Increased glycerol is not only an important carbon source for gluconeogenesis in liver, but the elevated blood glucose concentration in the glycerol-treated animals may have an important protein-sparing effect in part due to (i) providing a preferential fuel for liver gluconeogenesis, (ii) increasing insulin secretion and thereby further inhibiting breakdown of muscle protein, and (iii) countering the amino-acid mobilising effect of the stress hormone cortisol. In addition, carbohydrates and lipids have both been found to have nitrogen sparing effects in ruminants (Asplund *et al.* 1985; O'Kelly 1985), and cortisol has the opposite effect (Rang and Dale 1991).

It has been previously demonstrated that *Bos indicus* cattle rely to a greater extent on fat metabolism during fasting compared to *Bos taurus* cattle (O'Kelly 1985). Orally ingested glycerol follows the same carbohydrate metabolic pathway as endogenous

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glycerol from the breakdown of triglycerides. The ultimate fate of glycerol is dependant upon the individual's metabolic state; in starvation for example, glycerol is a primary fuel for liver gluconeogenesis, and the glucose formed is used by the brain (Freund *et al.* 1995). In addition, O'Kelly (1985) has suggested that *Bos indicus* animals utilized less muscle mass than *Bos taurus* animals during a 96 h fast. O'Kelly further argued that a high fat diet prior to fasting enhanced the suppression of muscle protein breakdown in both genotypes during fasting.

This heightened adaptation to a lipid fuel economy in times of nutritional stress may be exploited in animal production. Transportation stress and the associated elevation of cortisol concentrations predisposes animals to increased gluconeogenesis via the mobilization of amino acids from muscle and liver tissues, and the acceleration of lipid mobilization from fat stores (Guyton and Hall 2000). In a cortisol-induced gluconeogenic state, skeletal muscle protein supplies most of the carbon needed for net glucose synthesis (Lehninger *et al.* 1993). The resultant deleterious effects on carcass and meat quality have been well documented (Warriss 1990; Tarrant *et al.* 1992; Knowles 1999). Indeed all of the transported animals would have been in a gluconeogenic state in the present study, and the glycerol-treated animals had 30% higher cortisol levels compared to the other groups (Table 6.4).

Prophylactic glycerol administration may also antagonize cortisol's effects on the body's gluconeogenic and ketogenic amino acid pool as the alternate and preferential substrate for glucose production. The implications of this would be the preservation of carcass protein and a reduction in dark cutting beef. In contrast, the osmolyte betaine had little effect on increasing blood glucose compared to glycerol. There were no treatment group differences for total protein or albumin at 24 or 48 h. A time effect demonstrated an increase in total protein with increasing time in transit ($P = 0.01$).

Lactate, decreased in concentration with increased time transported ($P < 0.001$). Plasma concentrations of lactate for all the transported groups at 24 h were similar to those reported by other authors during short duration transport. Mitchell *et al.* (1988) transported *Bos indicus* x *Bos taurus* steers and heifers for 2 h and demonstrated an

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elevation of 4.00 ± 2.20 mmol/L for plasma lactate in the transported animals over the control group. Similarly, Schaefer *et al.* (1990) transported bulls for 6 h and demonstrated serum lactate to range from 3.36 ± 0.60 – 5.00 ± 0.59 mmol/L, between their treatment groups. Parker *et al.* (2003b) previously reported no difference in lactate concentrations between penned control steers and those subjected to long duration transport for 48 h, and hypothesized that this was due to the animals resting in transit during the last 24 h period. During the present study, individual animals started to lie down while the truck was in motion at 18 h. Resting in transit, presumably due to fatigue, has been reported to manifest itself in ruminants from 20 h in transit (Tarrant 1990; Tarrant *et al.* 1992; Knowles *et al.* 1999). This resting behaviour may have been sufficient to reduce plasma lactate concentrations in the transported steer groups at 48 h in the present study.

The deprivation of feed and water to all animals resulted in hematocrit values similar to those seen in long haul transportation studies (Knowles *et al.* 1999; Tarrant *et al.* 1992). However, a difference was detected between treatment groups. At 24 and 48 h, the penned Control animals had higher hematocrit values than all transported groups but were significantly different ($P = 0.04$) only to the Betaine group. Broom *et al.* (1996) demonstrated a similar phenomena in transported sheep, in which hematocrit was greater when the sheep were in a stationary unstressed condition. Similarly, restraint and isolation stress in sheep induced a significant decrease in hematocrit compared to handling alone (Parrott *et al.* 1987). Stressor induced changes in fluid compartments within the transported animals may be responsible for these changes (Broom *et al.* 1996). This result perhaps highlights the inconsistency of utilizing hematocrit as an indicator of hydration status in animals placed under stress.

Plasma cortisol concentrations are shown in Tables 6.3 and 6.4, and there were no significant differences between groups at 24 or 48 h, although in the glycerol-treated animals at 48 h the cortisol concentrations were 30% higher values than other groups. Differences among the groups may reflect the morning elevation in cortisol due to the circadian rhythm of the steers. However, the higher cortisol levels in the

glycerol-treated animals at 48 h may add to the already enhanced gluconeogenic state at 24 h.

6.3.4 Body Water

The Glycerol group had a greater body water content at 24 h than the control ($P = 0.05$) and transport ($P = 0.02$) groups. One animal contributed largely to the statistical difference seen in the glycerol group. The deletion of this outlier animal from the group would yield a non-significant result. Therefore, the body water data should be interpreted with caution. Human trials with glycerol have demonstrated a greater retention of fluid within the body and a delay in the loss of body water in tropical and temperate environs (Riedesel *et al.* 1987; Freund *et al.* 1995; Hitchins *et al.* 1999).

Riedesel *et al.* (1987) demonstrated in humans that glycerol with excess water could produce a state of hyper-hydration for 4 h. Freund *et al.* (1995) reported that glycerol increases fluid retention by reducing free water clearance. An increase in urination has been shown to be a contributing factor to dehydrating animals under stress. El-Nouty *et al.* (1980) demonstrated an increase in urine output under conditions of heat stress in cattle, and Parker *et al.* (2003a) demonstrated a diuresis in sheep with stress-like concentrations of cortisol. It would therefore be reasonable to hypothesize that osmolytes such as glycerol may attenuate the effects of dehydration in transported ruminants, however, our data appears equivocal. While the body water data supports a greater hydration effect for glycerol, liveweight does not reflect the expected higher value for the glycerol group. Our failure to concurrently demonstrate a higher liveweight for the glycerol group in support of the body water assay may lie in an increase in gut motility and hence a greater loss of GIT contents for the glycerol group. Indeed, the pharmacologic effects of glycerol in humans are known to include nausea, gastrointestinal cramps and vomiting (Wagner 1999). Preston and Kock (1973) concluded that urea space in the ruminant was a measure of empty body water (TBW less the water in the gastrointestinal tract). It may be that the dose rate of glycerol used in this study maintained empty body water in the steers but increased gastrointestinal emptying.

Hyper-hydration with osmolytes may only slow the development of water loss from the body as such. Perhaps a greater difference between both osmolyte treated groups would be seen in shorter transportation intervals. Gortel *et al.* (1992) commented that the carcass of the transported animal is of greater importance in detecting differences due to treatment or time transported. Further studies involving the use of osmolytes and the subsequent carcass appraisal on ruminants are recommended.

6.3.5 Liveweight

There was a treatment group x time interaction between 24 and 48 h ($P = 0.01$) indicating that the control group had lost less liveweight than the transported groups at 48 h (Table 6.5). A time effect for all treatment groups showed a decrease in liveweight with increased time off feed and water. Liveweight losses are similar to other reported values in long haul transportation studies (Camp *et al.* 1981; Fisher *et al.* 1999). Wythes *et al.* (1980) stated that liveweight losses from fasting and transport largely reflect changes in gut fill, and defecation and urination rates (Wythes *et al.* 1980). There was no differentiation due to treatment group or transportation during the first 36 h of feed and water deprivation for liveweight, suggesting that transportation stress *per se* did not have a significant effect on liveweight shrink. This is in contrast to the study by Cole *et al.* (1988) where transportation of 195 kg calves caused a significant increase in shrinkage, compared to feed and water deprivation alone.

6.4 Implications

The prophylactic treatment of *Bos indicus* steers with the osmolyte glycerol attenuated the loss of body water during transportation for 24 h but it did not appear to be effective at 48 h. However, the glycerol-linked enhanced gluconeogenic state persisted for 48 h. Elevated plasma glucose levels in the glycerol-treated animals would lead to higher insulin levels which in turn would inhibit the breakdown of muscle proteins, and also counter the amino-acid mobilising effect of increased cortisol concentrations. In conclusion, glycerol treatment resulted in hyperhydration, decreases in the energy deficit, and enhanced the gluconeogenic state of the animals

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that may lead to an insulin-linked sparing of muscle protein degradation during transportation of long duration. The implications of these findings would be the preservation of carcass protein and a reduction in dark cutting beef. Further studies are required to elucidate the benefit and underlying mechanisms of glycerol-treatment to minimize muscle wasting and promote the health of the animal during livestock transport

Table 6.3. Least squares means \pm SEM for TBW, cortisol, glucose, lactate, hematocrit, albumin and total protein in *Bos indicus* steers treated with osmolytes or not and subjected to road transportation for 24 h.

	Control	Transported	Glycerol	Betaine
TBW %	51.64 \pm 2.78 ^a	51.06 \pm 2.78 ^b	62.44 \pm 2.78 ^{a,b}	53.01 \pm 2.78
Cortisol, ng/mL	26.54 \pm 5.36	23.10 \pm 5.36	24.69 \pm 5.36	12.97 \pm 5.36
Glucose mmol/L	4.78 \pm 0.42 ^{a,c}	5.87 \pm 0.42	6.87 \pm 0.42 ^c	5.98 \pm 0.42
Lactate mmol/L	1.33 \pm 0.45	2.13 \pm 0.45	2.35 \pm 0.45	2.70 \pm 0.45
Hematocrit %	43.25 \pm 1.54 ^a	42.50 \pm 1.54	42.50 \pm 1.54	39.75 \pm 1.54 ^a
Albumin g/L	40.16 \pm 0.90	40.00 \pm 0.90	39.50 \pm 0.90	39.50 \pm 0.90
Tot Protein g/L	78.17 \pm 1.44	78.00 \pm 1.44	79.33 \pm 1.44	77.50 \pm 1.44
Urea mg/dL	38.48 \pm 2.09	38.39 \pm 2.09	35.77 \pm 2.09	41.54 \pm 2.09

Within a row, means with a common superscript letter differ. ^{a,b}P < 0.05, ^{c,d}P < 0.01

n = 6 per group

Table 6.4. Least squares means \pm SEM for TBW, cortisol, glucose, lactate, hematocrit, albumin and total protein in *Bos indicus* steers treated with osmolytes or not and subjected to road transportation for 48 h.

	Control	Transported	Glycerol	Betaine
TBW %	52.89 \pm 2.78	51.66 \pm 2.78	57.46 \pm 2.78	56.80 \pm 2.78
Cortisol, ng/mL	22.87 \pm 5.36	20.00 \pm 5.36	35.84 \pm 5.36	22.05 \pm 5.36
Glucose mmol/L	5.10 \pm 0.42 ^c	5.73 \pm 0.42	6.38 \pm 0.42 ^c	5.83 \pm 0.42
Lactate mmol/L	1.37 \pm 0.45	0.93 \pm 0.45	1.43 \pm 0.45	2.17 \pm 0.45
Hematocrit %	45.67 \pm 1.26 ^a	41.50 \pm 1.26	43.67 \pm 1.26	41.33 \pm 1.26 ^a
Albumin g/L	41.17 \pm 0.90	39.33 \pm 0.90	40.33 \pm 0.90	40.17 \pm 0.90
Total Protein g/L	81.33 \pm 1.44	78.83 \pm 1.44	82.00 \pm 1.44	80.17 \pm 1.44
Urea mg/dL	40.03 \pm 2.09	34.71 \pm 2.09	38.09 \pm 2.09	41.60 \pm 2.09

Within a row, means with a common superscript letter differ. ^{a,b}P < 0.05, ^{c,d}P < 0.01

n = 6 per group

Table 6.5. Least squares means \pm SEM for liveweight, and % body weight (BW) loss *Bos indicus* steers treated with osmolytes or not and subjected to road transportation for 48 h.

