liveexport

Management of pre-delivery stress in live export steers

Final Report

Project number LIVE.301

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1.0 ABSTRACT

There is an increased recognition of the need to minimise stress in animal production. This project sought to understand the effects of stress in transported cattle and attempted to minimise those effects by the use of novel oral supplements, fed prior to transportation. In addition, the project provided high quality postgraduate training for an MLA/Livecorp funded Research Fellow. The studies described here clearly demonstrate that offering electrolyte solutions to transported *Bos indicus* cattle is unlikely to reduce physiological stressors any more than water alone. Furthermore, the pre-transport treatment of cattle with the novel oral supplement SSF1* may be of significant benefit to their subsequent health and well-being, and warrants further investigation.

* Provisional Patent Application No.: 2004902760

2.0 EXECUTIVE SUMMARY

2.1 Introduction

There is increasing recognition of the need to minimise *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. 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. This project combined the resources, experience and expertise of the *Chief Investigator* with the focus of a dedicated *Research Fellow*, to investigate transportation *stress* in live export cattle, in significant depth, over the three year life of the *Research Fellow's* formal graduate studies program.

2.2 Project Objectives

The objectives of the project were: 1) *To enhance knowledge and understanding of the response of cattle to stressors typically associated with the collection and transportation for live export and their impacts on animal welfare and performance*; 2) *To determine the relationships between stress and dehydration, electrolyte and acid/base balance, plasma cortisol concentrations, a range of blood parameters, and cell mediated immunity*; 3) *To develop and validate a simple (crush-side) test that reflects an individual animal's response to the stress of a live export protocol and associated transportation*; 4) *To develop and test protocols to minimize production losses associated with stress in cattle for live export*; 5) *To develop and test treatments for cattle determined to be significantly stressed by the live export protocols*; and 6) *To provide high quality research training for a candidate enrolled in a formal graduate studies program.*

2.3 Significant Results

The studies undertaken in this project have: 1) Validated some new techniques for investigating the physiological responses of cattle to stress; 2) Quantified some of the physiological responses of cattle subjected to long-haul transportation and handling stress; 3) Identified a novel prophylactic treatment that may aid in reducing the effects of stress in cattle; and 4) Provided a quality research training experience.

Initial pen studies, utilizing a stress model in sheep demonstrated that well hydrated ruminants placed under stressful conditions respond with a diuresis (ie increased urination). Water deprivation has been touted as a significant stressor to ruminants. In *Bos indicus* steers, 60 h of water deprivation resulted in a significant increase in the concentration of cortisol in the plasma (generally regarded an indication of increased stress).

Evidence from the studies described here demonstrates that the concentration of potassium in plasma consistently decreases in ruminants under stress. In contrast plasma sodium, although elevated during water deprivation, does not yield a consistent response. Similarly, plasma magnesium and calcium are influenced more by water and feed deprivation than by the animal's physiological response to stress.

Transportation studies showed no differences in the pH of arterial blood from transported or non-transported steers, confirming that transportation stress causes no differences in the acid-base status of transported versus non-transported ruminants. Dehydration in the water deprived animals, both transported and non-transported, was shown to induce a mild metabolic acidosis. In the transported animals, there was a significant decrease in plasma concentrations of potassium, however, all other electrolytes measured did not differ between

* Provisional Patent Application No.: 2004902760

groups. As a result of this, the anion gap and strong ion group calculations also showed no differences 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.

Hydration strategies involved with the transportation process rely on the replacement of lost body water and electrolytes at the completion of the journey, ie after the welfare of the animals has been compromised. This project sought a novel approach to the problem of dehydration associated with transportation of steers. Prophylactic hyper-hydration steers was achieved during the first 24 h of transportation by the novel application of the oral supplement SSF1*, allowing a delay in TBW loss. This finding has significant welfare and production implications in that a prophylactic dose of SSF1* could assist the animal to remain hydrated longer in the export process.

As an important metabolic fuel source, SSF1* hypothetically may be a preferential supply of energy for cattle during times of transportation stress. In promoting gluconeogenesis and glycogen formation, SSF1* appears to have nitrogen-sparing effects in the ruminant, which may aid in the preservation of carcass protein and decrease dark cutting beef. It is possible that higher concentrations of plasma glucose may lead to greater steady state insulin concentrations, inhibiting muscle protein breakdown. Immunoprotective effects of the oral supplement SSF1* were also identified. The apparent ability of SSF1* treated steers to maintain white blood cell numbers in the face of significant stressors indicates a potential application for this treatment in limiting the physiological effects of stress by modulating the decrease in immunocompetence that is a feature of stress in cattle.

2.4 Conclusions

There are two significant outcomes from this project.

1) The data from this project challenges the current "Best Practice" management protocols for transported ruminants, in particular, the efficacy of electrolyte solutions administered pre- and(or) post-transportation to minimise effects of stress. Electrolyte solutions fed post-transportation would provide little benefit in correcting an animal's acid-base balance, compared to water alone.

2) Prophylactic pre-transport treatment of cattle with SSF1* could have a number of significant welfare and production benefits for cattle, and a provisional patent application has been filed on the novel application of this oral supplement (Provisional Patent Application No.: 2004902760). Pre-transport hyperhydration with the oral supplement SSF1* may: 1) Reduce the relative loss of body water during transportation. 2) Assist in delaying the catabolic effects of dehydration. 3) Promote gluconeogenesis and glycogen formation thus aiding in the preservation of carcass protein and decreasing the incidence of dark cutting meat. 4) Provide immunoprotective effects by enhancing lymphocyte function in the post-transport period.

These outcomes have the potential to benefit not just the livestock export and feedlot industries, but the entire meat and livestock industry, whenever ruminants are transported.

2.5 Recommendations for future actions

More research, including investigating genotype effect, is needed into the hyperhydration, metabolic and immunoprotective effects of SSF1*. This must be conducted in a timely manner if full patent protection of the novel use of this oral supplement is to be pursued.

* Provisional Patent Application No.: 2004902760

3.0 MAIN RESEARCH REPORT

3.1 Background

The research undertaken under this project dealt with the issue of stress in live export cattle, and the impact of stress during pre-delivery management on their performance. It focussed on the identification, treatment and prevention of animals that are *stressed* by the live export process to a significantly greater extent than their cohort and are therefore likely to be at greater risk of mortality and(or) poor performance. At the outset, it was assumed that *stress* was likely to be manifested as one or a combination of dehydration, dysfunction of electrolyte and(or) acid/base balance, reduced appetite and associated decreased growth rate and(or) compromised immuno-competance resulting in an increased susceptibility to disease. As such, the project fell under the category of Preparation and Delivery, in the joint R&D program for 1999-2000. Furthermore, it addressed a significant animal welfare issue.

Provision was made in the project for a postgraduate training scholarship under the Livestock Export Sector's Industry Learning Sub-Program.

3.2 Project Objectives

The objectives of the project were:

1) To enhance knowledge and understanding of the response of cattle to *stressors* typically associated with the collection and transportation for live export and their impacts on animal welfare and performance

2) To determine the relationships between *stress* and dehydration, electrolyte and acid/base balance, plasma cortisol concentrations, a range of blood parameters, and cell mediated immunity

3) To develop and validate a simple (crush-side) test that reflects an individual animal's response to the stress of a live export protocol and associated transportation

4) To develop and test protocols to minimize production losses associated with *stress* in cattle for live export

5) To develop and test treatments for cattle determined to be significantly stressed by the live export protocols

6) To provide high quality research training for a candidate enrolled in a formal graduate studies program

3.3 Methodology

There is increasing recognition of the need to minimise *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. 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 that is likely to be manifested as disturbances of the fluid, electrolyte and acid /base balance of these animals, or some other physiological parameter that can be measured. It was hypothesised that a relationship exists between fluid, electrolyte and acid/base balance, in particular, and stress in these animals. The result of this imbalance in blood homeostasis indicators may contribute to a lack of dry matter intake on the sea voyage

with body weight loss being a primary consequence. A further consequence of high and(or) prolonged exposure to such *stress* is likely to be a decline in immuno-competance and increased susceptibility to disease, as a result of elevated plasma cortisol levels, which should be measurable as changes in the white blood cell profile of the animal and compromised cell medicated immunity.

This project combined the resources, experience and expertise of the *Chief Investigator* at James Cook University (JCU) with the focus of a dedicated *Research Fellow*, to investigate transportation *stress* in live export cattle, in significant depth, over the three year life of the Research Fellow's formal graduate studies program. The project comprised a number of phases, as follows, some of which overlapped during the life of the program.

a) Literature Review - An extensive review of the literature was undertaken by the *Research Fellow* at the commencement of the project. This focused on livestock transport, stress, dehydration, electrolyte and acid/base balance, treatment and prevention, and was both an essential preliminary to further research and a formal requirement for the graduate studies program.

b) Intensive Pen Studies - Steers from JCU's *Fletcherview* Research Station were used to develop a model of stress in live export cattle. A steer model was chosen because steers represent the largest class of cattle that are exported and because they have inherently less variation than do other classes of cattle.

A series of studies were undertaken at JCU's cattle research facilities to investigate the relationships between *stress*, dehydration, rumen and plasma electrolytes, acid/base balance, plasma cortisol concentrations, haematocrit/packed cell volume (PCV), total leucocyte (white blood cell) count, differential leucocyte count, as well as water and food intake and liveweight gain in typical live export steers.

These studies were used to develop management protocols to minimize the detrimental effect of *stress* in cattle undergoing transportation for export.

c) Field Studies - Field studies were carried out to validate the technology developed in pen studies to identify *stressed* animals during transportation for export as well as to confirm the value of any treatment protocols developed, under actual live cattle export conditions.

Steers from JCU's *Fletcherview* Research Station, as well as those of a collaborating producer were utilized.

4.0 EXPERIMENTAL SCHEDULE

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

4.1.1 Introduction

The dilution principle technique for estimating total body water (TBW) has frequently been used to determine the body composition of animals *in vivo*. The principle is based on 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 be foreign to the body. The two most frequently used markers are urea and tritiated water (TOH). Although tritiated water consistently yields a high degree of accuracy, 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 cattle (Preston & Kock 1973; Kock & Preston 1979; De Campeneere *et al.* 2000) have indicated that urea can provide a similar degree of accuracy in the determination of TBW, in a shorter time frame.

4.1.2 Materials and Methods

Animals and management

Bos indicus steers (n = 6, 3 yr of age, 340 ± 20 kg mean BW) were placed into individual stalls and fed a commercial forage cube *ad libitum* for 3 d prior to the commencement of the experiment. Animals were weighed immediately before the procedures, TOH and urea space were calculated on this weight. All experimental procedures were reviewed and approved by the animal ethics committee at JCU (Approval No. A664-01).

Infusion and sampling procedures

On day 0, all animals were catheterized with a polyvinyl chloride tube inserted into the jugular vein under local anaesthetic. 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.

A bolus of approximately 500 µCi of TOH was injected into the jugular vein via the catheter, followed by 10 mL heparinised isotonic 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. 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 isotonic saline administered through the implanted catheters over a 2 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 isotonic saline solution 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.

TOH sample analysis

Plasma samples (0.5 mL) were deprotienised and agitated for 20 sec to ensure mixing prior to centrifugation at 200 x *g* at 4°Celsius for 20 minutes. Biodegradable counting scintillant (10 mL) was placed into scintillating glass tubes with 0.5 mL 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 Autoanalyser 2. 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)]. Total body water was recorded as the pool of water available to the urea molecule.

4.1.3 Results and Discussion

The time curve for TOH demonstrates equilibration to occur between 8 to 12 h after TOH administration (Figure 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, dependant on the species used, initial amount of TOH injected, the route of administration and fat composition of the animal (Seif 1972; Meissner 1976; Thornton and English 1978).