	Control	Transported	Glycerol	Betaine
Initial Lwt	331.50 \pm 11.31	323.00 \pm 11.31	314.83 \pm 11.31	329.58 \pm 11.31
Curfewed 12 h	322.83 \pm 10.72	311.17 \pm 10.72	302.42 \pm 10.72	314.00 \pm 10.72
% BW loss*	2.76 \pm 0.80	3.74 \pm 0.80	3.80 \pm 0.80	4.65 \pm 0.80
24 h transit	301.75 \pm 9.82	286.00 \pm 9.82	280.83 \pm 9.82	288.00 \pm 9.82
% BW loss*	8.98 \pm 0.90	11.50 \pm 0.90	10.70 \pm 0.90	12.50 \pm 0.90
48 h transit	293.42 \pm 9.76	277.67 \pm 9.67	268.50 \pm 9.67	277.83 \pm 9.67
% BW loss*	11.50 \pm 1.00	14.10 \pm 1.00	14.70 \pm 1.00	16.00 \pm 1.00

Within a row, means with a common superscript letter differ. ^{a,b}P < 0.05, ^{c,d}P < 0.01

n = 6 per group * % BW loss is an accumulative figure

CHAPTER SEVEN

GENERAL DISCUSSION

In Northern Australia cattle are required to be transported over vast distances to reach markets, abattoirs and ports. While the welfare of these animals has improved with the implementation of codes of conduct for road, rail and sea transportation of cattle, little physiological data is available for *Bos indicus* cattle to support or refute current recommendations made by these codes and other legislation. The studies described in this thesis identified some of the physiological mechanisms involved when ruminants are placed under long haul transportation and handling stress. The physiological mechanisms investigated included water, electrolyte, and acid-base balance.

The current 'best practice' management protocols for transported ruminants, involve the administration of electrolyte solutions pre- or post-transportation to minimize transit stress. These industry recommendations have been largely influenced by the experiments of Schaefer *et al.* (1988), Schaefer *et al.* (1990), Schaefer *et al.* (1992). Evidence from one transportation study (Chapter 5) challenges the use of electrolyte solutions to confer a physiological benefit to transported animals in the live export chain. This alone has the potential to save producers and exporters considerable costs.

A quantitative analysis of the animal's physiological status demonstrated no difference in the pH of arterial blood from transported or non-transported steers. This confirms other data, albeit on venous blood, that transportation stress causes no difference in the acid-base status of transported compared to non-transported ruminants (Schaefer *et al.* 1988, 1992). Dehydration in the water deprived animals, both transported and non-transported, was shown to induce a mild metabolic acidosis as a result of an increase in proteins that act as weak acids. All water-deprived animals showed a trend toward lowering the PCO₂ concentration of their blood in a bid to buffer against the acidosis resulting from the dehydration. In the transported animals, there was a significant decrease in plasma concentrations of potassium, however, all other electrolytes measured did not differ between groups. As a result

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of this the AG and strong ion group calculations also demonstrated no difference between groups. This would suggest that electrolyte solutions fed post-transportation would provide little benefit in correcting a steer's acid-base balance compared to water alone.

The experiment described here is the only study to utilize arterial blood to assess acid-base balance in *Bos indicus* steers after long haul transportation and handling. Most studies that have investigated the effects of transportation and handling stress in cattle have utilized venous blood. Good correlations were found for pH and HCO_3^- however, PCO_2 concentrations yielded poor correlations between arterial and venous blood (Appendix 3). This in part supports the work described in Chapter 5 and that of other authors who have utilized venous blood. It is conceded that arterial blood must be taken to provide a true assessment of the mechanisms associated with acid-base balance in animals. However, others have argued that due to the technical limitations of sampling arterial vessels, venous blood is preferentially used as it provides a reasonable estimate of the acid-base balance of the animal (Aguilera-Tejero *et al.* 2000). In addition, Stewart's quantitative approach has been validated in other species using venous blood samples (Weinstein *et al.* 1991).

Hydration strategies involved with the transportation process rely on the replacement of lost TBW and electrolytes at the completion of the journey, after the welfare of the animals has been compromised. The studies described here sought a novel approach to the problem of dehydration associated with transportation in *Bos indicus* steers. These studies evaluated the use of two osmolytes, glycerol and betaine, during the transportation process. Prophylactic hyper-hydration of *Bos indicus* steers was achieved during the first 24 h of transportation with the osmolyte glycerol (Chapter 6), allowing a delay in TBW loss. This finding has significant welfare and production implications in that a prophylactic dose of glycerol could assist the animal to remain hydrated longer in the export process. Furthermore, hydration status governs the metabolic state of an animal, in as much as hydration has an anabolic effect, then hyper-hydration with glycerol may assist in delaying the catabolic effects of dehydration.

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Glycerol is an important fuel source for gluconeogenesis and hypothetically may be a preferential supply of energy for *Bos indicus* cattle during times of transportation stress. The potential beneficial effects of glycerol administration to *Bos indicus* steers are outlined in Figure 7.1. Glycerol promotes gluconeogenesis and glycogen formation and these actions appear to have nitrogen-sparing effects in the ruminant. As a result this treatment may preserve carcass protein and reduce dark firm dry cutting beef. Further, glycerol may decrease the rate of urea synthesis by preserving carcass protein. The lower rate of urea formation would assist in conserving energy during times of transportation and feed deprivation, as the synthesis of urea is energetically expensive. It is possible that the higher concentrations of plasma glucose may lead to greater steady state insulin concentrations inhibiting muscle protein breakdown. More research is needed on the metabolic effects of glycerol during periods of heightened gluconeogenesis such as starvation and transit stress. Additionally, genotype comparisons are needed to assist in understanding the phenomena of preferential fuel sources during times of stress.

The experiment described here is the only study to utilize arterial blood to assess acid-base balance in *Bos indicus* steers after long haul transportation and handling. Most studies that have investigated the effects of transportation and handling stress in cattle have utilized venous blood. Good correlations were found for pH and HCO_3^- however, PCO_2 concentrations yielded poor correlations between arterial and venous blood (Appendix 3). This in part supports the work described in Chapter 5 and that of other authors who have utilized venous blood. While it is conceded that arterial blood must be taken to provide a true assessment of the mechanisms associated with acid-base balance in animals, others have argued that due to the technical limitations of sampling arterial vessels, venous blood is preferentially used as it provides a reasonable estimate of the acid-base balance of the animal (Aguilera-Tejero *et al.* 2000). In addition, Stewart's quantitative approach has been validated in other species using venous blood samples (Weinstein *et al.* 1991).

The measurement of water balance within the ruminant is problematic under field conditions. The standard measurement for TBW is a TOH dilution, but equilibrium in ruminants was found to occur approximately 12 h post-injection. Measurement of

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the urea space within the animal has also produced accurate determination of TBW within 12 – 15 min post-injection.

Positive correlations between TOH space and urea space were found in an initial validation experiment (Appendix 1). However, others have stated that the urea space is a measurement of empty body water (TBW less the water in the GIT) (Preston and Kock 1973). This is based upon an assumption that infused urea does not pass into the water of the gut and that the short time interval to sampling would exclude gut water from the urea space.

This would enable *Bos indicus* steers to utilize the water in the GIT as empty body water was depleted. Further to this, under stressful conditions a flux of water may occur within spaces in the body. This mechanism is well documented in cases of hypovolaemic compensated shock (Espiner 1987; Guyton and Hall 2000). It is conceivable that increased concentrations of AVP and AII within the body especially under conditions of water deprivation would result in a decrease in peripheral blood circulation. This would decrease the space available to the urea molecule when a urea dilution technique is used to measure body water. Associated with compensated shock are increases in water absorption from interstitial spaces and the GIT. This may explain the failure of the measured body water of the steers to decrease in experiments described in Chapters 4 and 6, in light of other hydration parameters indicating dehydration. Single indices of hydration status appear to yield inconsistent results when stressors are applied. Therefore a model in which body water, liveweight, osmolality and plasma proteins are incorporated should be investigated in pursuit of an accurate assessment of hydration status of an animal under stress.

Initial pen studies, utilizing a stress model based upon cortisol infusions (Chapter 3), demonstrated that well hydrated ruminants placed under stressful conditions respond with a diuresis. This diuresis was not associated with a decrease in TBW in the animals involved. In this experiment, the length of time that cortisol was infused was not sufficient to induce a significant decrease in body water, in the cortisol treated animals. It would appear that these animals were able to draw on water reserves within the GIT to maintain their body water. While water deprivation alone

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induced a 13% decrease in TBW at 72 h, water deprivation with cortisol infusion resulted in the maintenance of TBW throughout the experimental period.

Water deprivation has been touted as a significant stressor to ruminants (Atkinson 1992). However, the studies described here have revealed that water deprivation for 72 h is not a prototypical stressor that will activate the HPA axis in Merino sheep (Chapter 3). However, 60 h of water deprivation resulted in a significant increase in cortisol concentration for *Bos indicus* steers (Chapter 4). The response of the HPA axis to water deprivation appears to be dependant on the degree to which an animal elevates the water retentive hormones AVP and AII. Under the influence of transportation and water deprivation the HPA axis failed to replicate an increase in the cortisol concentration in the plasma of transported *Bos indicus* steers (Appendix 2). These animals demonstrated an initial peak in cortisol concentration followed by a gradual decline, highlighting the inconsistent and complex interactions associated with the HPA axis.

In the experiment with *Bos indicus* steers described in Chapter 4, it appeared that the RAA axis was suppressed under the influence of high concentrations of cortisol, however, AVP concentrations remained unaffected. The data produced indicated that elevation of sodium concentration in the plasma of the animals was the predominant factor in the suppression of AII associated with excess cortisol infusion. Hypernatremia has been implicated for the disruption of the nexus between the renin-angiotensin system and aldosterone in other ruminants (Blair-West *et al.* 1972; Ben Goumi *et al.* 1993).

The increase in the sodium content in the urine of a dehydrated animal is a homeostatic mechanism that allows sodium balance to be maintained within the body (McKinley *et al.* 1983). These studies concur with those of McKinley *et al.* (1983), that a natriuresis does in fact occur. However, the total amount of sodium excreted per day actually decreases with total urinary volume as dehydration ensues. Schaefer *et al.* (1992) evacuated the bladder of cattle post-transport and found that a natriuresis occurred in transported cattle. These authors extrapolated the data to support the inclusion of electrolyte solutions containing sodium to minimize the physiological stress on the animals. The data presented by Schaefer *et al.* (1992),

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demonstrates the animal's natriuretic mechanism only and highlights the need for in-depth sampling regimens over longer periods of time to qualify a physiological interpretation.

Evidence from four studies (described in Chapters 3, 4, 5 and 6) demonstrates that plasma potassium consistently decreases in an animal when stress is simulated by injection of cortisol or by transportation and handling. This concurs with Hutcheson and Cole (1984), in which they recommended a 20% increase in the potassium content of receiving diets in feedlot cattle. In contrast, plasma sodium although elevated during water deprivation does not yield a consistent response due to stress. Similarly plasma magnesium and calcium are influenced more by water and feed deprivation than the HPA axis.

In conclusion, the studies reported in this thesis increased the level of understanding of the physiological responses of *Bos indicus* steers placed under long-haul transportation and handling stress. The physiological data has contributed to our knowledge of water, electrolyte and acid-base balance during times of stress. Current management protocols relating to the efficacy of electrolyte solutions in minimizing the physiological stress imposed upon these animals have been tested and refuted. The strategic application of osmolytes administered pre-transportation has been investigated with promising results for the live export industry. Finally, a number of areas of research have been identified that will further contribute to our understanding of the physiological responses of *Bos indicus* steers to transportation and handling stress, leading to significantly improved management practices and enhance animal welfare.

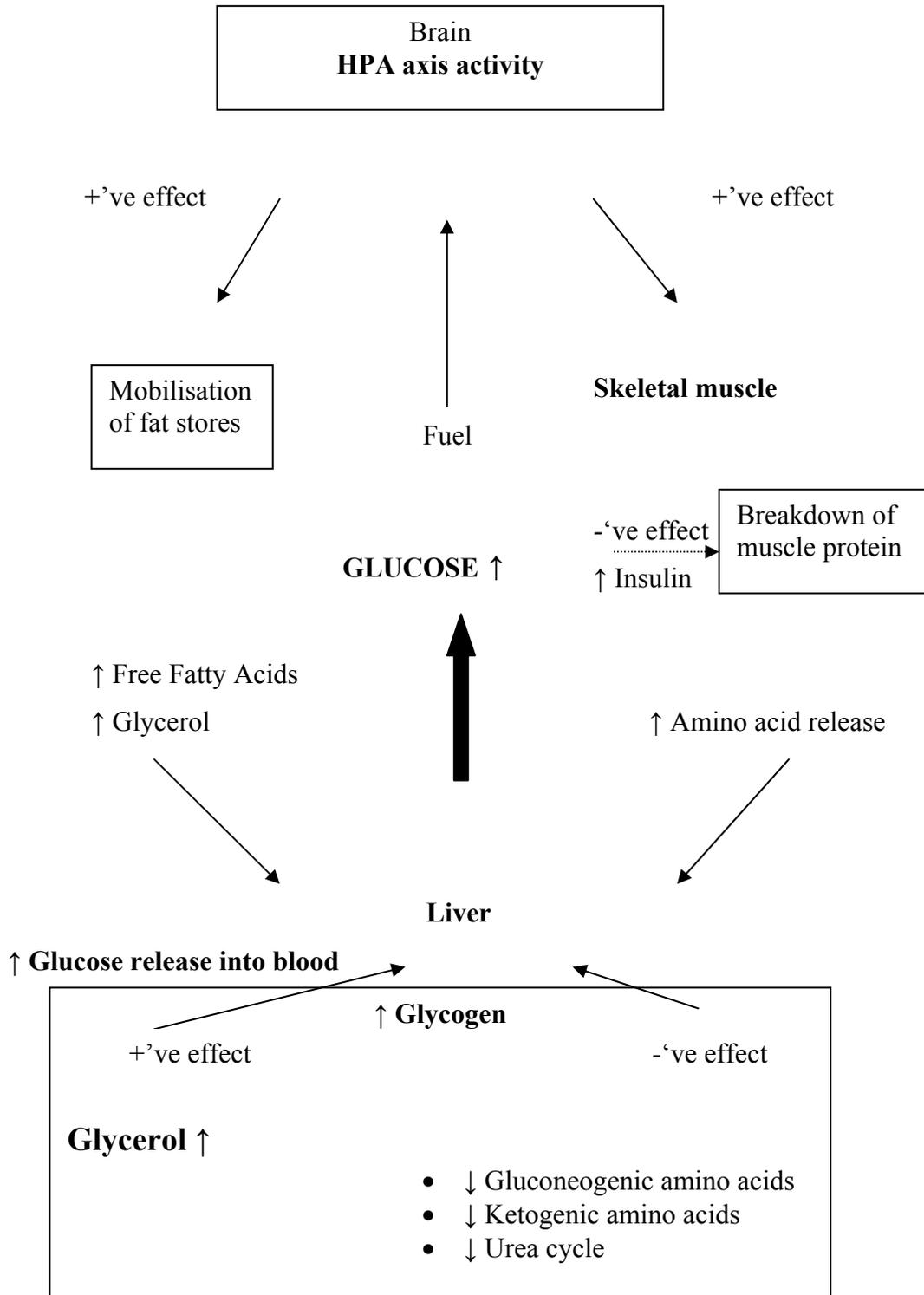


Figure 7.1. Schematic of the potential beneficial effects of glycerol prophylactic treatment during long haul transportation in *Bos indicus* steers.

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APPENDIX 1

A comparison of urea and tritiated water space to determine total body water in *Bos indicus* steers.

Introduction

The dilution principle technique for estimating TBW has frequently been used to determine the body composition of animals in vivo (Little and Morris, 1972; Hammond *et al.* 1984; Mitchell and Steele, 1987; Waltner *et al.* 1994; De Campeneere *et al.* 2000). The principle is based upon a marker being able to diffuse rapidly and homogeneously over the total water compartment of the body. The marker should not be toxic or metabolized, have any physiological effect and preferably not foreign to the body (De Campeneere *et al.* 2000; Guyton 2000). The two most frequently used markers are urea and tritiated water. Tritiated water consistently yields a high degree of accuracy. However, the time required to obtain complete dispersion throughout the body is 6 to 12 h. A further consideration is the lack of salvage value of the animal from the use of radioactive isotopes. Studies in pigs (Mitchell and Steele 1987) and cattle (Preston and Kock 1973; Kock and Preston 1979; De Campeneere *et al.* 2000) have indicated that urea can provide a similar degree of accuracy in determining TBW in a shorter time frame

Materials and Methods

Animals and management

Bos indicus steers (n = 6, 3 yr of age, 340 ± 20 kg mean BW) were halter broken, placed into individual stalls and fed a commercial forage cube (ME 8.5 MJ/kg DM, CP 12.5% and crude fiber 31.1%; cane fiber products, Brandon, QLD, Australia) *ad libitum* for 3 d prior to the commencement of the experiment. Animals were weighed immediately before the procedures, TOH and urea space was calculated on this weight. All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (Approval No. A664-01).

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Infusion and sampling procedures

On day 0, all animals were catheterized with a poly-vinyl chloride tube (OD 2.0 mm x ID 1.0 mm; Critchley Electrical Products Pty Ltd, Silverwater, NSW, Australia) inserted into the jugular vein under local anesthetic. Food and water was withdrawn from the animals while the assays were performed. On day 0, 10 mL of blood was collected from all animals as a background sample and decanted into 10 mL tubes containing lithium heparin (Disposable Products Pty Ltd, Adelaide, SA, Australia).

A bolus of approximately 500 μCi of tritiated water was injected into the jugular vein via the catheter followed by 10 mL heparinised saline (15000 IU heparin \bullet L⁻¹ 0.9% saline). The syringe was weighed before and immediately after infusion to determine the exact amount of TOH injected. At 4, 8, 12, 16, 20 and 24 h post-bolus injection, a 10 mL blood sample was collected from all animals in tubes containing lithium heparin (Disposable Products Pty Ltd, Adelaide, SA, Australia). Blood samples were immediately placed into an ice water slurry then centrifuged at 200 x g for 15 min and plasma poured off within 2 h and frozen (-20° C) for analysis.

Animals were allowed access to water and feed for 24 h before the urea dilution was performed. Each animal had a solution of 20% (wt/vol) urea dissolved in 0.9% (wt/vol) saline administered through the implanted catheters over a two min period. The volume injected was calculated to provide 130 mg of urea/kg BW. The catheter was flushed with 10 mL of isotonic saline followed by 10 mL of heparinized saline solution (35,000 IU/L of 0.9% saline) to prevent clotting between samplings. At 0 min a background sample was taken pre-infusion. Blood sampling continued at 3 min intervals post-urea infusion until 30 min. Blood samples were collected into 10 mL tubes containing lithium heparin (Disposable Products Pty Ltd, Adelaide, SA, Australia).

TOH sample analysis

Plasma samples (0.5 mL) were deproteinised with the addition of 0.1 mL of perchloric acid. Samples were agitated for 20 sec to ensure mixing prior to centrifugation at 200 x g at 4°C for 20 min. Biodegradable counting scintillate (BCS) ® (Amersham

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America) (10 mL) was placed into scintillating glass tubes with 0.5mL of the supernatant and mixed by hand. Samples were performed in duplicate and placed into a beta radiation liquid scintillating counter for subsequent analysis.

Urea –N analysis

Plasma urea-N was analysed with a technicon auto-analyser 2 (Bran + Leubbe Pty Ltd., Homebush, NSW, Australia) according to technicon auto analyzer method SE40001FD4. Samples were performed in duplicate. The following formula was used to calculate urea space as a percentage of BW (Kock and Preston 1979):

Urea space (%) = [volume infused (mL) x concentration of solution (mg urea nitrogen/dL)]/[plasma urea –N/liveweight (kg)]. TBW was recorded as the pool of water available to the urea molecule.

Results and Discussion

The time curve for TOH demonstrates equilibration to occur between 8 to 12 h after TOH administration (Figure A1.1). This is consistent with Little and Morris, (1972) who demonstrated 8 to 10 h was required for TOH equilibration in steers. There is considerable variation among TOH equilibration time reported by other authors. This appears to be dependant on the species used, initial amount of TOH injected, the route of administration and fat composition of the animal. Meissner (1976) utilized 0.4 mCi in sheep and found TOH space to equilibrate at 4 to 6 h. Thornton and English (1978) injected aliquots of 800 mCi into 15 d old calves and found the mean equilibration time to be 56 min. Others using sub-cutaneous injections in cows found equilibration to occur at 4 d (Seif 1972).

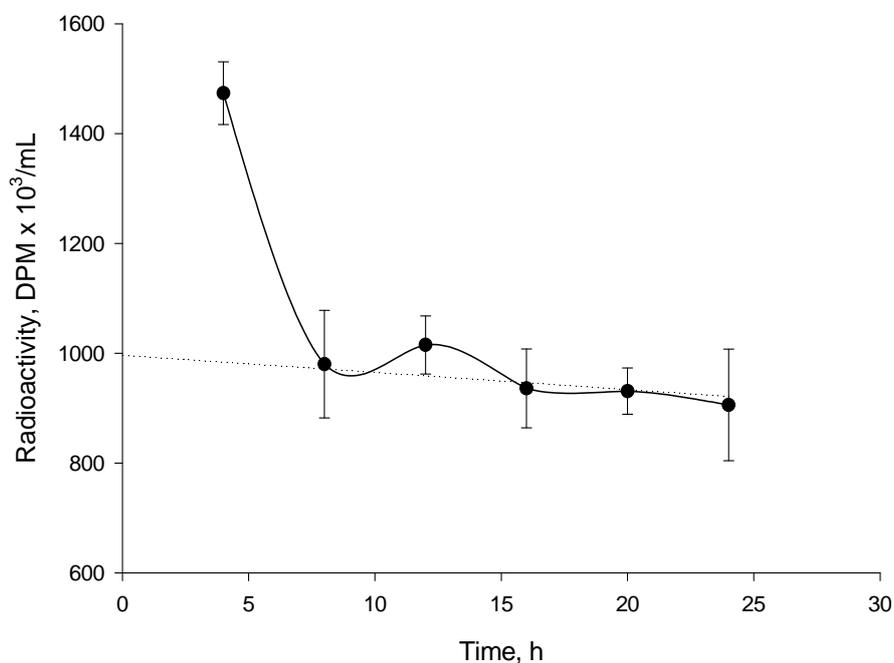


Figure A1.1. Mean \pm SEM change in radioactivity in six *Bos indicus* steers after infusion with 500 μ Ci/steer.