The urea time curve for the six steers appeared to equilibrate between 12 to 15 minutes post-infusion (Figure 2). Preston and Kock (1973) concluded that although urea mixes in the total body water 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 r2 = 0.89) in double muscled bulls.

Implications

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 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 gastrointestinal tract and that of the rest of the body using *in vivo* techniques is difficult to obtain. Compartmental models have been utilized by others to separate water in the gastrointestinal tract from total body water 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.

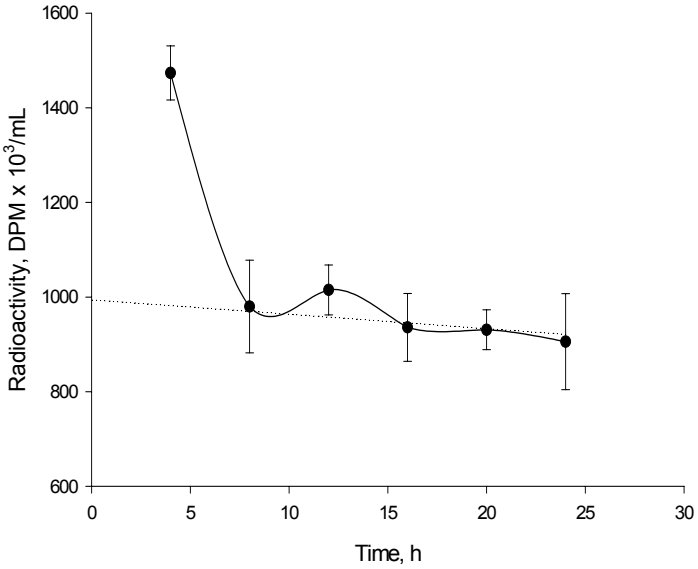

Figure 1. Mean ± SEM change in radioactivity in six *Bos indicus* steers after infusion with 500 µCi/steer

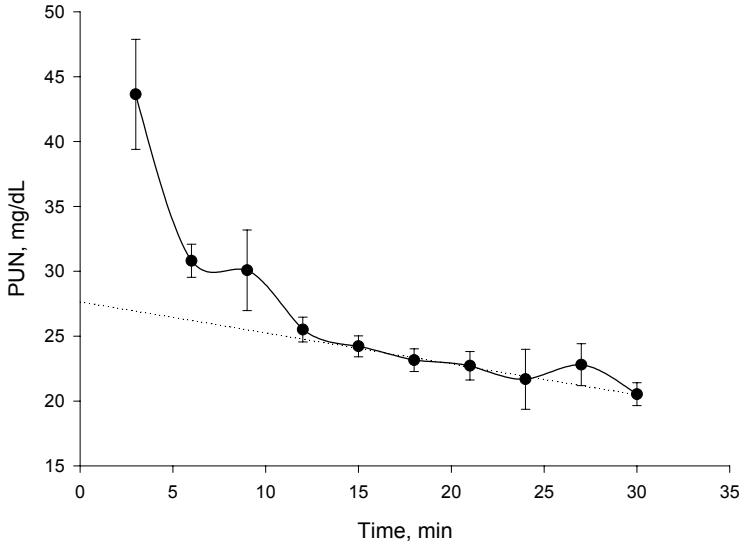

Figure 2. Mean ± SEM change in plasma urea nitrogen (PUN) in six *Bos indicus* steers after infusion with 130 mg urea/kg BW

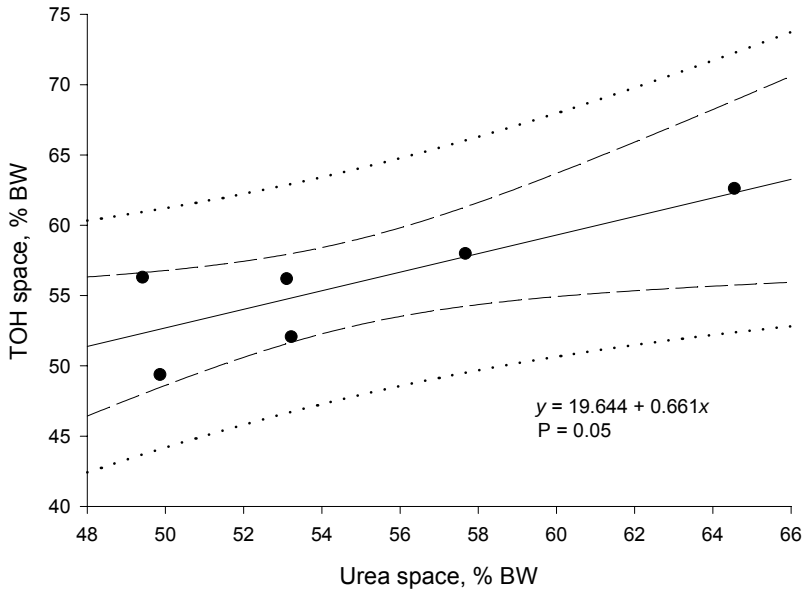

Figure 3. Relation between urea space and TOH space in six *Bos indicus* steers. Dashed lines depict confidence interval of 95%. Dotted lines depict predicted intervals.

4.3 Dehydration in stressed ruminants may be the result of a cortisol induced diuresis

4.3.1 Introduction

Researchers have endeavoured to discover the physiological changes that occur when animals are exposed to stressors by utilizing models that mimic the effects of the hypothalamo-pituitary-adrenal (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 principle stress hormone associated with the activation of the HPA axis, and has been shown to induce pathophysiological changes to the immune, metabolic and reproductive systems of animals.

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 adrenocorticotrophic hormone 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 in ruminants induce a diuresis that contributes to water loss in excess of electrolyte loss. Merino sheep were used as the ruminant model to test the initial hypothesis.

4.3.2 Materials and Methods

Animals and management

Twenty four, 18 mo Merino wethers (37.0 $\pm$ 0.94 kg mean body weight) were allocated to metabolism crates at random and fed oaten chaff *ad libitum* for 10 d prior to the commencement of the experiment. The mean daily wet bulb temperatures during the experimental period for days 0, 1, 2, 3 were 22.2^{o}C, 22.8^{o}C, 22.9^{o}C and 22.9^{o}C, respectively. There was no significant difference between the wet bulb temperatures for the acclimatization period or the experimental period. All experimental procedures were reviewed and approved by the animal ethics committee at JCU (Approval No. A664-01).

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 = 4); 3) no water/cortisol (n = 6); and 4) water/cortisol (n = 4). On day 0, all animals were catheterized with a poly-vinyl chloride tube 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 0.1mg/kg BW/hr of hydrocortisone suspended in isotonic saline administered at a rate of 0.1mL/kg BW/hr, 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 at the commencement of the experiment.

Sample collection

On day 0, 10 mL of blood was manually collected from all treatment groups into lithium heparin containing tubes and continued at 3 h intervals for 72 h. Intakes of water and feed were measured daily. Total urine excreted was collected, measured and sub-sampled 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 x *g* for 15 min and plasma poured off within two hours and frozen (-20°C) for analysis at a later date. Plasma cortisol concentration was measured using a radioimmunoassay kit.

Urea space measurements

Urea space was determined on days 0, 1, 2, 3 for each animal using the technique described by Preston and Kock (1973). The following formula was used to calculate urea space as a percentage of live weight.

Urea space % = [Volume infused (mL) * concentration of solution (mg urea-N/dL)] / [Plasma Urea Nitrogen / live weight in kg]. Total body water was recorded as the pool available to the urea molecule.

Urea and Electrolyte measurement

Plasma urea nitrogen was analyzed with a Technicon Autoanalyzer 2. 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.

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 the SPSS 10 software package (SPSS 2001). Four animals had to be withdrawn from the experiment, one for scours and three for blocked catheter lines.

4.3.3 Results and Discussion

Plasma cortisol concentration

The infusion rate chosen in this study appeared to offer a physiological dose rate, when mean plasma cortisol concentrations (Figure 4) 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. 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.

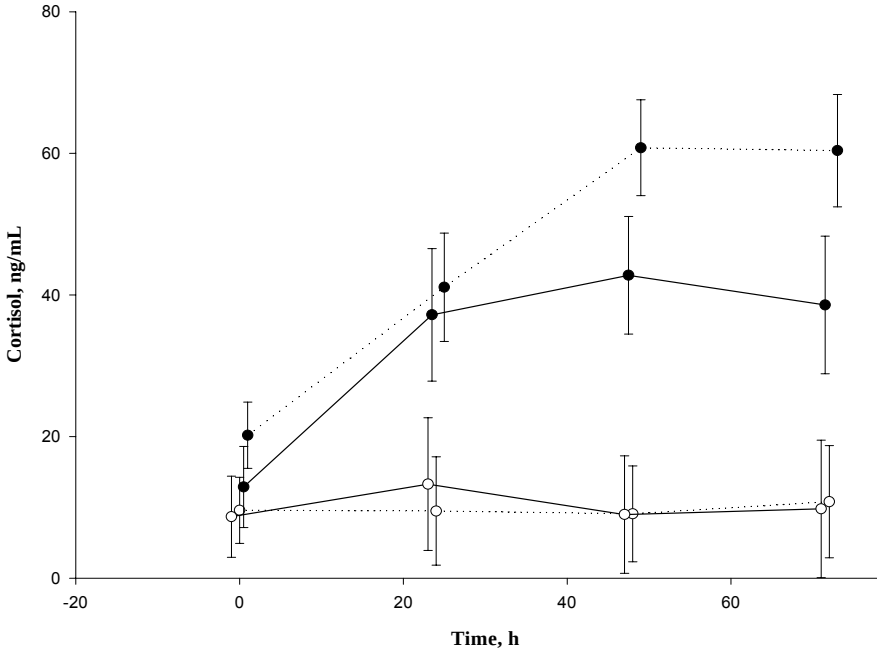

Figure 4. Plasma cortisol concentrations (mean ± SEM) at 0, 24, 48 and 72 h for four groups of sheep in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

Body Water

There was no change in body water (Figure 5) 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% ± 4.2% and 53.2% ± 5.1%, respectively), before losing 13% by 72 h (40.2% ± 5.7%). A time x water interaction demonstrated body water loss for the no water groups between 24 and 48 hours (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 (total body water 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.

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 6). 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

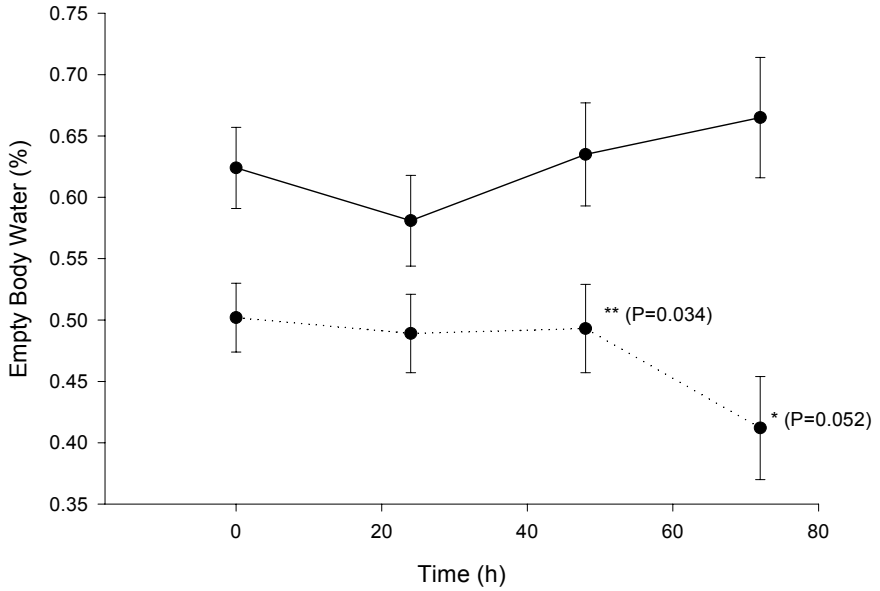

Figure 5. Empty body water (mean ± SEM) at 24, 48 and 72 h for two groups of sheep which were either water deprived (dotted line) or given ad libitum access to water (solid line).

water is unavailable (Phillips *et al.* 1991; Atkinson 1992). The mechanism by which cortisol induces a diuresis is still under debate. In cattle ,El-Nouty *et al.* (1977) demonstrated 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 due 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. 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 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 and increasing the secretion and efficacy of atrial natriuretic peptide, both of which enhance water excretion. This response may explain why the greatest contributing factor to the two-way and three-way interaction involving cortisol seen in this 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 it's 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, in the presence of *ad libitum* water, that glucocorticoids promoted a diuresis by increasing the glomerular filtration rate.