The urea time curve for the six steers appeared to equilibrate between 12 to 15 min post-infusion (Figure A1.2). Preston and Kock (1973) concluded that although urea mixes in the TBW within 9 to 10 min the relation between urea space determined at 12 or 15 min after urea infusion yields greater correlations between urea space and empty body water. Hammond *et al.* (1984) measured urea space in steers at 12 min post-injection with a good correlation between empty body water and urea space ($r = 0.96$; $P = 0.001$). Meissner (1976) suggested an equilibration point between 14 to 20 min in sheep. DeCampeneere *et al.* (2000) demonstrated 24 min post infusion yielded the highest correlation between actual and predicted empty body water ($adj\ r^2 = 0.89$) in double muscled bulls.

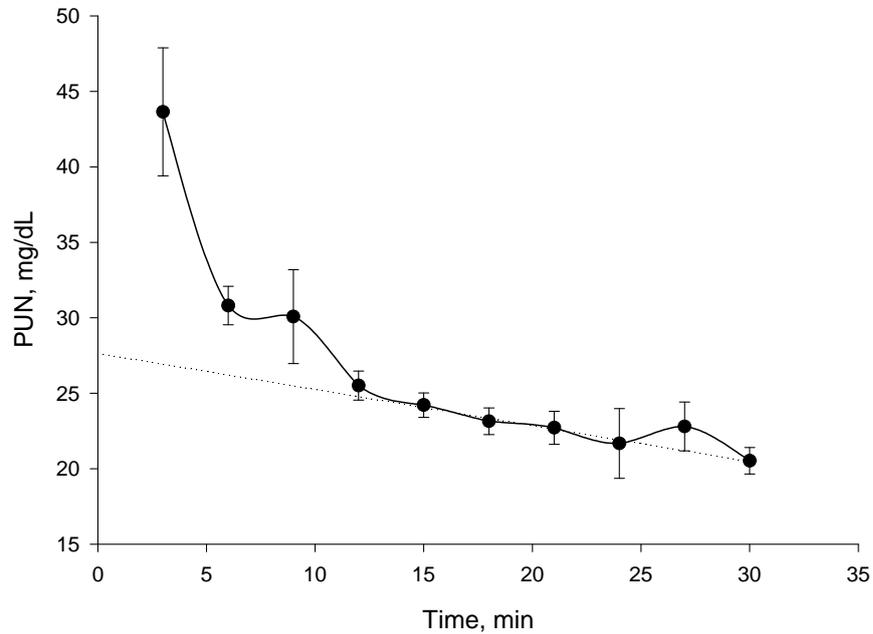


Figure A1.2. Mean \pm SEM change in plasma urea nitrogen (PUN) in six *Bos indicus* steers after infusion with 130 mg urea/kg BW.

A linear relationship existed between TOH space at 12 h and the urea space at 15 min post-infusion in the sampled steers ($r = 0.81$; $P = 0.05$) (Figure A1.3). Validating the use of the urea dilution technique as an accurate measure of an animals body water. It should be noted however that accurate separation of water from the GIT and that of the rest of the body using *in vivo* techniques is difficult to obtain. Arnold and Trenkle (1986) quoting Byers (1979) stated that compartmental models have been utilized to separate water in the GIT from TBW utilizing deuterium oxide. However, Hammond *et al.* (1984) stated that this procedure is more cumbersome than the urea dilution technique. Further to this Arnold and Trenkle (1986) found that compartmental analysis failed to estimate the amount of water in rumen contents separate from the remainder of water in the body of Holstein steers.

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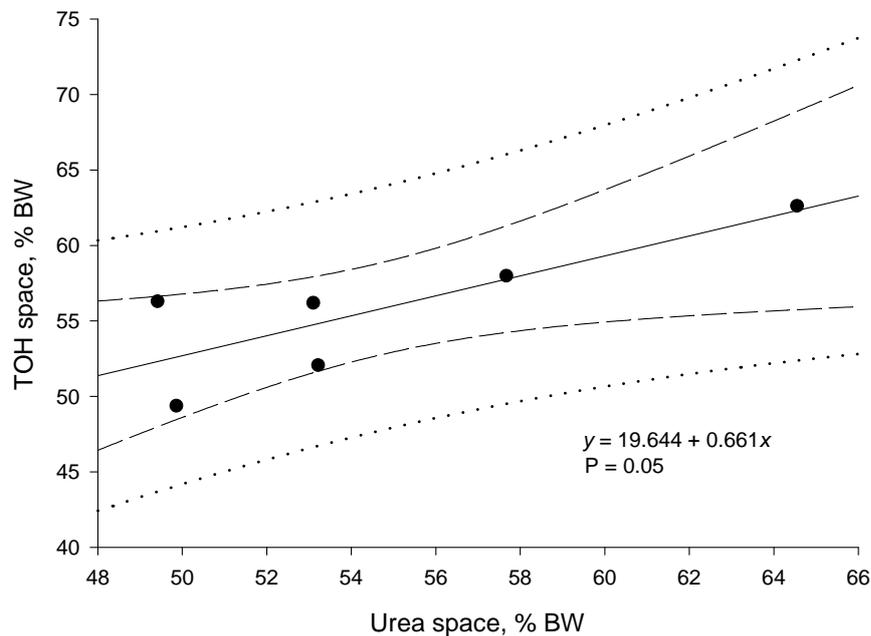


Figure A1.3. Relation between urea space and TOH space in six *Bos indicus* steers. Dashed lines depict confidence interval of 95%. Dotted lines depict predicted intervals.

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APPENDIX 2

Cortisol response to transportation during short and long haul transportation in *Bos indicus*

Introduction

The concentration of cortisol in plasma is widely used as an indicator of stress. However, few reports have determined the in transit reaction of ruminants to transportation stress. Infrequent or no sampling during transport may not allow for discrimination between the effects of transportation and those of unloading. Parrott *et al.* (1998) and Smith and Dobson (2002), with the latter traveling in the rear of a moving heavy vehicle, obtained in transit data of sheep subjected to transportation of 31 and 2 h respectively. This study was conducted to determine the in transit hypothalamo-pituitary-adrenal (HPA) axis response to transportation in *Bos indicus* steers during short haul transportation.

Materials and Methods

This transportation study was conducted using 4, 2.5 yr old *Bos indicus* steers (340.75 ± 12.79 kg BW). All animals were bilaterally catheterized with a poly-vinyl chloride tube (OD 2.0 mm x ID 1.0 mm; Critchley Electrical Products Pty Ltd, Silverwater, NSW, Australia) inserted into the jugular vein under local anesthetic prior to loading. Steers were placed into adjustable stalls in the rear of a body truck (1.56m²/animal). Blood sampling commenced one h after all animals were connected to their respective main sampling lines that flowed into the cabin of the truck. Sampling continued at 20-min intervals until the conclusion of the study. Animals were sampled while the truck remained stationary for 120 min, then during transport for 2 periods each of 120 min and a total distance of 350 km. At the conclusion of the transportation phase, the steers were kept on board the stationary truck and sampled for a further 120 min.

Blood samples were kept in an ice slurry whilst in transit and were centrifuged at 200 x g for 20 min upon returning to the laboratory at the conclusion of the transport phase. Plasma cortisol concentrations were measured using a radioimmunoassay kit (Spectria cortisol ¹²⁵I-coated tube kit, Orion Corp., Espoo, Finland).

Statistical analysis

A one-way repeated measures ANOVA was performed based on the areas under the time curve, integrated using the multiple-application trapezoidal rule for 4 x 2 h time periods; 1. Pre-transport (stationary in the truck); 2. First 2 h transport period; 3. Second 2 h transport period and; 4. Post-transport (stationary in the truck). Data presented in Figure A2.1 is the arithmetic mean \pm SE.

Results and Discussion

There was no difference ($P = 0.156$) due to time period on the cortisol concentration of the transported steers. The numerical differences seen in Table A2.1 and Figure A2.1 are limited by the statistical power of the test ($n = 4$) and as such any lack of differences should be interpreted cautiously. A greater number of steers may have resulted in significant differences between time periods.

Table A2.1. Mean \pm SEM for area under the cortisol time curve for *Bos indicus* steers during stationary and transportation periods. Time period effects are not significantly different ($P = 0.156$).

Time period	Mean Area \pm SEM
Pre-transit (stationary)	269.43 \pm 77.21
1 st transport period	550.89 \pm 163.09
2 nd transport period	775.64 \pm 265.21
Post-transit (stationary)	424.65 \pm 107.94

$n = 4$

The possibility exists for an acute stress response due to a novel housing environment such as the stalls in the rear of the truck. This novelty may have compounded the effects of transport on cortisol concentrations in the present study. However, the animals had considerable experience in being handled, stalled, catheterized and blood sampled prior to undertaking the study. This was evident from the cortisol concentrations obtained after one h of standing on the truck post-catheterization. Basal cortisol concentrations for steers have been reported to range from 0.5 to 9.0

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ng/mL (Grandin 1997). Data from this laboratory has demonstrated variations in diurnal cortisol concentrations in stalled *Bos indicus* steers to vary from 7.25 to 36ng/mL over a 90 h time period. Initial plasma cortisol concentrations in this study concur with that of our past experience, that basal concentrations were measured prior to transportation (7.25 to 18 ng/mL).

Although there was no statistical difference between either of the two h time periods during transport, plasma cortisol concentrations demonstrated a trend in response to transport, reaching a peak at 120 min into transit (Figure A2.1). The area under the time curve for the second two h period was numerically greater than all other time periods. Locatelli *et al.* (1989) suggested that a habituation response occurs within the HPA axis to prolonged trucking in cattle. Habituation is a waning of a response, which can still be shown, to a constant or repeated stimulus and may involve complex cognitive processes. It is not the simple adaptive responses suggested by Locatelli *et al.* (1989) due to fatigue of effectors such as muscles or adrenal output (Broom and Johnson 1993).

Adaptive responses in cortisol may be due to intrinsic control mechanisms primarily designed to prevent a prolonged increase in cortisol (Smith and Dobson 2002). Four h of transportation in the present study was not sufficient to induce an adaptive response in which cortisol concentrations during transportation reflected the pre-transport concentrations.

Trunkfield and Broom (1990), quoting Kent (1977), stated that a rapid decline to pre-transport levels following unloading was found in transported calves and concluded that blood samples should be taken during transit, in order to detect any increase in cortisol concentrations. We observed a decline in cortisol concentrations within 40 min of stopping transportation, similar to Locatelli *et al.* (1989) and Smith and Dobson, (2002) who demonstrated a return to basal cortisol concentrations in calves and sheep within 30 min of unloading from a simulated transport and transport event respectively.

Researchers to date have been limited in large ruminant research by the interpretation of a single cortisol concentration in assessing animal welfare. As such, the interpretation of a single cortisol concentration taken post-transport justifiably comes

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under review due to the differing adaptive responses available to livestock when placed under stress. While adaptation responses may exist during transportation, a different stressor post-transport, such as handling and blood sampling may induce a sensitized response or conversely due to sensory adaptation or a learned response, may respond to a new stimulus with hypo-sensitivity (Broom and Johnson 1993). The data of Warriss *et al.* (1995) is typical of previous transport stress methods in which blood samples were taken after transportation. Sampling immediately after 5, 10, and 15 h of transportation resulted in a decrease in cortisol concentration in 341 kg steers (72, 45, and 37 ng/mL) respectively. Warriss *et al.* (1995) offered one interpretation of their data as an adaptation response, in that, the animals perceived the novelty of transport as less stressful and were recovering from the early stages of transport as time *en-route* increased. Further to this it may be possible that the data reflects differing states of sensitivity of the HPA axis to an additional stress of blood sampling. Prior to the development of the current model to assess in transit HPA axis responses, data collected post-transport created a moot point for authors to argue their respective interpretations within the limitations of the methods.

There is a clear trend from this preliminary study that the plasma cortisol response adapts to a transportation event. The effect of this response on subsequent reactivity of the HPA axis to new novel stressors post-transport is yet to be elucidated.