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 adrenocorticotrophic hormone (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).

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 the HPA axis on aldosterone secretion, other aldosterone secretagogues (angiotensin II and plasma potassium) have specific actions on the adrenal glomerulosa alone, and do not stimulate glucocorticoids. Although in well hydrated animals elevated concentrations of cortisol

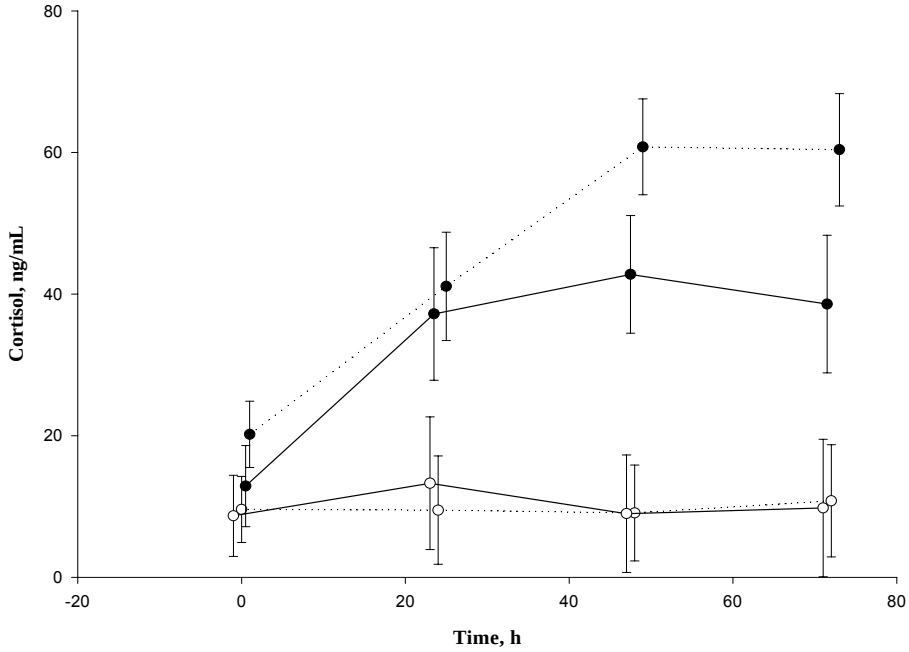

Figure 6. Plasma cortisol concentrations (mean ± SEM) at 0, 24, 48 and 72 h for four groups of sheep in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

induces a diuresis, it would appear from the results of this study that the principle effect of cortisol on the ruminant body is to protect and maintain water balance in times of stress.

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

1). There was also a time effect for decreasing feed intake between 48 and 72 h for all groups ($P < 0.001$).

Table 1. 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]
		Water Intake, kg/d		
24		2.08 ± 0.49		2.45 ± 0.44
48		2.84 ± 0.31		3.01 ± 0.28
72		2.58 ± 0.28		2.49 ± 0.25
		Feed Intake, kg/d as-fed		
24	0.69 ± 0.13	0.86 ± 0.14	0.67 ± 0.14	0.86 ± 0.14
48	0.49 ± 0.05	0.73 ± 0.59	0.48 ± 0.59	0.83 ± 0.59
72	0.23 ± 0.82\	0.45 ± 0.09	0.28 ± 0.90	0.59 ± 0.09

[a]n=6

[b]n=4

Although mean daily wet bulb temperature increased by 0.7^0C 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 the wet bulb temperature on water and feed intake, the wet bulb temperature dropped significantly for nine hours per day allowing night-time 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.

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 7). There were no differences between groups at 72 h for total urinary sodium output.

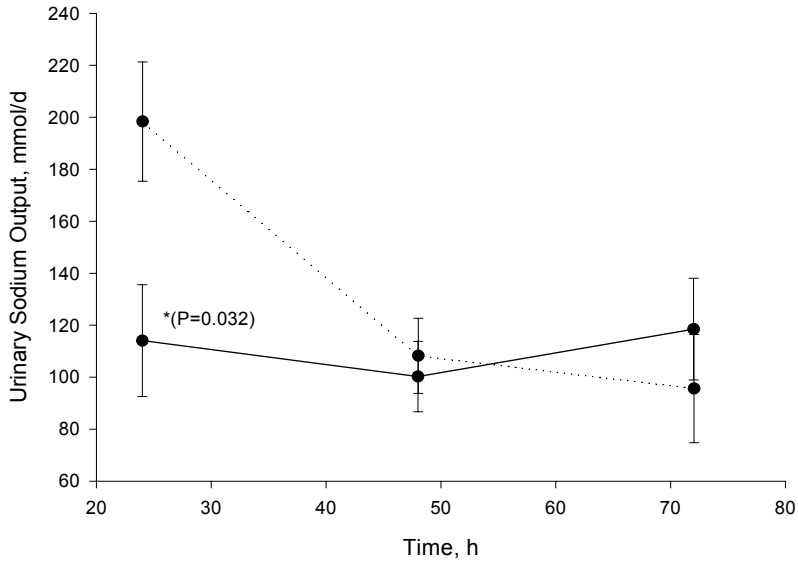

Figure 7. Total urine sodium output (mean ± SEM) at 24, 48 and 72 h for two groups of sheep in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

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.

There was a significant water x 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 8) 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).

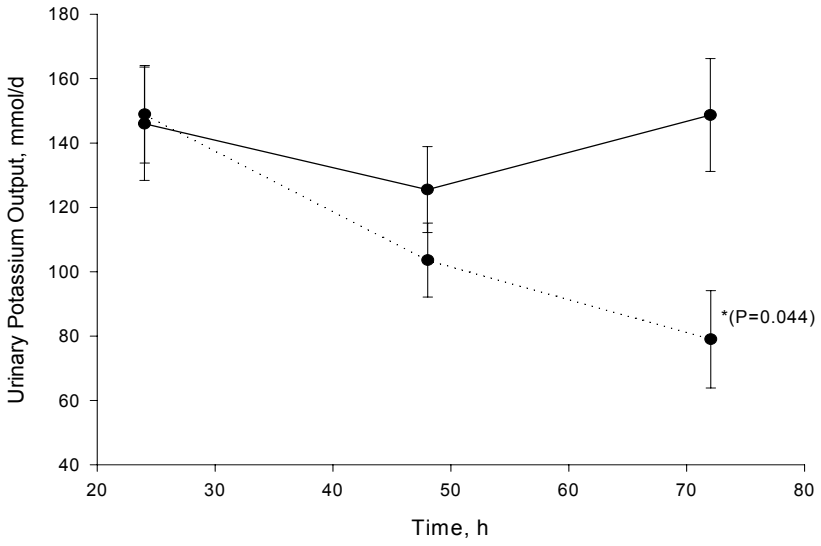

Figure 8. Total urine potassium output (mean ± SEM) at 24, 48 and 72 h for two groups of sheep which were either water deprived (dotted line) or given ad libitum access to water (solid line).

There was a water x 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 9). 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 calciotrophic hormones are similar for calcium and magnesium and are said to influence magnesium in the kidney, affecting reabsoption (Saris *et al.* 2000). The action of cortisol is said to induce a negative calcium balance by decreasing calcium absorption in the gastrointestinal tract 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.

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 10). 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.

There was a trend toward a time x cortisol interaction on plasma potassium concentrations from 0 to 24 h (*P* = 0.078) (Figure 11), 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

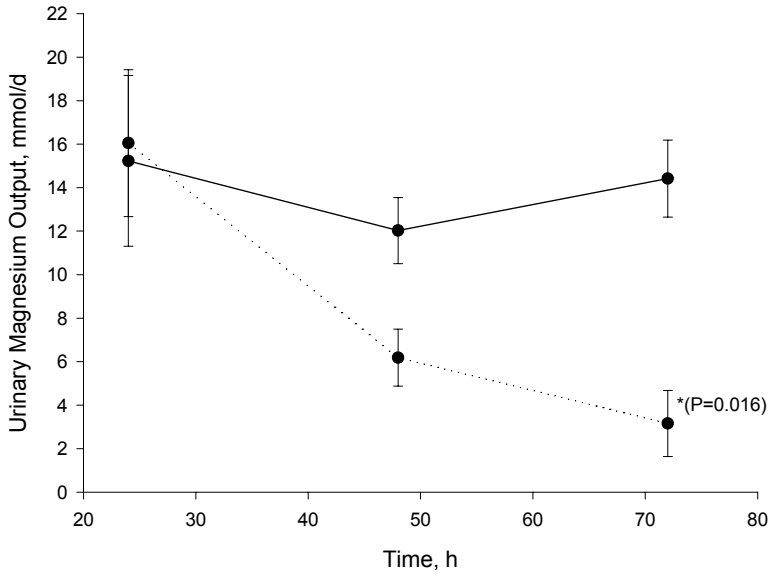

Figure 9. Total urine magnesium output (mean ± SEM) at 0, 24, 48 and 72 h for two groups of sheep which were either water deprived (dotted line) or given ad libitum access to water (solid line).

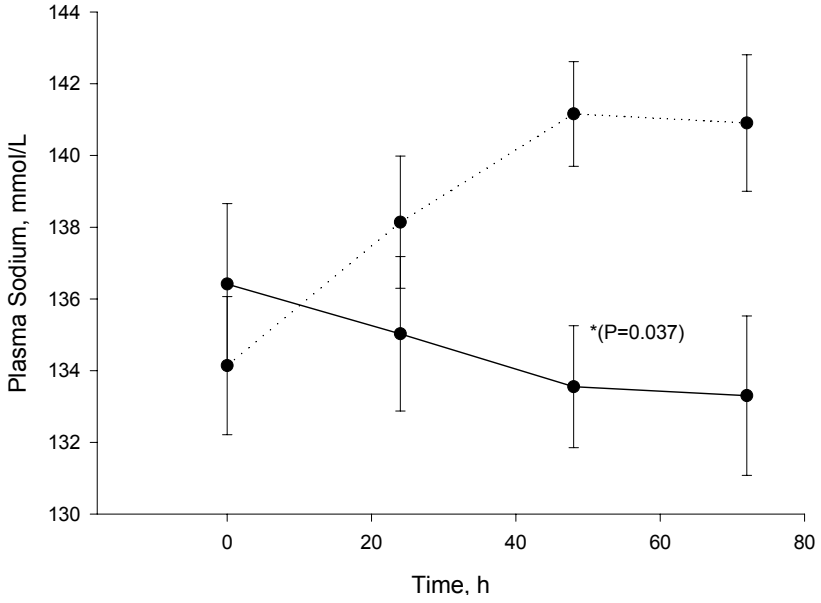

Figure 10. Plasma sodium concentration (mean ± SEM) at 0, 24, 48 and 72 h for two groups of sheep which were either water deprived (dotted line) or given ad libitum access to water (solid line).

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 have resulted in a similar outcome to that seen in this 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).

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, Galyean *et al.* (1981) demonstrated no difference in plasma sodium concentration compared with unstressed controls, in steers subjected to fasting or transportation and fasting stress.