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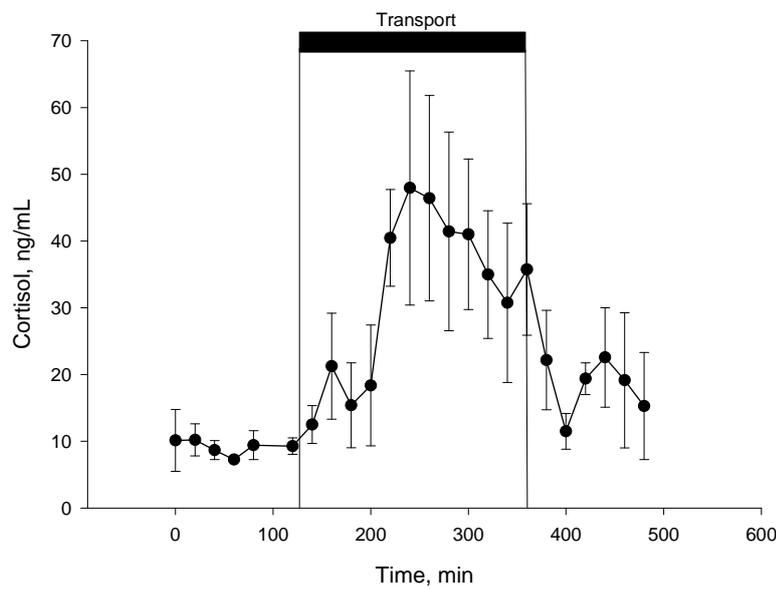


Figure A2.1. Mean \pm SEM plasma cortisol concentrations in four *Bos indicus* steers confined to stalls on a stationary body truck for two periods of two h separated by four h under transportation. Points represent sampling intervals of 20 min.

APPENDIX 3

Blood gas correlations between auricular arterial and jugular venous blood in normal *Bos indicus* steers

Introduction

The acid-base status of an animal can be easily assessed with three measurements from an arterial blood sample, pH, bicarbonate concentration (HCO_3), and the partial pressure of carbon dioxide (pCO_2) (Guyton and Hall 2000). Arterial blood sampling of large ruminants in the field has been found to be technically arduous (Mirakhur *et al.* 1985). The collection of blood from the auricular artery, although claimed by others as simple and free from any risk to animal or operator (Fisher *et al.* 1980), has also been found to be difficult due to the size of the lumen (Nagy *et al.* 2002). Furthermore, success in sampling the auricular artery is heavily dependent on the temperament of the animal. Cattle with limited experience in handling or with a poor temperament, when suitably restrained to attempt auricular sampling may react in such a way that a meaningful sample is not possible due to respiratory and or metabolic aberrations.

These difficulties have led to an acceptance that an adequate understanding of the changes involved in acid-base homeostasis may be provided by using venous blood (Schaefer *et al.* 1992, Weinstein *et al.* 1991, Aguilera-Tejero *et al.* 2000). There are limited data on the correlations between arterial and venous blood in *Bos indicus* cattle. This study seeks to validate the correlations between arterial and venous blood pH, pCO_2 , and HCO_3 in *Bos indicus* cattle.

Materials and Methods

Animals and management

Twenty-seven 2yr old *Bos indicus* steers (193 ± 21.47 kg mean BW) were halter broken and taught to lead. The steers were fed a commercial forage cube (ME 8.5 MJ/kg DM, CP 12.5% and crude fiber 31.1%; cane fiber products, Brandon, QLD, Australia) *ad libitum* for 14 d prior to the commencement of the experiment.

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At sampling, all animals were forced into a race, captured in a cattle head bail and restrained. A halter was placed on the individual animals and their heads were then restrained to the side with an attendant holding the head while samples were taken.

When cattle are captured and restrained, abnormal physiological reactions to the restraint may be expressed as an increase or decrease in respiration rates.

Subsequently, the blood gas parameters of the animal become affected. While the possibility exists for an abnormal measurement in blood gas parameters to have taken place in an animal from this study, it is unlikely when the current results are compared to those of other authors (Fisher *et al.* 1980; Mirakhur *et al.* 1985). Further to this, the animals used in the present study were accustomed to being handled, and behaved in a quiet and amicable manner when sampled.

All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (Approval No. A730-02).

Sample collection

At –30 min to sampling animals were catheterised with a poly-vinyl chloride tube (OD 2.0 mm x ID 1.0 mm; Critchley Electrical Products Pty Ltd, Silverwater, NSW, Australia) inserted into the jugular vein under local anesthetic. The catheter was then flushed with 10 mL of heparinised saline (15000 IU heparin • L⁻¹ 0.9% saline). Prior to sampling 15 mL of blood was drawn through the venous catheter and discarded. A venous blood sample was then taken utilising a 2 mL blood gas syringe containing lithium heparin (Sarstedt Australia, Technology Park, SA, Australia). Simultaneously to sampling the venous blood a 22 G (0.9 x 25 mm) intra-arterial catheter (Optiva, Johnson and Johnson Int., Obst, Belgium) was utilized with a 2 mL blood gas syringe containing lithium heparin (Sarstedt Australia, Technology Park, SA, Australia) to sample arterial blood gases. Arterial blood samples for blood gas analysis were obtained from the caudal auricular artery (Riley and Thompson 1978). Blood gas syringes were capped and placed into an ice water slurry for immediate analysis of blood gases. All blood gas assays were performed within 0.5 h of collection. Arterial and venous blood pH, pCO₂, and HCO₃⁻ were measured using a blood gas analyser (Ciba Corning Model 278, Bayer Diagnostics, Brisbane, Australia).

Statistical analysis

Regression analyses were performed using SPSS 10 software package (SPSS, IL, USA).

Results and Discussion

There was a good correlation ($r = 0.80$; $P = 0.01$) between the pH values for the auricular artery and jugular vein. The regression equation between the pH values for arterial blood (y) and jugular blood (x) was $y = 1.796 + 0.765x$ (Figure A3.1).

Mirakhur *et al.* (1985) produced similar correlations between the jugular vein and auricular artery ($r = 0.86$) in *Bos indicus* bullocks. The mean pH values for the arterial and venous blood were 7.46 ± 0.014 and 7.41 ± 0.014 respectively reflecting the slight acidosis expected in the venous blood. The animals used in the present study were well hydrated and had access to *ad libitum* feed prior to sampling, however, different pH values may be expected in a pathological state. Schaefer *et al.* (1992) have reported lower pH values (7.35 ± 0.014) in venous blood of bulls subjected to transportation, but this result may have been the result of higher lactate concentrations in these bulls or an elevation of plasma proteins due to dehydration.

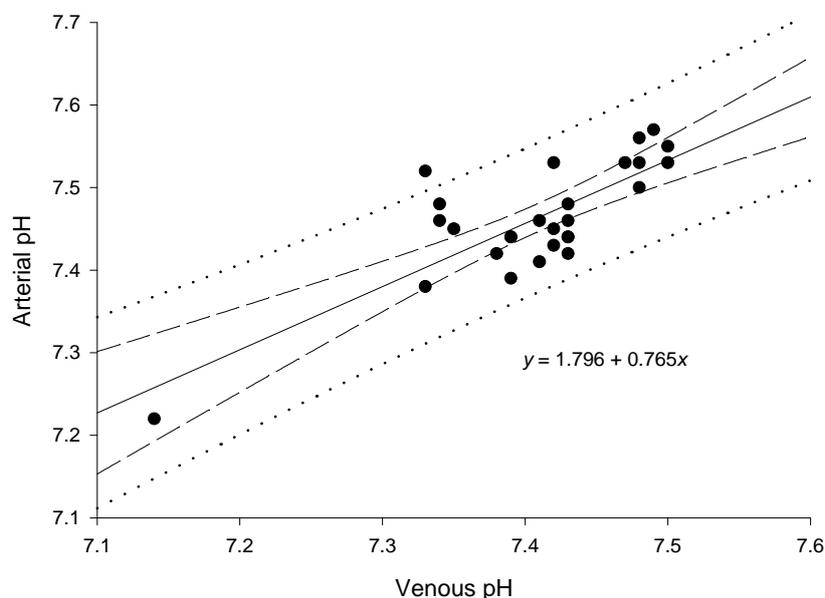


Figure A3.1. Correlation between pH in auricular arterial and jugular venous blood in *Bos indicus* steers. With fitted line (—), 95% confidence interval (- -) and prediction limits (····).

Bicarbonate concentrations also demonstrated a good relationship between values for arterial and venous blood ($r = 0.86$; $P = 0.01$) (Figure A3.2). This relationship was greater than that reported by Mirakhur *et al.* (1985), who recorded HCO_3 concentrations in the auricular artery and jugular vein at 24.1 ± 2.3 and 24.8 ± 1.9 mmol/L, respectively ($r = 0.70$; $P < 0.05$). The steers in the present study demonstrated HCO_3 concentrations in both arterial and venous blood of 29.57 ± 1.07 and 31.71 ± 1.05 , respectively.

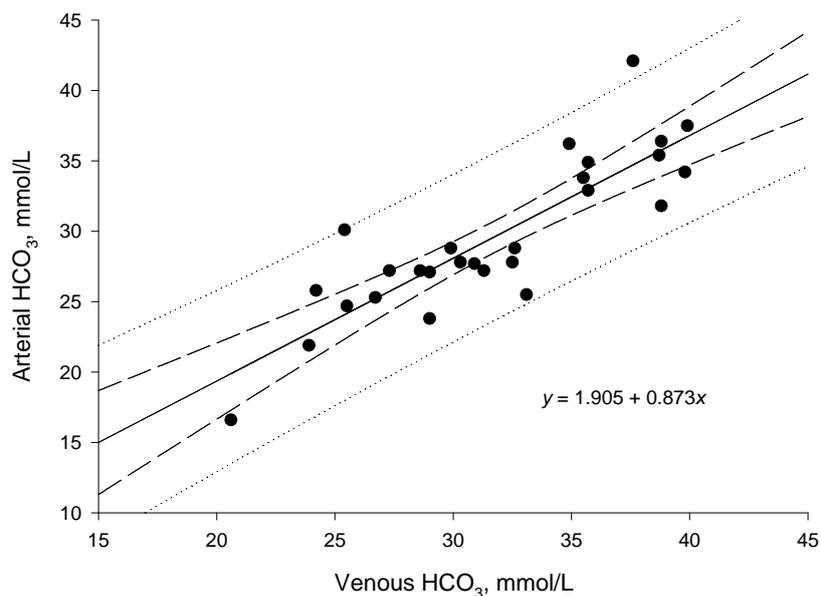


Figure A3.2. Correlation between bicarbonate concentration in auricular arterial and jugular venous blood in *Bos indicus* steers. With fitted line (—), 95% confidence interval(- -) and prediction limits (····).

Partial pressure of carbon dioxide yielded a poor relationship between arterial and venous blood ($r = 0.364$; $P = 0.06$) (Figure A3.3). Mirakhur *et al.* (1985) demonstrated a similar relationship ($r = 0.39$) in pCO_2 values. The mean values for pCO_2 in the steers in the present study were 41.54 ± 0.94 and 50.61 ± 1.19 mm/Hg in the arterial and venous blood samples, respectively. Normal arterial blood is believed to have a pCO_2 value of 40 mm/Hg and venous blood slightly higher due to a greater diffusion of CO_2 (Guyton and Hall, 2000). The animals used in this study therefore reflect normal pCO_2 values for *Bos indicus* steers.

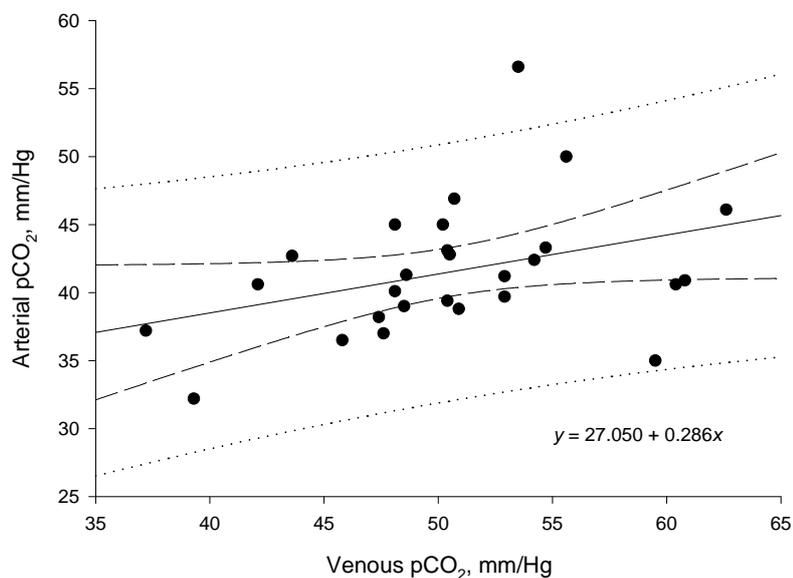


Figure A3.3. Correlation between pCO₂ concentrations in auricular arterial and jugular venous blood in *Bos indicus* steers. With fitted line (—), 95% confidence interval (- -), and prediction limits (····).

Conclusion

It would appear that, in normal *Bos indicus* steers at rest, pH and HCO₃ in arterial and venous blood, are well correlated. The pCO₂ of arterial and venous blood, however, does not yield favorable correlations. These results support, at least in part, the acceptance of researchers to utilise venous blood samples to examine changes in the acid base status of cattle.

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APPENDIX 4

ANOVA TABLES

CHAPTER 3 ANOVA TABLES

Table A4.3.1. Repeated measures ANOVA of interactions between time, water and cortisol for TBW in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	1.601 ⁻⁰²	6.875	0.018
	24 h - 48 h	1	1.708 ⁻⁰²	6.823	0.018
	48 h – 72 h	1	1.307 ⁻⁰²	0.906	0.354
Time * Water	0 h – 24 h	1	4.907 ⁻⁰³	2.107	0.165
	24 h - 48 h	1	1.337 ⁻⁰²	5.341	0.034
	48 h – 72 h	1	6.301 ⁻⁰²	4.371	0.052
Time * Cortisol	0 h – 24 h	1	5.810 ⁻⁰⁴	0.249	0.624
	24 h - 48 h	1	4.099 ⁻⁰⁵	0.016	0.900
	48 h – 72 h	1	3.700 ⁻⁰³	0.257	0.619
Time * Cortisol * Water	0 h – 24 h	1	7.767 ⁻⁰⁴	0.333	0.571
	24 h - 48 h	1	1.335 ⁻⁰³	0.533	0.475
	48 h – 72 h	1	2.604 ⁻⁰²	1.807	0.197
Error (Time)	0 h – 24 h	17	2.329 ⁻⁰³		
	24 h - 48 h	17	2.503 ⁻⁰³		
	48 h – 72 h	17	1.441 ⁻⁰²		

Table A4.3.2. Repeated measures ANOVA of interaction between time, water and cortisol for total urine output in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	24 h - 48 h	1	1071486.156	17.294	0.001
	48 h – 72 h	1	838.810	0.011	0.918
Time * Water	24 h - 48 h	1	0.922	0.000	0.997
	48 h – 72 h	1	249363.235	3.260	0.089
Time * Cortisol	24 h - 48 h	1	725797.192	11.714	0.003
	48 h – 72 h	1	279167.150	3.650	0.073
Time * Cortisol *	24 h - 48 h	1	318744.809	5.145	0.037
Water	48 h – 72 h		10797.278	0.141	0.712
Error (Time)	24 h - 48 h	17	61958.242		
	48 h – 72 h	17	76481.037		

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Table A4.3.3. Repeated measures ANOVA of interaction between time, water and cortisol for water intake in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	24 h - 48 h	1	3.940	12.409	0.010
	48 h - 72 h	1	1.361	3.027	0.125
Time * Water	24 h - 48 h	0			
	48 h - 72 h	0			
Time * Cortisol	24 h - 48 h	1	9.203 ⁻⁰²	0.290	0.607
	48 h - 72 h	1	0.147	0.328	0.585
Time * Cortisol * Water	24 h - 48 h	0			
	48 h - 72 h	0			
Error (Time)	24 h - 48 h	7	0.317		
	48 h - 72 h	7	0.450		

Table A4.3.4. Repeated measures ANOVA of interaction between time, water and cortisol for total feed intake in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	24 h - 48 h	1	0.404	3.911	0.064
	48 h - 72 h	1	1.224	22.391	0.000
Time * Water	24 h - 48 h	1	7.061 ⁻⁰²	0.683	0.420
	48 h - 72 h	1	4.592 ⁻⁰³	0.084	0.775
Time * Cortisol	24 h - 48 h	1	1.899 ⁻⁰²	0.184	0.674
	48 h - 72 h	1	1.322 ⁻⁰²	0.242	0.629
Time * Cortisol * Water	24 h - 48 h	1	9.058 ⁻⁰³	0.088	0.771
	48 h - 72 h		3.623 ⁻⁰⁴	0.007	0.936
Error (Time)	24 h - 48 h	17	0.103		
	48 h - 72 h	17	5.466 ⁻⁰²		

Table A4.3.5. Repeated measures ANOVA of interaction between time, water and cortisol for total urinary sodium output in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	24 h - 48 h	1	55217.751	10.099	0.006
	48 h - 72 h	1	166.423	0.050	0.826
Time * Water	24 h - 48 h	1	173.123	0.032	0.861
	48 h - 72 h	1	12417.923	3.735	0.070
Time * Cortisol	24 h - 48 h	1	29741.030	5.440	0.032
	48 h - 72 h	1	4862.770	1.463	0.243
Time * Cortisol * Water	24 h - 48 h	1	633.421	0.116	0.738
	48 h - 72 h		1897.217	0.571	0.460
Error (Time)	24 h - 48 h	17	5467.469		
	48 h - 72 h	17	3324.945		

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Table A4.3.6. Repeated measures ANOVA of interaction between time, water and cortisol for total urinary potassium output in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	24 h - 48 h	1	22099.073	4.884	0.041
	48 h – 72 h	1	9.989	0.004	0.950
Time * Water	24 h - 48 h	1	3141.620	0.694	0.416
	48 h – 72 h	1	11645.425	4.730	0.044
Time * Cortisol	24 h - 48 h	1	11160.013	2.466	0.135
	48 h – 72 h	1	32.853	0.013	0.909
Time * Cortisol *	24 h - 48 h	1	4429.083	0.979	0.336
Water	48 h – 72 h		430.181	0.175	0.681
Error (Time)	24 h - 48 h	17	4524.960		
	48 h – 72 h	17	2462.256		

Table A4.3.7. Repeated measures ANOVA of interaction between time, water and cortisol for total urinary magnesium output in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	24 h - 48 h	1	872.430	4.850	0.042
	48 h – 72 h	1	2.025	0.096	0.760
Time * Water	24 h - 48 h	1	226.605	1.260	0.277
	48 h – 72 h	1	149.752	7.124	0.016
Time * Cortisol	24 h - 48 h	1	508.903	2.829	0.111
	48 h – 72 h	1	8.141	0.387	0.542
Time * Cortisol *	24 h - 48 h	1	30.059	0.167	0.688
Water	48 h – 72 h		0.236	0.011	0.917
Error (Time)	24 h - 48 h	17	179.890		
	48 h – 72 h	17	21.022		

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Table A4.3.8. Repeated measures ANOVA of interactions between time, water and cortisol for plasma potassium concentration in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	1.039	7.276	0.017
	24 h - 48 h	1	0.171	2.815	0.114
	48 h – 72 h	1	2.985 ⁻⁰²	0.410	0.532
Time * Water	0 h – 24 h	1	0.450	3.153	0.096
	24 h - 48 h	1	1.115 ⁻⁰³	0.018	0.894
	48 h – 72 h	1	3.385 ⁻⁰³	0.046	0.832
Time * Cortisol	0 h – 24 h	1	0.511	3.579	0.078
	24 h - 48 h	1	7.149 ⁻⁰²	1.180	0.294
	48 h – 72 h	1	2.802 ⁻⁰²	0.385	0.544
Time * Cortisol * Water	0 h – 24 h	1	6.715 ⁻⁰²	0.470	0.503
	24 h - 48 h	1	2.121 ⁻⁰²	0.350	0.563
	48 h – 72 h	1	8.464 ⁻⁰²	1.162	0.298
Error (Time)	0 h – 24 h	17	0.143		
	24 h - 48 h	17	6.058 ⁻⁰²		
	48 h – 72 h	17	7.283 ⁻⁰²		

Table A4.3.9. Repeated measures ANOVA of interactions between time, water and cortisol for plasma sodium concentration in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	31.541	0.636	0.438
	24 h - 48 h	1	10.993	0.616	0.445
	48 h – 72 h	1	1.169	0.067	0.800
Time * Water	0 h – 24 h	1	134.045	2.703	0.121
	24 h - 48 h	1	93.185	5.224	0.037
	48 h – 72 h	1	5.128 ⁻⁰⁵	0.000	0.999
Time * Cortisol	0 h – 24 h	1	13.827	0.279	0.605
	24 h - 48 h	1	6.945	0.389	0.542
	48 h – 72 h	1	16.429	0.935	0.349
Time * Cortisol * Water	0 h – 24 h	1	5.644	0.114	0.741
	24 h - 48 h	1	46.575	2.611	0.127
	48 h – 72 h	1	28.539	1.623	0.222
Error (Time)	0 h – 24 h	17	49.594		
	24 h - 48 h	17	17.839		
	48 h – 72 h	17	17.579		

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Table A4.3.10. Repeated measures ANOVA of interactions between time, water and cortisol for plasma magnesium concentration in Merino wethers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	1.662 ⁻⁰²	3.003	0.104
	24 h - 48 h	1	2.513 ⁻⁰³	0.615	0.445
	48 h – 72 h	1	3.270 ⁻⁰²	5.921	0.028
Time * Water	0 h – 24 h	1	7.385 ⁻⁰³	1.335	0.266
	24 h - 48 h	1	2.513 ⁻⁰³	0.615	0.445
	48 h – 72 h	1	3.878 ⁻⁰⁴	0.070	0.795
Time * Cortisol	0 h – 24 h	1	5.654 ⁻⁰³	1.022	0.328
	24 h - 48 h	1	8.667 ⁻⁰³	2.120	0.166
	48 h – 72 h	1	3.080 ⁻⁰³	0.558	0.467
Time * Cortisol * Water	0 h – 24 h	1	1.038 ⁻⁰³	0.188	0.671
	24 h - 48 h	1	8.667 ⁻⁰³	2.120	0.166
	48 h – 72 h	1	3.205 ⁻⁰⁶	0.001	0.981
Error (Time)	0 h – 24 h	17	5.533 ⁻⁰³		
	24 h - 48 h	17	4.089 ⁻⁰³		
	48 h – 72 h	17	5.522 ⁻⁰³		

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CHAPTER 4 ANOVA TABLES

Table A4.4.1. Oneway ANOVA for plasma cortisol concentration in *Bos indicus* steers.