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

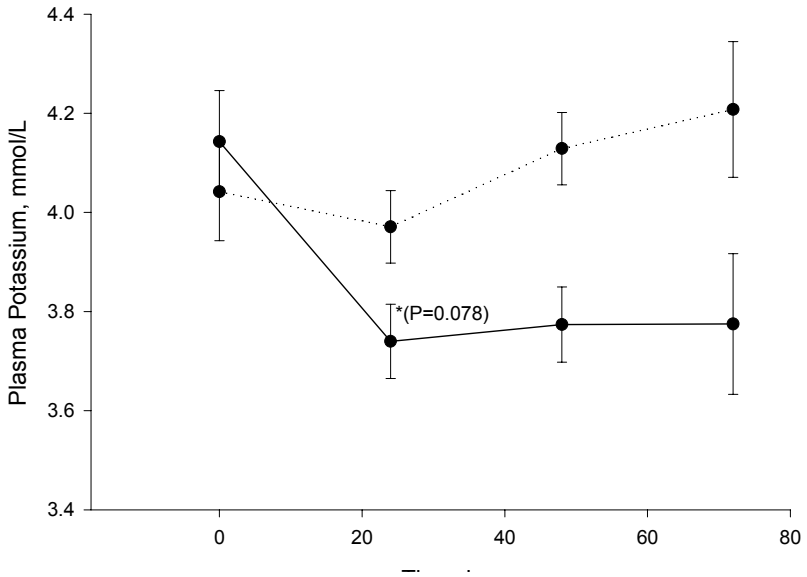

Figure 11. Plasma potassium concentration (mean ± SEM) at 0, 24, 48 and 72 h for two groups of sheep in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

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, seventy-two hours 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.

The results of this experiment are published in full in the Journal of Animal Science, as follows:

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

4.4 Excess cortisol interferes with a principle mechanism of resistance to dehydration in Bos indicus steers

4.4.1 Introduction

Ruminants exposed to the stressors of transport and handling respond with an activation of the sympatho-adrenal-medullary (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.

In the previous experiment, we adapted a stress model based upon cortisol infusions from Macfarlane *et al*. (2000) to investigate the effects of excess cortisol infusions on water balance using a Merino sheep model (Parker *et al.* 2003a). As a consequence of this work, it appeared that the stress hormone cortisol, had the capacity to interfere with a principle mechanism of resistance to dehydration. Cortisol has been implicated in inhibiting the effects of arginine vasopressin in dogs (Baas *et al.* 1984) and there is evidence to suggest elevated ACTH and glucocorticoids also inhibit the renin-angiotensin-aldosterone (RAA) axis (Coghlan *et al.* 1979). In this experiment we applied the sheep model described in Experimant B to cattle. The 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.

4.4.2 Materials and Methods

Animals and management

Bos indicus steers (n = 32, 2 yr of age, 193 $\pm$ 21.47 kg mean BW) were fitted with canvas urine collectors 21 d prior to initiation of experimentation to minimize stress during sample collection. The steers were allocated to individual stalls at random and fed a commercial forage cube *ad libitum* for 14 d prior to the commencement of the experiment All experimental procedures were reviewed and approved by the animal ethics committee at JCU (Approval No. A664-01).

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 polyvinyl chloride tube inserted into the jugular vein under local anaesthetic. All animals allocated to the two cortisol groups were given 0.1mg/kg BW/h of hydrocortisone suspended in isotonic saline administered at a rate of 0.1mL/kg BW/h, 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.

Sample collection

On day 0, 20 mL of blood was collected from all treatment groups and decanted into 2 x 10mL tubes containing lithium heparin. The catheters were then flushed with 10mL of heparinized isotonic saline to prevent clotting between sampling periods. The sampling regimen continued at 6 h intervals for 90 h. Total urine excreted was collected and measured every 24 h. 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.

Urea, electrolyte and metabolite measurement

Analysis of Na and K in steer plasma was conducted using ion selective electrodes (Lablyte 830 electrolyte analyzer). Plasma Ca, P, Cl, total protein, and albumin were analyzed spectrophotometrically using a Cobas-Mira Autoanalyzer. Plasma urea nitrogen was analyzed with a Technicon Autoanalyzer 2. Heamatocrit was measured using a micro-centrifuge. Plasma osmolality was measured using an automatic osmometer.

Plasma cortisol concentration was measured using a standard radioimmunoassy kit. Plasma AVP and AII concentrations were assayed using a radioimmunoassay method by Austin Biomedical services. 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). Total body water was recorded as the pool available to the urea molecule.

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. Least squares means and standard errors have been presented. 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 12) 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.

4.4.3 Results and Discussion

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. This result was unexpected given that the dose rate was extrapolated on a per kg basis from that used for sheep in the previous experiment.

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 12). 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 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 this 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.

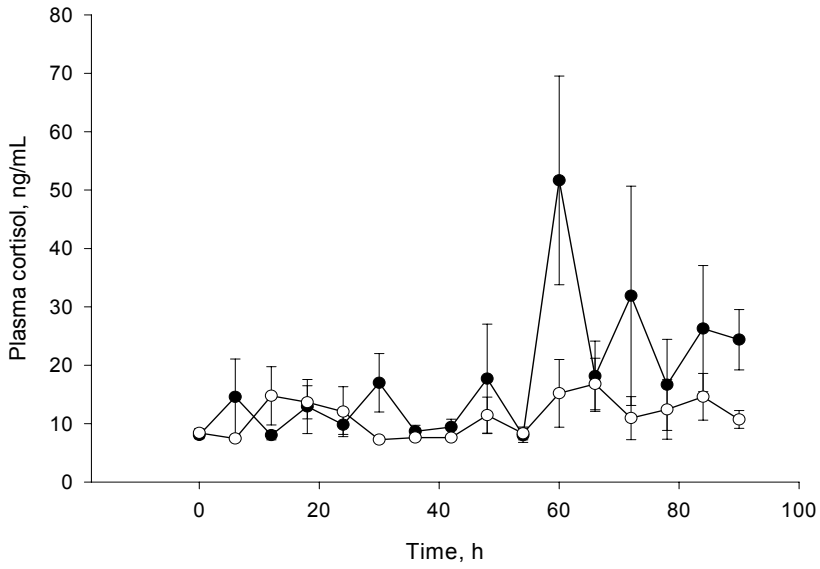

Figure 12. Plasma cortisol concentration (mean ± SEM) at 6 h intervals for 90 h in the no water/no-cortisol (●) and water/no-cortisol (○) steer groups. The no-water/no-cortisol group demonstrated a group X time interaction (P = 0.028) toward increasing the area under the plasma cortisol concentration curve from 60 –90 h compared to the water/no-cortisol group.

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 13), 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 this 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 $\pm$ 4.60 pg/mL and 30.38 $\pm$ 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 $\pm$ 2.2 pg/mL that did not change for the rest of their water deprivation period of 14 d (5.7 $\pm$ 2.2 pg/mL).

Arginine vasopressin may stimulate ACTH secretion and potentiate the response to corticotrophin releasing factor (Redekopp *et al*. 1985; Redekopp *et al*. 1986). 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 this study. Water deprivation, however, had a consistent effect in increasing AVP concentrations.

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 14).

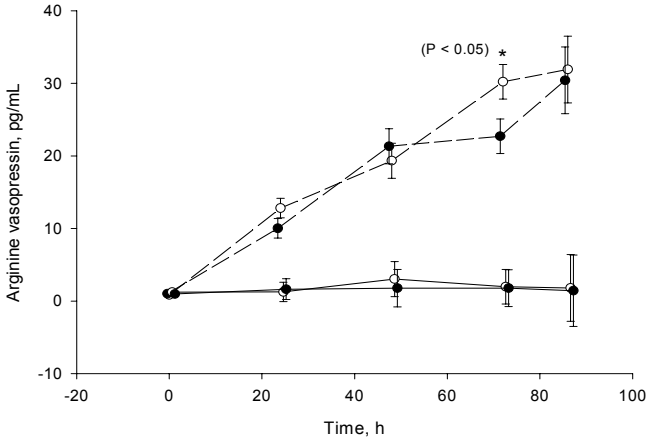

Figure 13. Plasma concentrations of Arginine Vasopressin (AVP) in *Bos indicus* steers (mean ± SEM) at 0, 24, 48, 72 and 90 h for hour groups of steers in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dashed line) or given *ad libitum* access to water (solid line). * Denotes a water-x-time interaction (P<0.05).

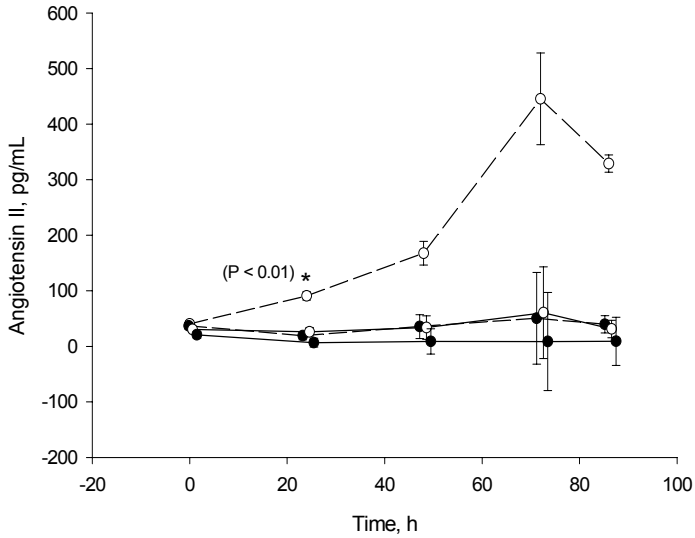

Figure 14. Plasma concentrations of Angiotensin II (AII) in *Bos indicus* steers (mean ± SEM) at 0, 24, 48, 72 and 90 h for four groups of steers in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dashed line) or given *ad libitum* access to water (solid line). * Denotes a cortisol-x-water-x-time interaction (P<0.01).

The RAA axis remains a principle mechanism in the resistance to dehydration. 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. Angiotensin II 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 this 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).

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 15). 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.

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 16). The decrease in osmolality at 72 h for all groups may have been due to fluctuations in water compartments within the animal. An elevation of total protein and albumin is indicative of heamoconcentration due to dehydration. The elevation of plasma proteins was not found in this study and changes across time were similar for all groups for total protein and albumin.

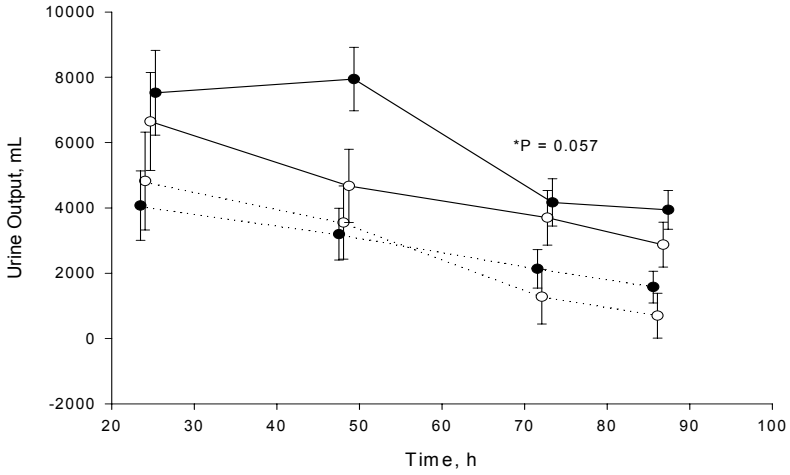

Figure 15. Total urine output (mean ± SEM) at 24, 48, 72 and 90 h for four groups of steers which were given an injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

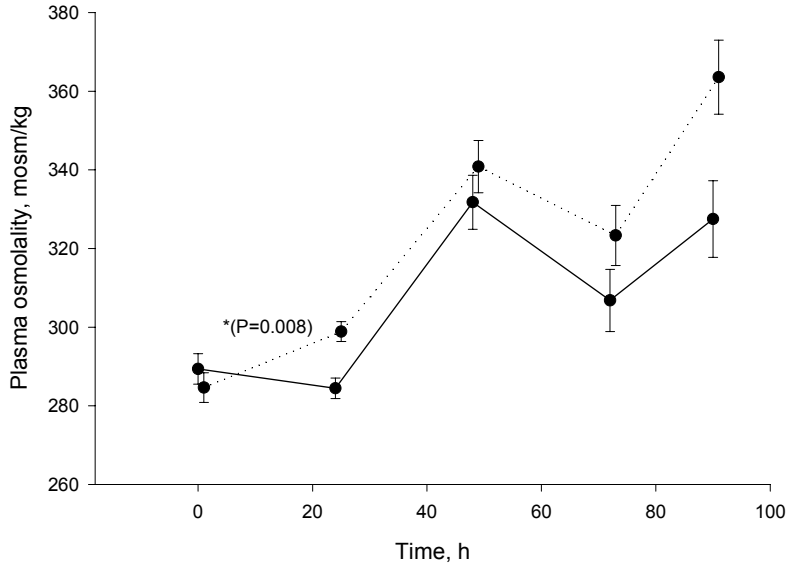

Figure 16. Plasma osmolality (mean ± SEM) at 0, 24, 48, 72 and 90 h for two groups of steers that were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

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 lesser hematocrit as compared with the water deprived groups at 90 h (Figure 17). Hematocrit data did not indicate dehydration in any group.

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 have previously demonstrated 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 is supported by a time x water effect on plasma osmolality in the water-deprived groups. In contrast to this the hematocrit, total protein, and albumin data indicated no difference among groups in this 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 and analytical errors were minimized in this 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.

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 18), 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 this study, water deprivation alone had a greater effect on plasma Na concentration.

A cortisol x time interaction was detected between 0 and 24 h (P = 0.001) of the treatment period demonstrating that cortisol infusion induced a decrease in plasma K at 24 h of treatment (Figure 19). 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.

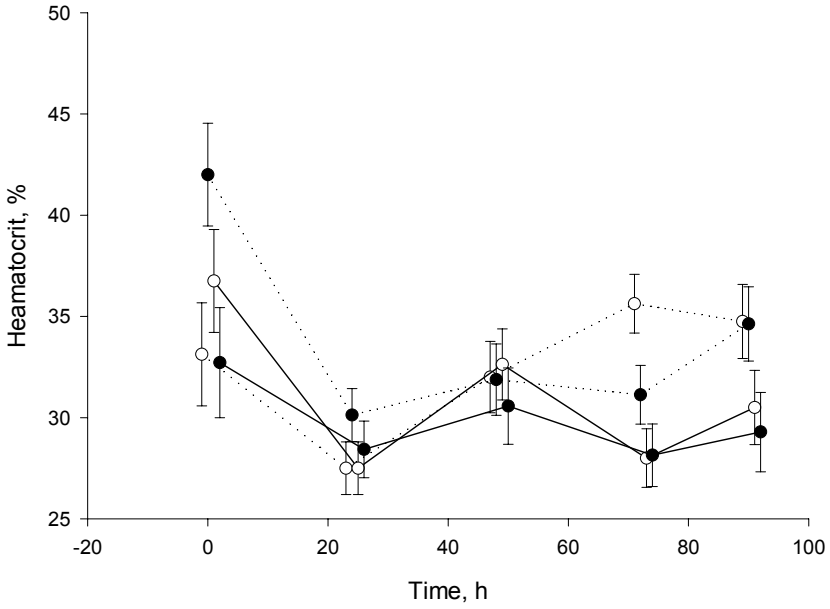

Figure 17. Haematocrit (mean ± SEM) at 24, 48, 72 and 90 h for four groups of steers which were given an injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given *ad libitum* access to water.

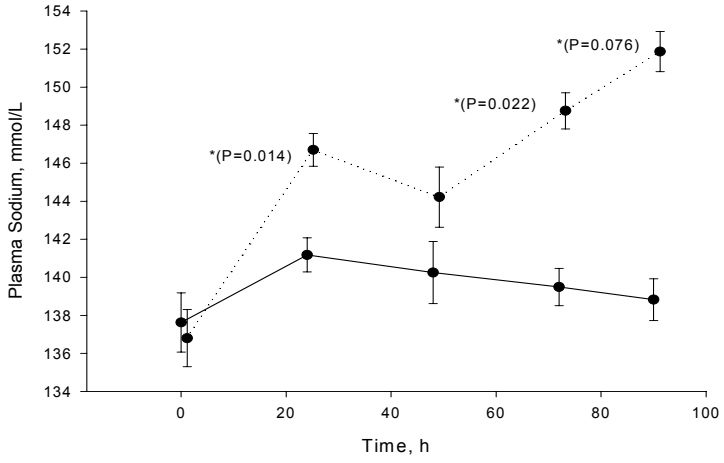

Figure 18. Plasma sodium concentration (mean ± SEM) at 0, 24, 48, 72 and 90 h for two groups of steers that were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

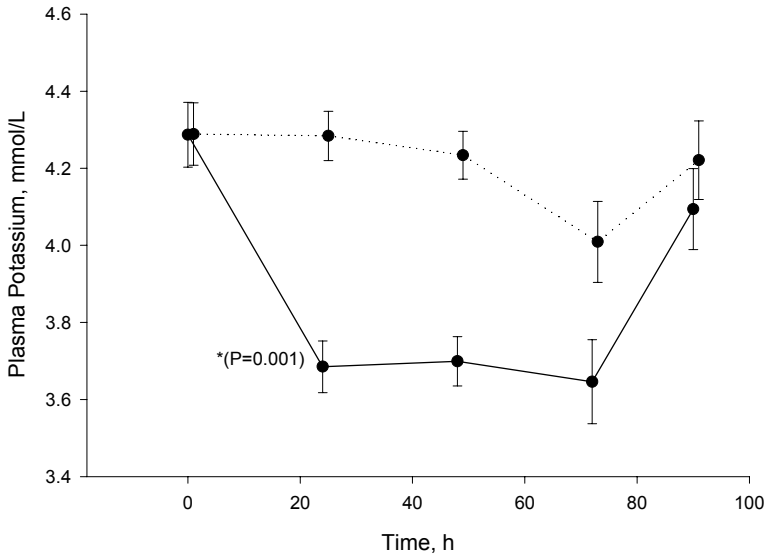

Figure 19. Plasma potassium concentration (mean ± SEM) at 0, 24, 48, 72 and 90 h for two groups of steers in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

Plasma Ca concentrations had water x time interactions 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 20). The reduced feed intake by water deprivation and the subsequent effects on gastrointestinal motility and absorption in the water-deprived groups did not have a significant effect until 90 h. The plasma Ca concentration of the no-water/no-cortisol group were 1.75 mmol/L ± 0.12 mmol/L at 90 h of the experimental period which falls below the range for calcium in cattle (2.00 – 2.62 mmol/L) (Blood and Radostits 1989), although all other groups were within the normal range.

Water x 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 21). Plasma concentrations and interactions of Cl followed Na concentrations in plasma. All groups were within normal ranges for plasma Cl in cattle (95 –110 mmol/L) (Blood and Radostits 1989).

A 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 22). The plasma P concentration of the cortisol groups remained within normal limits for cattle (1.30 – 2.25 mmol/L) (Blood and Radostits 1989).

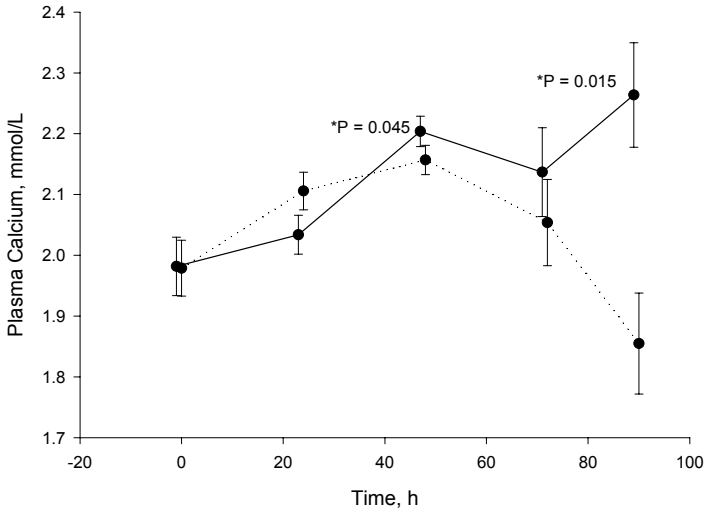

Figure 20. Plasma calcium (mean ± SEM) at 0, 24, 48, 72 and 90 h for two groups of steers that were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

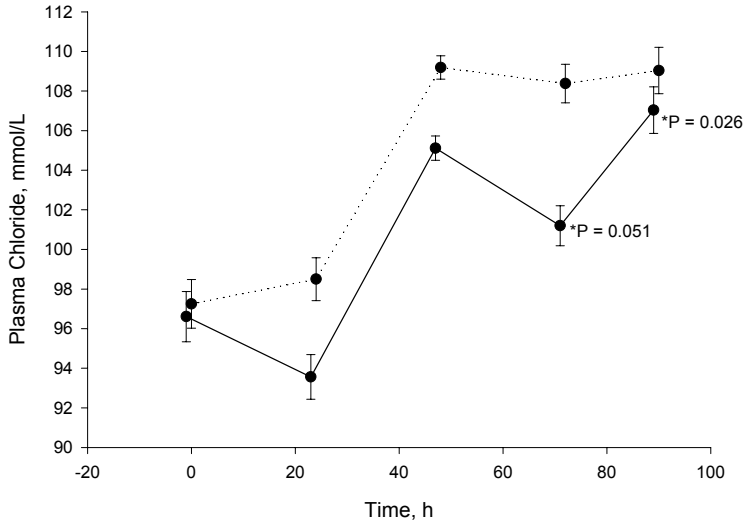

Figure 21. Plasma chloride concentration (mean ± SEM) at 0, 24, 48, 72 and 90 h for two groups of steers that were either water deprived (dotted line) or given *ad libitum* access to water (solid line).

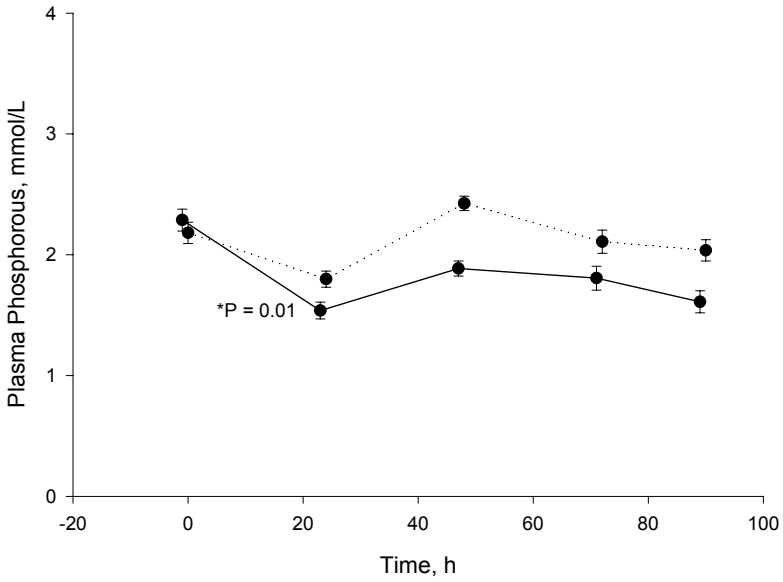

Figure 22. Plasma phosphorous concentration (mean ± SEM) at 0, 24, 48, 72 and 90 h for two groups of steers in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

Water and Feed Intake

The animals offered water demonstrated a time effect, decreasing their water intake between 24 and 48 h (P = 0.016) before increasing their intake from 48 and 72 h (P=0.000) (Table 2). However, the watered groups did not alter their feed intake 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 this 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.