Source	df	Mean Square	F	Sig.
Between Groups	1	10629669283	6.548	0.023
Within Groups	14	1623293299		
Total	15			

Table A4.4.2. Repeated measures ANOVA of interactions between time, water and cortisol for plasma Arginine vasopressin concentration in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	571.633	46.90	0.000
	24 h - 48 h	1	751.727	19.404	0.000
	48 h – 72 h	1	242.229	6.164	0.020
	72 h – 90 h	1	151.182	1.354	0.255
Time * Water	0 h – 24 h	1	649.843	53.317	0.000
	24 h - 48 h	1	487.856	12.593	0.001
	48 h – 72 h	1	342.866	8.724	0.006
	72 h – 90 h	1	190.286	1.704	0.203
Time * Cortisol	0 h – 24 h	1	3.491	0.286	0.597
	24 h - 48 h	1	18.922	0.488	0.491
	48 h – 72 h	1	137.066	3.488	0.073
	72 h – 90 h	1	65.481	0.586	0.450
Time * Cortisol * Water	0 h – 24 h	1	13.616	1.117	0.300
	24 h - 48 h	1	78.963	2.038	0.165
	48 h – 72 h	1	214.929	5.469	0.027
	72 h – 90 h	1	74.272	0.665	0.422
Error (Time)	0 h – 24 h	27	12.188		
	24 h - 48 h	27	38.741		
	48 h – 72 h	27	39.299		
	72 h – 90 h	27	111.650		

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Table A4.4.3. Repeated measures ANOVA of interactions between time, water and cortisol for plasma Angiotensin II concentration in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	2008.174	4.969	0.034
	24 h - 48 h	1	20607.281	6.326	0.018
	48 h – 72 h	1	196966.272	5.914	0.022
	72 h – 90 h	1	47114.229	1.015	0.323
Time * Water	0 h – 24 h	1	3616.596	8.949	0.006
	24 h - 48 h	1	13349.876	4.098	0.053
	48 h – 72 h	1	136863.518	4.109	0.053
	72 h – 90 h	1	18640.397	0.402	0.532
Time * Cortisol	0 h – 24 h	1	16891.260	41.797	0.000
	24 h - 48 h	1	8448.952	2.594	0.119
	48 h – 72 h	1	1162092.146	4.867	0.036
	72 h – 90 h	1	35362.756	0.762	0.390
Time * Cortisol * Water	0 h – 24 h	1	6881.969	17.029	0.000
	24 h - 48 h	1	5820.915	1.787	0.192
	48 h – 72 h	1	107710.459	3.234	0.083
	72 h – 90 h	1	11205.080	0.242	0.627
Error (Time)	0 h – 24 h	27	404.123		
	24 h - 48 h	27	3257.429		
	48 h – 72 h	27	33306.231		
	72 h – 90 h	27	46397.119		

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Table A4.4.4. Repeated measures ANOVA of interactions between time, water and cortisol for total urine output in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	24 h – 48 h	1	12619851.923	3.488	0.088
	48 h - 72 h	1	60164964.103	17.971	0.001
	72 h – 90 h	1	4396898.077	1.513	0.242
Time * Water	24 h – 48 h	1	324051.923	0.089	0.771
	48 h - 72 h	1	1843856.41	0.551	0.472
	72 h – 90 h	1	6031.410	0.002	0.964
Time * Cortisol	24 h – 48 h	1	7147644.231	1.953	0.188
	48 h - 72 h	1	2323856.410	0.694	0.421
	72 h – 90 h	1	351975.00	0.121	0.734
Time * Cortisol * Water	24 h – 48 h	1	3664667.308	1.001	0.337
	48 h - 72 h	1	14818502.564	4.426	0.057
	72 h – 90 h	1	304308.333	0.105	0.752
Error (Time)	24 h – 48 h	12	3659702.083		
	48 h - 72 h	12	3347955.556		
	72 h – 90 h	12	2906413.194		

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Table A4.4.5. Repeated measures ANOVA of interactions between time, water and cortisol for plasma osmolality in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	673.717	1.972	0.172
	24 h - 48 h	1	61514.794	79.206	0.000
	48 h – 72 h	1	13928.286	12.493	0.001
	72 h – 90 h	1	28691.057	14.148	0.001
Time * Water	0 h – 24 h	1	2835.786	8.301	0.008
	24 h - 48 h	1	222.414	0.286	0.597
	48 h – 72 h	1	430.355	0.386	0.540
	72 h – 90 h	1	2953.264	1.456	0.238
Time * Cortisol	0 h – 24 h	1	5.441	0.016	0.901
	24 h - 48 h	1	448.035	0.577	0.454
	48 h – 72 h	1	44.889	0.040	0.842
	72 h – 90 h	1	169.074	0.083	0.775
Time * Cortisol * Water	0 h – 24 h	1	0.200	0.001	0.981
	24 h - 48 h	1	17.173	0.022	0.883
	48 h – 72 h	1	1374.406	1.233	0.277
	72 h – 90 h	1	1872.867	0.924	0.345
Error (Time)	0 h – 24 h	27	9224.214		
	24 h - 48 h	27	20969.232		
	48 h – 72 h	27	30101.464		
	72 h – 90 h	27	54755.107		

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Table A4.4.6. Repeated measures ANOVA of interactions between time, water and cortisol for hematocrit in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	1860.002	39.678	0.000
	24 h - 48 h	1	352.863	19.316	0.000
	48 h – 72 h	1	33.717	1.266	0.270
	72 h – 90 h	1	75.863	6.447	0.017
Time * Water	0 h – 24 h	1	30.347	0.647	0.428
	24 h - 48 h	1	2.001	0.110	0.743
	48 h – 72 h	1	190.355	7.149	0.013
	72 h – 90 h	1	2.001	0.170	0.683
Time * Cortisol	0 h – 24 h	1	3.192	0.068	0.796
	24 h - 48 h	1	63.449	3.474	0.073
	48 h – 72 h	1	9.165	0.344	0.562
	72 h – 90 h	1	17.587	1.494	0.232
Time * Cortisol * Water	0 h – 24 h	1	242.847	5.181	0.031
	24 h - 48 h	1	0.104	0.006	0.940
	48 h – 72 h	1	83.389	3.132	0.088
	72 h – 90 h	1	63.449	5.392	0.028
Error (Time)	0 h – 24 h	27	46.877		
	24 h - 48 h	27	18.268		
	48 h – 72 h	27	26.628		
	72 h – 90 h	27	11.768		

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Table A4.4.7. Repeated measures ANOVA of interactions between time, water and cortisol for plasma sodium concentration in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	1396.203	31.174	0.000
	24 h - 48 h	1	89.855	2.125	0.156
	48 h – 72 h	1	110.334	3.010	0.094
	72 h – 90 h	1	46.229	1.440	0.241
Time * Water	0 h – 24 h	1	310.756	6.939	0.014
	24 h - 48 h	1	18.60	0.440	0.513
	48 h – 72 h	1	216.606	5.910	0.022
	72 h – 90 h	1	110.282	3.435	0.076
Time * Cortisol	0 h – 24 h	1	131.887	2.945	0.098
	24 h - 48 h	1	10.632	0.251	0.620
	48 h – 72 h	1	6.706 ⁻⁰³	0.000	0.989
	72 h – 90 h	1	14.947	0.466	0.501
Time * Cortisol * Water	0 h – 24 h	1	2.731	0.061	0.807
	24 h - 48 h	1	48.691	1.152	0.293
	48 h – 72 h	1	10.583	0.289	0.595
	72 h – 90 h	1	14.947	0.466	0.501
Error (Time)	0 h – 24 h	27	44.787		
	24 h - 48 h	27	42.279		
	48 h – 72 h	27	36.653		
	72 h – 90 h	27	32.103		

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Table A4.4.8. Repeated measures ANOVA of interactions between time, water and cortisol for plasma potassium concentration in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	2.847	14.992	0.001
	24 h - 48 h	1	1.010 ⁻⁰²	0.116	0.736
	48 h – 72 h	1	0.594	2.921	0.099
	72 h – 90 h	1	3.357	10.031	0.004
Time * Water	0 h – 24 h	1	0.191	1.004	0.325
	24 h - 48 h	1	0.149	1.716	0.201
	48 h – 72 h	1	6.108 ⁻⁰²	0.300	0.588
	72 h – 90 h	1	0.221	0.661	0.423
Time * Cortisol	0 h – 24 h	1	2.743	14.442	0.001
	24 h - 48 h	1	3.148 ⁻⁰²	0.363	0.552
	48 h – 72 h	1	0.227	1.116	0.300
	72 h – 90 h	1	0.433	1.294	0.265
Time * Cortisol * Water	0 h – 24 h	1	0.219	1.153	0.292
	24 h - 48 h	1	1.020 ⁻⁰²	0.118	0.734
	48 h – 72 h	1	2.538 ⁻⁰²	0.125	0.727
	72 h – 90 h	1	3.980 ⁻⁰²	0.119	0.733
Error (Time)	0 h – 24 h	27	0.190		
	24 h - 48 h	27	8.676 ⁻⁰²		
	48 h – 72 h	27	0.203		
	72 h – 90 h	27	0.335		

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Table A4.4.9. Repeated measures ANOVA of interactions between time, water and cortisol for plasma calcium concentration in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	0.249	4.653	0.040
	24 h - 48 h	1	0.379	15.089	0.001
	48 h – 72 h	1	0.224	2.916	0.099
	72 h – 90 h	1	3.951 ⁻⁰²	0.322	0.575
Time * Water	0 h – 24 h	1	4.273 ⁻⁰²	0.800	0.379
	24 h - 48 h	1	0.110	4.415	0.045
	48 h – 72 h	1	9.902 ⁻⁰³	0.129	0.723
	72 h – 90 h	1	0.821	6.700	0.015
Time * Cortisol	0 h – 24 h	1	0.106	1.976	0.171
	24 h - 48 h	1	2.831 ⁻⁰²	1.137	0.296
	48 h – 72 h	1	0.232	3.015	0.094
	72 h – 90 h	1	4.607 ⁻⁰²	0.376	0.545
Time * Cortisol * Water	0 h – 24 h	1	1.789 ⁻⁰²	0.335	0.568
	24 h - 48 h	1	2.176 ⁻⁰³	0.087	0.770
	48 h – 72 h	1	3.843 ⁻⁰⁴	0.005	0.944
	72 h – 90 h	1	2.806 ⁻⁰²	0.229	0.636
Error (Time)	0 h – 24 h	27	5.341 ⁻⁰²		
	24 h - 48 h	27	2.490 ⁻⁰²		
	48 h – 72 h	27	7.695 ⁻⁰²		
	72 h – 90 h	27	0.123		

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Table A4.4.10. Repeated measures ANOVA of interactions between time, water and cortisol for plasma chloride concentration in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	24.877	0.526	0.475
	24 h - 48 h	1	3820.863	154.037	0.000
	48 h – 72 h	1	172.968	9.665	0.004
	72 h – 90 h	1	395.075	13.825	0.001
Time * Water	0 h – 24 h	1	142.464	3.011	0.094
	24 h - 48 h	1	5.794	0.234	0.633
	48 h – 72 h	1	74.571	4.167	0.051
	72 h – 90 h	1	158.281	5.539	0.026
Time * Cortisol	0 h – 24 h	1	65.843	1.392	0.248
	24 h - 48 h	1	300.863	12.129	0.002
	48 h – 72 h	1	16.968	0.948	0.339
	72 h – 90 h	1	76.730	2.685	0.113
Time * Cortisol * Water	0 h – 24 h	1	150.877	3.189	0.085
	24 h - 48 h	1	43.242	1.743	0.198
	48 h – 72 h	1	8.867 ⁻⁰²	0.005	0.944
	72 h – 90 h	1	5.542 ⁻⁰³	0.000	0.989
Error (Time)	0 h – 24 h	27	47.308		
	24 h - 48 h	27	24.805		
	48 h – 72 h	27	17.896		
	72 h – 90 h	27	28.577		

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Table A4.4.11. Repeated measures ANOVA of interactions between time, water and cortisol for plasma phosphorous concentration in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	0 h – 24 h	1	9.896	69.980	0.000
	24 h - 48 h	1	7.333	63.107	0.000
	48 h – 72 h	1	1.218	12.344	0.002
	72 h – 90 h	1	0.553	4.201	0.050
Time * Water	0 h – 24 h	1	4.130 ⁻⁰²	0.292	0.593
	24 h - 48 h	1	2.180 ⁻⁰²	0.188	0.668
	48 h – 72 h	1	5.054 ⁻⁰²	0.512	0.480
	72 h – 90 h	1	2.676 ⁻⁰²	0.203	0.656
Time * Cortisol	0 h – 24 h	1	1.033	7.302	0.012
	24 h - 48 h	1	0.603	5.188	0.031
	48 h – 72 h	1	0.432	4.381	0.046
	72 h – 90 h	1	0.118	0.900	0.351
Time * Cortisol * Water	0 h – 24 h	1	1.478 ⁻⁰⁴	0.001	0.974
	24 h - 48 h	1	3.585 ⁻⁰²	0.308	0.583
	48 h – 72 h	1	0.136	1.381	0.250
	72 h – 90 h	1	2.136 ⁻⁰²	0.162	0.690
Error (Time)	0 h – 24 h	27	0.141		
	24 h - 48 h	27	0.116		
	48 h – 72 h	27	9.869 ⁻⁰²		
	72 h – 90 h	27	0.132		

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Table A4.4.12. Repeated measures ANOVA of interactions between time, water and cortisol for water intake in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	-24 h – 24 h	1	82.184	1.983	0.171
	24 h - 48 h	1	141.990	6.610	0.016
	48 h – 72 h	1	917.554	37.071	0.000
	72 h – 90 h	1	887.439	40.562	0.000
Time * Water	-24 h – 24 h	1	82.184	1.983	0.171
	24 h - 48 h	1	141.990	6.610	0.016
	48 h – 72 h	1	917.554	37.071	0.000
	72 h – 90 h	1	887.439	40.562	0.000
Time * Cortisol	-24 h – 24 h	1	1.693	0.041	0.841
	24 h - 48 h	1	24.680	1.149	0.293
	48 h – 72 h	1	2.906	0.117	0.735
	72 h – 90 h	1	11.473	0.524	0.475
Time * Cortisol * Water	-24 h – 24 h	1	1.693	0.041	0.841
	24 h - 48 h	1	24.680	1.149	0.293
	48 h – 72 h	1	2.906	0.117	0.735
	72 h – 90 h	1	11.473	0.524	0.475
Error (Time)	-24 h – 24 h	27	41.452		
	24 h - 48 h	27	21.480		
	48 h – 72 h	27	24.751		
	72 h – 90 h	27	21.878		

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Table A4.4.13. Repeated measures ANOVA of interactions between time, water and cortisol for feed intake in *Bos indicus* steers.

Source	Time	df	Mean Square	F	Sig.
Time	-24 h – 24 h	1	11.545	5.790	0.023
	24 h - 48 h	1	2.552	0.861	0.362
	48 h – 72 h	1	0.398	0.232	0.634
	72 h – 90 h	1	11.286	2.781	0.107
Time * Water	-24 h – 24 h	1	18.691	9.373	0.005
	24 h - 48 h	1	15.067	5.084	0.032
	48 h – 72 h	1	7.050 ⁻⁰²	0.041	0.841
	72 h – 90 h	1	28.437	7.006	0.013
Time * Cortisol	-24 h – 24 h	1	0.498	0.250	0.621
	24 h - 48 h	1	5.096	1.719	0.201
	48 h – 72 h	1	1.112	0.649	0.427
	72 h – 90 h	1	8.720 ⁻⁰²	0.021	0.885
Time * Cortisol * Water	-24 h – 24 h	1	0.480	0.241	0.628
	24 h - 48 h	1	0.519	0.175	0.679
	48 h – 72 h	1	0.490	0.286	0.597
	72 h – 90 h	1	0.195	0.048	0.828
Error (Time)	-24 h – 24 h	27	1.994		
	24 h - 48 h	27	2.964		
	48 h – 72 h	27	1.713		
	72 h – 90 h	27	4.059		

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CHAPTER 5 ANOVA TABLES

Table A4.5.1. Univariate ANOVA for plasma total weak acids concentration in *Bos indicus* steers transported for 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	2856.483	3018.497	0.000
Group	2	11.124	11.755	0.001
Error	16	0.946		
Total	19			

Table A4.5.2. Univariate ANOVA for arterial blood pH in *Bos indicus* steers transported for 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	1017.291	0.657	0.000
Group	2	7.916 ⁻⁰⁴	844551.64	0.532
Error	16	1.205 ⁻⁰³		
Total	19			

Table A4.5.3. Univariate ANOVA for arterial blood PCO₂ in *Bos indicus* steers transported for 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	27623.931	1669.025	0.000
Group	2	81.072	4.898	0.022
Error	16	16.551		
Total	19			

Table A4.5.4. Univariate ANOVA for arterial blood HCO₃ in *Bos indicus* steers transported for 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	13483.704	1243.910	0.000
Group	2	20.120	1.856	0.188
Error	16	10.840		
Total	19			

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Table A4.5.5. Univariate ANOVA for plasma total protein concentration in *Bos indicus* steers transported for 48 h.

Source	Df	Mean Square	F	Sig.
Intercept	1	102106.041	3450.925	0.000
Group	2	600.506	20.296	0.000
Error	16	29.588		
Total	19			

Table A4.5.6. Univariate ANOVA for plasma albumin concentration in *Bos indicus* steers transported for 48 h.

Source	Df	Mean Square	F	Sig.
Intercept	1	273.170	3444.003	0.000
Group	2	0.691	8.715	0.003
Error	16	7.932 ⁻⁰³		
Total	19			

Table A4.5.7. Univariate ANOVA for plasma lactate concentration in *Bos indicus* steers transported for 48 h.

Source	Df	Mean Square	F	Sig.
Intercept	1	7.794	87.093	0.000
Group	2	4.781 ⁻⁰²	0.534	0.596
Error	16	8.948 ⁻⁰²		
Total	19			

Table A4.5.8. Univariate ANOVA for plasma SID in *Bos indicus* steers transported for 48 h.

Source	Df	Mean Square	F	Sig.
Intercept	1	41917.622	581.453	0.000
Group	2	19.942	0.277	0.762
Error	16	72.091		
Total	19			

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Table A4.5.9. Univariate ANOVA for plasma AG in *Bos indicus* steers transported for 48 h.

Source	Df	Mean Square	F	Sig.
Intercept	1	5725.390	64.519	0.000
Group	2	4.212	0.047	0.954
Error	16	88.739		
Total	19			

Table A4.5.10. Univariate ANOVA for plasma phosphorous concentration in *Bos indicus* steers transported for 48 h.

Source	Df	Mean Square	F	Sig.
Intercept	1	119.122	487.347	0.000
Group	2	0.807	3.302	0.063
Error	16	0.244		
Total	19			

Table A4.5.11. Univariate ANOVA for plasma calcium concentration in *Bos indicus* steers transported for 48 h.

Source	Df	Mean Square	F	Sig.
Intercept	1	103.238	4013.320	0.000
Group	2	6.264 ⁻⁰²	2.435	0.119
Error	16	2.572 ⁻⁰²		
Total	19			

Table A4.5.12. Univariate ANOVA for plasma chloride concentrations in *Bos indicus* steers transported for 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	182096.163	6877.501	0.000
Group	2	43.499	1.643	0.224
Error	16	26.477		
Total	19			

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Table A4.5.13. Univariate ANOVA for plasma sodium concentration in *Bos indicus* steers transported for 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	361052.673	13474.744	0.000
Group	2	5.240	0.196	0.824
Error	16	26.795		
Total	19			

Table A4.5.14. Univariate ANOVA for plasma potassium concentration in *Bos indicus* steers transported for 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	311.041	2823.645	0.000
Group	2	0.488	4.434	0.029
Error	16	0.110		
Total	19			

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CHAPTER 6 ANOVA TABLES

Table A4.6.0. Two way ANOVA for plasma potassium concentration in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	979.215	4084.028	0.000
Group	4	0.406	1.693	0.166
Time	1	0.881	3.674	0.061
Group (Time)	4	0.171	0.713	0.587
Error	50	0.240		
Total	60			

Table A4.6.1. Two way ANOVA for plasma magnesium concentration in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	32.871	10900.012	0.000
Group	4	1.043 ⁻⁰²	3.459	0.014
Time	1	8.402 ⁻⁰³	2.786	0.101
Group (Time)	4	1.498 ⁻⁰³	0.497	0.738
Error	50	3.016 ⁻⁰³		
Total	60			

Table A4.6.2. Two way ANOVA for plasma calcium concentration in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	345.648	24395.812	0.000
Group	4	5.777 ⁻⁰²	4.078	0.006
Time	1	1.204 ⁻⁰²	0.850	0.361
Group (Time)	4	6.138 ⁻⁰³	0.433	0.784
Error	50	1.417 ⁻⁰²		
Total	60			

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Table A4.6.3. Two way ANOVA for plasma chloride concentration in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	599000.417	46931.346	0.000
Group	4	23.208	1.818	0.140
Time	1	144.150	11.294	0.001
Group (Time)	4	7.358	0.577	0.681
Error	50	12.763		
Total	60			

Table A4.6.4. Two way ANOVA for plasma glucose concentration in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	2081.526	2006.870	0.000
Group	4	4.588	4.423	0.004
Time	1	0.171	0.165	0.687
Group (Time)	4	0.243	0.234	0.918
Error	50			
Total	60			

Table A4.6.5. Two way ANOVA for plasma total protein concentration in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	375566.817	30149.865	0.000
Group	4	14.442	1.159	0.340
Time	1	84.017	6.745	0.012
Group (Time)	4	2.392	0.192	0.941
Error	50	12.457		
Total	60			

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Table A4.6.6. Two way ANOVA for plasma lactate concentration in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	224.654	181.265	0.000
Group	4	3.109	2.509	0.053
Time	1	13.538	10.923	0.002
Group (Time)	4	1.953	1.576	0.195
Error	50	1.293		
Total	60			

Table A4.6.7. Two way ANOVA for hematocrit in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	85311.603	9001.884	0.000
Group	4	26.128	2.757	0.041
Time	1	8.003	0.844	0.364
Group (Time)	4	4.428	0.467	0.759
Error	50	9.477		
Total	60			

Table A4.6.8. Two way ANOVA for TBW in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	174495.663	3761.823	0.000
Group	4	135.356	2.918	0.030
Time	1	6.584	0.142	0.708
Group (Time)	4	40.501	0.873	0.487
Error	50	46.386		
Total	60			

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Table A4.6.9. Two way repeated measures ANOVA of interaction between time and treatment group for body weight loss in *Bos indicus* steers transported for 24 and 48 h.

Source	Time	df	Mean Square	F	Sig.
Time	BW1-BW2	1	3990.533	80.362	0.000
	BW2-BW3	1	16850.700	818.657	0.000
	BW3-BW4	1	3244.800	501.773	0.000
Time * Group	BW1-BW2	4	46.637	0.939	0.458
	BW2-BW3	4	29.554	1.436	0.251
	BW3-BW4	4	27.383	4.235	0.009
Error (Time)	BW1-BW2	25	49.657		
	BW2-BW3	25	20.583		
	BW3-BW4	25	6.467		

BW1 = Initial BW, BW2 = fasted BW, BW3 = 24 h BW, BW4 = 48 h BW

Table A4.6.10. Two way ANOVA for venous blood bicarbonate in *Bos indicus* steers transported for 24 and 48 h.

Source	df	Mean Square	F	Sig.
Intercept	1	38671.757	13481.981	0.000
Group	4	7.770	2.709	0.043
Time	1	6.427	2.241	0.142
Group (Time)	4	3.382	1.179	0.334
Error	50	2.868		
Total	60			