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, however, it does not affect the circulating concentrations of arginine vasopressin. Plasma electrolytes in this study demonstrated 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.

The results of this experiment are published in full in the Journal of Animal Science, as follows:

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

Table 2. 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±1.95		18.09±2.08
24		16.44±1.36		14.36±1.46
48		10.36±1.58		11.86±1.69
72		21.86±1.28		22.14±1.37
90		9.94±1.25		12.64±1.33
	Feed intake, kg/d, as fed			
0	3.96±0.28	4.63±0.28	4.13±0.28	4.74±0.30
24	2.83±0.45	4.80±0.45	2.48±0.45	4.91±0.48
48	2.14±0.57	5.25±0.57	2.35±0.57	6.43±0.61
72	2.04±0.52	5.50±0.52	2.13±0.52	6.05±0.56
90	2.26±0.49	3.96±0.49	2.61±0.49	4.46±0.53

[a]n = 8

[b]n = 7

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

4.5.1 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). 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 would be provided by using venous blood (Schaefer *et al.* 1992, Weinstein *et al.* 1995, Aguilera-Tejero *et al*. 2000). However, there are limited data on the correlations between arterial and venous blood in cattle. This study was conducted to validate the correlations between arterial and venous blood pH, pCO_2, and HCO_3 in *Bos indicus* cattle.

4.5.2 Materials and Methods

Animals and management

Twenty-seven 2 yr old *Bos indicus* steers (193 $\pm$ 21.47 kg mean BW) were halter broken and taught to lead. The steers were fed a commercial forage cube *ad libitum* for 14 d prior to the commencement of the experiment.

At sampling, all animals were forced into a race, captured in a cattle head bail and restrained. 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 this 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 polyvinyl chloride tube inserted into the jugular vein under local anaesthetic. The catheter was then flushed with 10 mL of heparinised 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. Simultaneously to sampling the venous blood a 22 G intra-arterial catheter was utilized with a 2 mL blood gas syringe containing lithium heparin to sample arterial blood gases (Figure 23). 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, pCO_2, and HCO_3^- were measured using a blood gas analyser.

Statistical analysis

Regression analyses were performed using SPSS 10 software package.

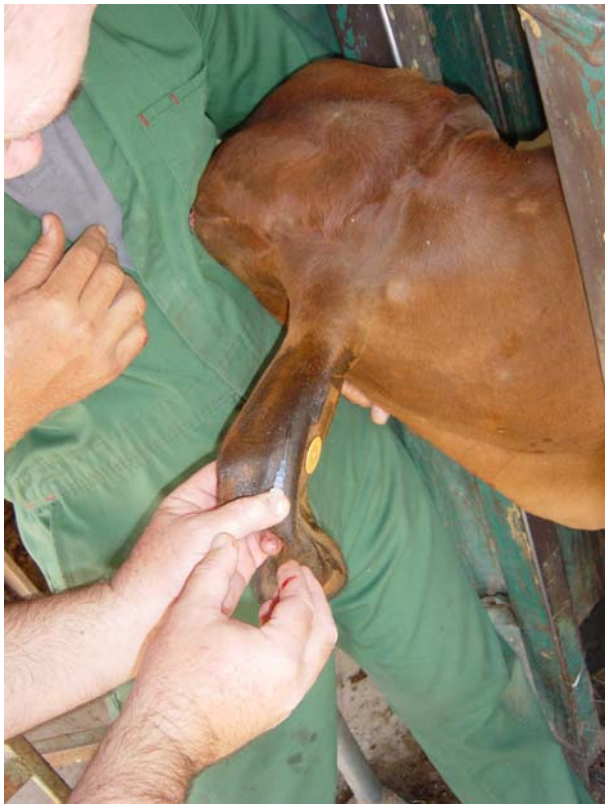

Figure 23. 22 G intra-arterial catheter inserted into the caudal auricular artery of a *Bos indicus* steer.

4.5.3 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 + 0.765*x* (Figure 24). 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 this 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, however, this may have been the result of higher lactate concentrations in these bulls or an elevation of plasma proteins due to dehydration.

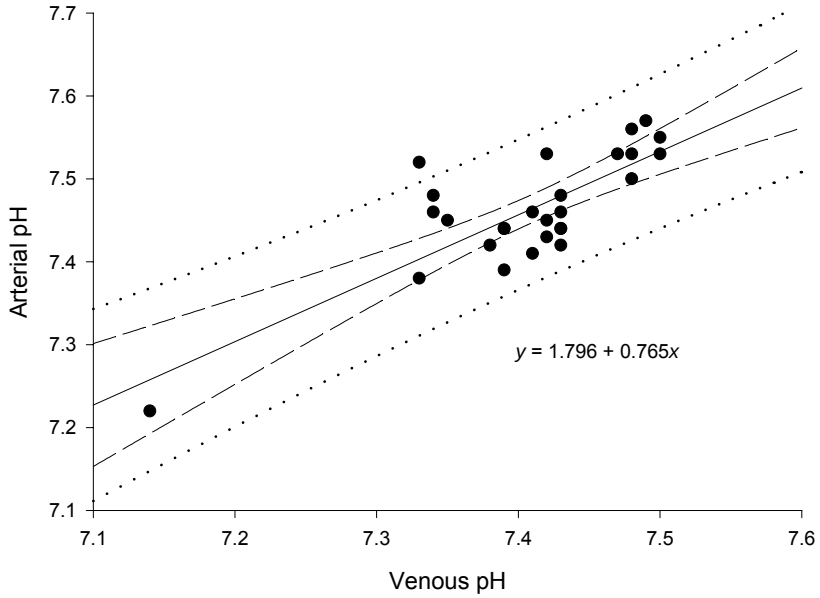

Figure 24. Correlation between pH in auricular arterial and jugular venous blood in *Bos indicus* steers. With fitted line (—), 95% confidence interval (- -) and prediction limits (····).

Bicarbonate concentrations also demonstrated a good relationship between values for arterial and venous blood (r = 0.86; P = 0.01) (Figure 25). 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 this study demonstrated HCO_3 concentrations in both arterial and venous blood of 29.57 ± 1.07 and 31.71± 1.05 respectively. Partial pressure of carbon dioxide yielded a poor relationship between arterial and venous blood (r = 0.364; P = 0.06) (Figure 26). 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 this 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.

Implications

It would appear that, in normal *Bos indicus* steers at rest, pH and HCO_3 in arterial and venous blood, are well correlated. The pCO_2 of arterial and venous blood, however, does not yield favourable 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

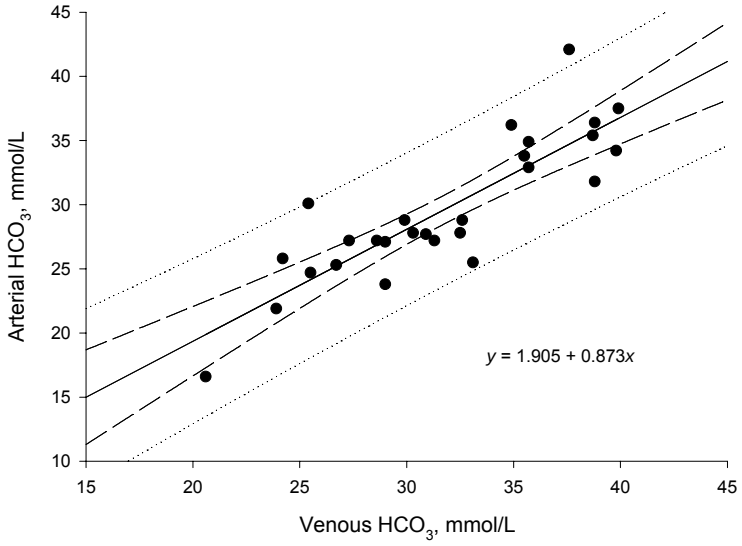

Figure 25. Correlation between bicarbonate concentration in auricular arterial and jugular venous blood in *Bos indicus* steers. With fitted line (—), 95% confidence interval(- -) and prediction limits (····).

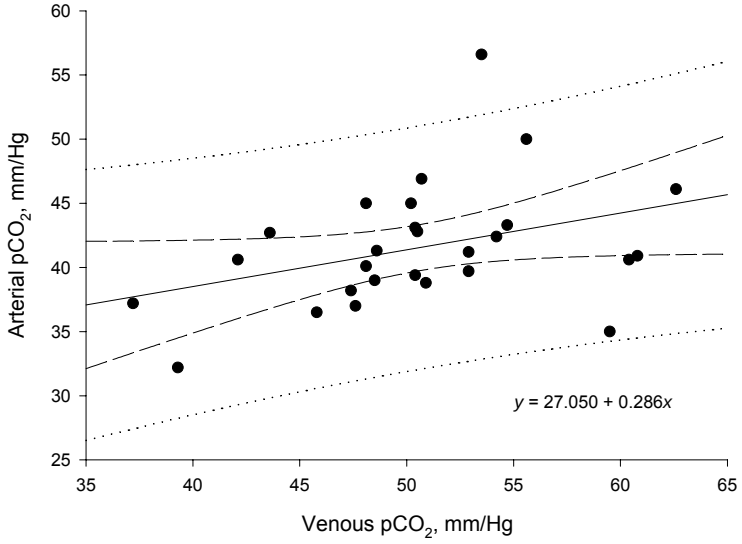

Figure 26. Correlation between pCO_2 concentrations in auricular arterial and jugular venous blood in *Bos indicus* steers. With fitted line (—), 95% confidence interval (- -), and prediction limits (····).

4.6 Cortisol response to transportation in Bos indicus steers

4.6.1Introduction

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 travelling in the rear of a moving heavy vehicle, obtained in transit data of sheep subjected to transportation of 31 h and 2 h, respectively. This study was conducted to determine the in-transit HPA axis response to transportation in *Bos indicus* steers during transportation.

4.6.2 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 polyvinyl chloride tube inserted into the jugular vein under local anaesthetic prior to loading. Steers were placed into adjustable stalls in the rear of a body truck (1.56m^2/animal). Blood sampling commenced one hour 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 240 min and a 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. All experimental procedures were reviewed and approved by the animal ethics committee at JCU (Approval No. A730-02).

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).

4.6.3 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 3 and Figure 27 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.

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 this 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 hour of standing on the truck post-catheterization. Basal cortisol concentrations for steers have been reported to range from 0.5 to 9.0 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 36 ng/mL over a 90 h time period. Initial plasma cortisol concentrations in this study concur with our past experience that basal concentrations were measured prior to transportation (7.25 to 18 ng/mL).

Table 3. Mean ± 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 ± SEM
Pre-transit (stationary)	269.43 ± 77.21
1st transport period	550.89 ± 163.09
2nd transport period	775.64 ± 265.21
Post-transit (stationary)	424.65 ± 107.94

n = 4

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 minutes into transit (Figure 27). The area under the time curve for the second two hour 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 hours of transportation in this 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 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 adaption 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.

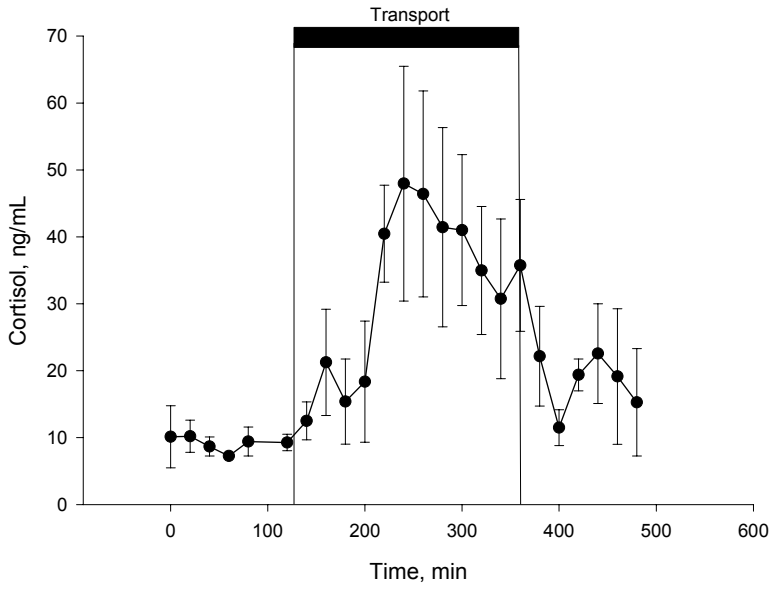

Figure 27. Mean ± SEM plasma cortisol concentrations in four *Bos indicus* steers confined to stalls on a stationary body truck for two periods of two h separated by four h under transportation. Points represent sampling intervals of 20 min.

Implications

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.

4.7 Quantitative analysis of acid base balance in Bos indicus steers subjected to transportation of long duration

4.7.1 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 (Phillips *et al.* 1991) *en route*, and greater carcass shrink (Schaefer *et al.* 1992), while it is also accepted that animals dehydrate with increasing transit time (Sinclair *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), however, 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.

4.7.2 Materials and Methods

Animals and management

Nineteen, 2 yr old *Bos indicus* steers (276 ± 14.65 kg mean BW) were 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 = 6); and 3) Transported, offered no feed or water for 12 h and then transported for 48 h (n = 5). Animals in the control group were offered a commercial dietary cube: ME 8.5 MJ, CP 12%, CF 31.1% per kg/DM. Animals in the feed and water deprived and trucked groups had their water and feed 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 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 8 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 0.86 m^2/animal.

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 this study were accustomed to being handled, and behaved in a quiet and amicable manner when sampled. The mean daily wet bulb temperatures during the experimental period for days 0, 1 and 2 were 21.1^{o}C, 19.6^{o}C and 20.7^{o}C respectively. 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 JCU (Approval No. A730-02).

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; and 1 x 2.5 mL tube containing fluoride oxalate. 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 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.

A 22 G (0.9 x 25 mm) intra-arterial catheter was utilized with a 2 mL blood gas syringe containing lithium heparin 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.

Measurement

Arterial blood pH, partial pressure of carbon dioxide (pCO_2) and bicarbonate (HCO_3^-) were measured using a blood gas analyzer. Plasma concentrations of Na and K were measured using ion selective electrodes. Sodium and K samples were completed on singular samples and quality control samples were performed every 10 samples. Lactate, P, albumin, total protein, Ca and Cl concentrations in plasma were measured using a Mira Autoanalyzer with standard enzymatic and spectrophotometric kits.

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

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. Multiple comparison tests were undertaken using Tukey's honestly significant difference test, where the level of significance was set at $P < 0.05$.

4.7.3 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 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 4. 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 this 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 pCO_2 compared to the control animals ($P = 0.023$). However, the pCO_2 values for the transported animals did not differ from those of the control group ($P = 0.126$). A lowering of the pCO_2 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 $[HCO_3]$, results in a low pH that stimulates respiration. This produces a low pCO_2, 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 then it is the renal system, which reverts the pH back to normal via the reabsorption and production of HCO_3^- and secretion of H^+ ions. It is difficult to state which compensatory system contributed to the differences seen in $[pCO_2]$ in the water and feed deprived and transported groups. However, we would speculate that, due to the duration of feed and water deprivation and transit, at 48 h it is a metabolic system that is influencing $[pCO_2]$.

There were no differences in HCO_3^- concentrations in the treatment groups and all groups remained within normal limits for cattle (20 – 30mmol/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 anerobic 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 this study, failed to elevate lactate

concentrations to 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 collection of blood for lactate analysis, during the studies of Schaefer *et al.* (1988) was not recorded as using an anti-glycolytic agent in the collection tubes. As such, the lactate data reported by Schaefer *et al.* (1988) especially the pre-transport values may be artefactually elevated, due to this pre-analytical error. If the analysis of plasma lactate by Schaefer *et al*. (1988) were collected in tubes containing lithium heparin, then, 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. Although we cannot confirm that all transported animals rested in transit in our study, resting may have been sufficient to decrease lactate concentration in the transported steers compared to that expressed in the other groups housed in pens. This author (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 to this, the animals used in this 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, however, this 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, however the changes, as in this study, were small and within physiological limits.

The anion gap (AG) is a diagnostic concept that demonstrates the difference between unmeasured anions and unmeasured cations. 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 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 strong ion difference 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).

Table 4. Least squares means ± SEM for blood pH, blood gases, plasma lactate, electrolytes, albumin, total protein, anion gap (AG), strong ion difference (SID) and total weak acids (Atotal) 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 ± 0.01a	7. 46 b± 0.01a	7. 46 ± 0.02a
PCO2 mm Hg	42.63 ± 1.45a	36.08 ± 1.66b	37.82 ± 1.82a,b
HCO3 mmol/L	28.87 ± 1.16a	25.47 ± 1.34a	27.08 ± 1.47a
Lactate mmol/L	0.59 ± 0.11a	0.75 ± 0.12a	0.62 ± 0.13a
Na+ mmol/L	139.48 ± 1.83a	141.13 ± 2.11a	140.72 ± 2.32a
K+ mmol/L	4.41 ± 0.12a	4.10 ± 0.14a,b	3.86 ± 0.15b
Ca++ mmol/L	2.30 ± 0.06a	2.48 ± 0.07a	2.34 ± 0.07a
Cl- mmol/L	97.75 ± 1.82a	102.67 ± 2.10a	98.80 ± 2.30a
PO4— mmol/L	2.17 ± 0.18a	2.80 ± 0.20a	2.69 ± 0.22a
AG mEq/L	17.26 ± 3.33a	17.10 ± 3.85a	18.70 ± 4.21a
SID mEq/L	49.02 ± 3.00a	45.80 ± 3.47a	48.73 ± 3.80a
Total Protein g/L	64.13 ± 1.92d	81.33 ± 2.43e	78.60 ± 2.22e
Albumin g/L	35.12 ± 1.00a,d	41.17 ± 1.15a,e	39.60 ± 1.26b,d
Atotal mEq/L	11.07 ± 0.34d	13.46 ± 0.40e	12.95 ± 0.44e

a, b, c within 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.

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 total body water 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 b-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, however, this difference 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.

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 this study, that the water and feed deprived group incurred a greater deviation from the control group in some of the parameters measured compared to the transported group, in spite of the winter wet bulb temperatures for the region being mild for tropically adapted cattle. 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.

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 by the respiratory and renal systems decreasing the pCO_2 concentration in arterial blood. Plasma electrolytes were selectively altered, however, the strong ion difference 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.

The results of this experiment are published in full in the Journal of Animal Science, as follows:

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

4.8 Physiological and metabolic effects of prophylactic treatment with the oral supplements SSF1* or Betaine on Bos indicus steers during long duration transportation

4.8.1 Introduction

Prolonged periods of water deprivation, as occur 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. The administration of oral supplements 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. The effects of two organic oral supplements; betaine and SSF1* on the total body water, electrolyte, glucose and acid-base balance of *Bos indicus* steers subjected to transportation of 48 h were investigated. The working hypothesis was that prophylactic treatment of steers with oral supplements 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.

4.8.2 Materials and Methods

Animals and management

Twenty four, 2.5 yr old *Bos indicus* steers (321.00 $\pm$ 4.60 kg mean BW) were 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) SSF1®, dosed with SSF1® (2 g/kg BW), deprived of feed and water and transported for 48 h (n = 6) and; 4) Betaine, dosed with betaine (Betafin 80 g/steer) deprived of feed and water then transported for 48 h (n = 6). All animals were dosed using a naso-gastric tube and all treatments had 500 mL of distilled water added to aid the flow of the product through the tube. The Control and the Transported groups 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. All animals in the study were assessed for temperament using the Flight Time Test developed by CSIRO (Burrow and Corbet 2000).

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 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 m^2/animal.

* Provisional Patent Application No.: 2004902760

At sampling, all animals were forced into a race where the animals were captured in a cattle head bail and restrained. 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 this study were accustomed to being handled, and behaved in a quiet and amicable manner when sampled.

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 5 reflect this incident where for the 24 h analysis for blood gas measurements; n = 4/group, except the control group where n = 5. The mean daily wet bulb temperatures during the experimental period for the transit period were 19.8^0C and 19.5^0C, 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 JCU (Approval No. A730-02).

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; 1 x 2.5 mL tube containing fluoride oxalate, and a 2 mL blood gas syringe containing lithium heparin 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. Five days after the end of transportation, a further 5 mL of blood was manually collected by jugular venipuncture from all groups, for total white blood cell count. A thin blood smear was made immediately, for subsequent differential white blood cell count .

Measurement

Venous blood pH, partial pressure of carbon dioxide (pCO_2) and bicarbonate (HCO_3^-) were measured using a blood gas analyzer. Plasma concentrations of Na and K were measured using ion selective electrodes. Sodium and K samples were completed on singular samples and quality control samples were performed every 10 samples. Lactate, glucose, albumin, total protein, P, Ca, Mg, and Cl concentrations in plasma were measured using a Mira Autoanalyzer with standard enzymatic and spectrophotometric kits. Plasma urea N was analysed with a Technicon Autoanalyser 2.

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

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

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urea/kg live weight. 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.

Statistical analysis

A two way ANOVA, with the main effects of time (24 h and 48 h) and group (Control, Transported, SSF1® or Betaine) and an interaction effect of group nested within time were analyzed statistically using SPSS 10 software package. Quantitative variables (plasma electrolytes, albumin, total protein, glucose, hematocrit, osmolality, and blood gases) were independently sampled. Liveweight and accumulated BW loss (Table 9) were analyzed using a two way repeated measures ANOVA, with the main effects of time (24 and 48 h) and group (control, transported, SSF1® or betaine) and the interaction effect of group x time using SPSS 10. Total and differential white blood cell counts at 5 days post-transport were analysed for the effect of group, by one way ANOVA. Least squares means and standard errors are presented. Multiple comparison tests within the factors were performed using Tukey's honestly significant difference test. Differences were considered significant for $P < 0.05$.

4.8.3 Results and Discussion

Temperament

Mean flight time of all animals in the study was 1.00 ± 0.09 sec (mean $\pm$ SEM) ranging from 0.39 to 4.62 sec. Mean flight times did not differ between treatment groups, nor did they influence any of the parameters reported here.

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, 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 5). 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 SSF1® 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 SSF1® 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 $[HCO_3]/[CO_2]$, thereby maintaining blood pH. Indeed, this is evident in all groups withheld off feed and water in this 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 SSF1® group may be that it is due to 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 bringing 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 SSF1® 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 8), the urea concentration was lower in the SSF1® 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

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would be of particular importance during nitrogen- or energy-limiting situations such as starvation or during livestock transport.

There were no differences in AG between groups at 24 or 48 h (Table 5). 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).

Plasma Electrolytes

Plasma electrolytes are presented in Table 6. 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. Although K remained within normal limits for cattle (Blood and Radostits 1991), this result lies in contrast with other authors who demonstrated a decrease in K with increasing time in transit. A lack of feed intake or the activation of the hypothalamo-pituitary-adrenal axis has been reported to decrease the concentration of K in plasma associated with animals in the marketing process (Schaefer *et al.* 1990; 1994; Parker *et al.* 2003a,b).

The Transported, SSF1®, and Betaine groups had lower plasma concentrations of Mg than the Control group at 24 h (P = 0.02). 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. Concentrations of Ca in plasma of the Control group was higher than the SSF1® (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.

Metabolites

The SSF1® group maintained greater plasma glucose concentrations than the Control group (P = 0.01). Increased SSF1® is not only an important carbon source for gluconeogenesis in liver, but the elevated blood glucose concentration in the SSF1® treated animals may have an important protein-sparing effect in part due to (i) providing a preferential fuel for liver gluconeogensis, (ii) increasing insulin secretion and thereby further inhibiting breakdown of muscle protein, and (iii) counter the amino-acid mobilising effect of the stress hormone cortisol. In addition, carbohydrates and lipids have both been found to have nitrogen sparring effects in ruminants (Asplund *et al.* 1985; O'Kelly 1985), and cortisol has the opposite effect.

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 SSF1®

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follows the same carbohydrate metabolic pathway as endogenous SSF1® from the breakdown of triglycerides. The ultimate fate of SSF1® is dependant upon the individual's metabolic state. 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.

Table 5 Least squares means ± 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	SSF1	Betaine
		24 h		
PH	7.40 ± 0.02	7.38 ± 0.02	7.38 ± 0.02	7.35 ± 0.02
pCO_2 mm/Hg	42.72 ± 1.70^{a,d}	47.18 ± 1.90	51.08 ± 1.90^{d}	51.70 ± 1.90^{a}
HCO_3 mmol/L	26.44 ± 0.78^{b}	27.93 ± 0.85	30.38 ± 0.85^{b}	28.33 ± 0.85
Anion Gap meq/L	11.73 ± 2.82	18.08 ± 3.15	13.84 ± 3.15	20.98 ± 3.15
SID meq/L	35.88 ± 2.25	41.94 ± 2.25	44.46 ± 2.25	44.79 ± 2.25
A_{total} meq/L	12.38 ± 0.29	12.45 ± 0.33	12.26 ± 0.33	11.93 ± 0.33
$[HCO_3] : [CO_2]$	20.66 ± 0.73	19.75 ± 0.82	19.82 ± 0.82	18.50 ± 0.82
		48 h		
PH	7.41 ± 0.02	7.42 ± 0.02	7.38 ± 0.02	7.37 ± 0.02
pCO_2 mm/Hg	42.92 ± 1.55	42.50 ± 1.55^{a}	47.12 ± 1.55	48.18 ± 1.55^{a}
HCO_3 mmol/L	27.10 ± 0.69	27.68 ± 0.69	27.88 ± 0.69	27.78 ± 0.69
Anion Gap meq/L	15.47 ± 2.58	18.00 ± 2.58	18.42 ± 2.58	16.78 ± 2.58
SID meq/L	41.20 ± 2.25	44.75 ± 2.25	44.87 ± 2.25	42.40 ± 2.25
A_{total} meq/L	13.18 ± 0.27	12.68 ± 0.27	12.76 ± 0.27	12.42 ± 0.27
$[HCO_3] : [CO_2]$	21.05 ± 0.67:1	21.74 ± 0.67:1	19.91 ± 0.67:1	19.36 ± .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

pCO_2 = partial pressure of carbon dioxide

SID – Strong Ion Difference

HCO_3 = bicarbonate concentration

A_{total} = Total weak acids

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Table 6. The effects of oral supplement treatment or not prior to transit on serum electrolytes after 24 h and 48 h of transit

Group	Pen	Transported	SSF1	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 ± 0.05a	2.45 ± 0.05	2.36 ± 0.05a	2.42 ± 0.05
Magnesium mmol/L	0.81 ± 0.02a,b	0.74 ± 0.02a	0.75 ± 0.02	0.71 ± 0.02b
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 ± 0.05a	2.37 ± 0.05	2.34 ± 0.05a	2.45 ± 0.05
Magnesium mmol/L	0.77 ± 0.02a	0.69 ± 0.02a	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,bP < 0.05, c,dP < 0.01

n = 6 per group

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 gluconeogenisis via the mobilization of amino acids from muscle and liver tissues, and the acceleration of lipid mobilization from fat stores. 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 *et al.* 1999). Indeed all of the transported animals would have been in a gluconeogenic state in this study, and the SSF1® treated animals had 30% higher cortisol levels compared to the other groups (Table 8).

Prophylactic SSF1® 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 oral supplement betaine had little effect on increasing blood glucose compared to SSF1®. There were no treatment group differences for total protein or

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albumin at 24 or 48 h. A time effect was demonstrated for total protein to increase with time (P = 0.01).

Lactate demonstrated a time effect (P < 0.001), decreasing in concentration with increased time transported. 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 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 this 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 this 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). 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 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 7 and 8, and there were no significant differences between groups at 24 or 48 h, although in the SSF1® 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 SSF1® treated animals at 48 h may add to the already enhanced gluconeogenic state at 24 h.

White Blood Cells

Total white blood cell and lymphocyte counts are shown in Table 7. Steers that were treated with SSF1 pre-transport did not show a decrease in white cell counts that was observed in non-treated steers 5 days after transportation. This maintenance of blood white cells was reflected in an increase in lymphocyte numbers. The ability of SSF1 treated steers to maintain white blood cell numbers in the face of significant stressors indicates a potential application for this treatment in limiting the physiological effects of stress by modulating the decrease in immunocompetence that is a feature of stress in cattle.

Body Water

The SSF1® group had a greater body water content at 24 h than the control (P = 0.05) and transport (P = 0.02) groups. However, one animal contributed largely to the statistical difference seen in the SSF1® group for body water. Therefore, the body water data should be interpreted with caution.

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Table 7. Total white blood cell and lymphocyte counts (mean ± SEM, x 10^6/L), 5 days post-transport, for *Bos indicus* steers treated with oral supplements or not and subjected to road transportation for 48 h.

	Control	Transported	SSF1®	Betaine
Total white blood cells	10.57 ± 0.35[a]	7.00 ± 0.60[b]	10.33 ± 1.03[a]	8.00 ± 0.99[b]
Lymphocytes	5.72 ± 0.64[a]	3.60 ± 0.48[b]	7.30 ± 0.69[c]	4.30 ± 0.63[a]

[a,b,c] Row means with unlike superscripts differ significantly (P<0.001)

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) using a model system, demonstrated a diuresis in sheep with stress-like concentrations of cortisol. It would therefore be reasonable to hypothesize that oral supplements such as SSF1® 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 SSF1®, liveweight does not reflect the expected higher value for the SSF1® group. Our failure to concurrently demonstrate a higher liveweight for the SSF1® 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 SSF1® group. Preston and Kock (1973) concluded that urea space in the ruminant was a measure of empty body water (total body water less the water in the gastrointestinal tract). It may be that the dose rate of SSF1® used in this study maintained empty body water in the steers but increased gastrointestinal emptying.

Hyper-hydration with oral supplements may only slow the development of water loss from the body as such. Perhaps a greater difference between both oral supplement 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 oral supplements and the subsequent carcass appraisal on ruminants are recommended.

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 9). 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 defeacation 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 Cole *et al*. (1988) study where transportation of 195 kg calves caused a significant increase in shrinkage, compared to feed and water deprivation alone.

Implications

The prophylactic treatment of *Bos indicus* steers with the oral supplement SSF1® attenuated the loss of body water during transportation for 24 h but it did not appear to be effective at 48 h. However, the SSF1®-linked enhanced glyconeogenic state persisted for 48 h. Elevated plasma glucose levels in the SSF1® treated animals would lead to higher insulin levels which in turn would

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inhibit the breakdown of muscle proteins, and also counter the amino-acid mobilising effect of increased cortisol concentrations. In conclusion, SSF1® treatment resulted in hyperhydration, decreases in the energy deficit, and confered an immuno-stimulatory effect on the animals, and enhanced the gluconeogenic state that may lead to an insulin-linked sparring of mucle 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 SSF1® treatment to minimize muscle wasting and promote the health of the animal during livestock transport.

Table 8. Least squares means ± SEM for TBW, cortisol, glucose, lactate, hematocrit, albumin and total protein in *Bos indicus* steers treated with oral supplements or not and subjected to road transportation for 24 h.

	Control	Transported	SSF1	Betaine
TBW %	51.64 ± 2.78[a]	51.06 ± 2.78[b]	62.44 ± 2.78[a,b]	53.01 ± 2.78
Cortisol, ng/mL	26.54 ± 5.36	23.10 ± 5.36	24.69 ± 5.36	12.97 ± 5.36
Glucose mmol/L	4.78 ± 0.42[a,c]	5.87 ± 0.42	6.87 ± 0.42[c]	5.98 ± 0.42
Lactate mmol/L	1.33 ± 0.45	2.13 ± 0.45	2.35 ± 0.45	2.70 ± 0.45
Hematocrit %	43.25 ± 1.54[a]	42.50 ± 1.54	42.50 ± 1.54	39.75 ± 1.54[a]
Albumin g/L	40.16 ± 0.90	40.00 ± 0.90	39.50 ± 0.90	39.50 ± 0.90
Tot Protein g/L	78.17 ± 1.44	78.00 ± 1.44	79.33 ± 1.44	77.50 ± 1.44
Urea mg/dL	38.48 ± 2.09	38.39 ± 2.09	35.77 ± 2.09	41.54 ± 2.09

Within a row, means with a common superscript letter differ. a,bP < 0.05, c,dP < 0.01

n = 6 per group

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Table 9. Least squares means ± SEM for TBW, cortisol, glucose, lactate, hematocrit, albumin and total protein in *Bos indicus* steers treated with oral supplements or not and subjected to road transportation for 48 h.

	Control	Transported	SSF1	Betaine
TBW %	52.89 ± 2.78	51.66 ± 2.78	57.46 ± 2.78	56.80 ± 2.78
Cortisol, ng/mL	22.87 ± 5.36	20.00 ± 5.36	35.84 ± 5.36	22.05 ± 5.36
Glucose mmol/L	5.10 ± 0.42[c]	5.73 ± 0.42	6.38 ± 0.42[c]	5.83 ± 0.42
Lactate mmol/L	1.37 ± 0.45	0.93 ± 0.45	1.43 ± 0.45	2.17. ± 0.45
Hematocrit %	45.67 ± 1.26[a]	41.50 ± 1.26	43.67 ± 1.26	41.33 ± 1.26[a]
Albumin g/L	41.17 ± 0.90	39.33 ± 0.90	40.33 ± 0.90	40.17 ± 0.90
Total Protein g/L	81.33 ± 1.44	78.83 ± 1.44	82.00 ± 1.44	80.17 ± 1.44
Urea mg/dL	40.03 ± 2.09	34.71 ± 2.09	38.09 ± 2.09	41.60 ± 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 10. Least squares means ± SEM for liveweight, and % body weight (BW) loss *Bos indicus* steers treated with oral supplements or not and subjected to road transportation for 48 h.

	Control	Transported	SSF1	Betaine
Initial Lwt	331.50 ± 11.31	323.00 ± 11.31	314.83 ± 11.31	329.58 ± 11.31
Curfewed 12 h	322.83 ± 10.72	311.17 ± 10.72	302.42 ± 10.72	314.00 ± 10.72
% BW loss*	2.76 ± 0.80	3.74 ± 0.80	3.80 ± 0.80	4.65 ± 0.80
24 h transit	301.75 ± 9.82	286.00 ± 9.82	280.83 ± 9.82	288.00 ± 9.82
% BW loss*	8.98 ± 0.90	11.50 ± 0.90	10.70 ± 0.90	12.50 ± 0.90
48 h transit	293.42 ± 9.76	277.67 ± 9.67	268.50 ± 9.67	277.83 ± 9.67
% BW loss*	11.50 ± 1.00	14.10 ± 1.00	14.70 ± 1.00	16.00 ± 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

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4.9 Pre-treatment with a novel oral supplement will minimize the stress response of steers during transportation

4.9.1 Introduction

At the request of MLA/Livecorp, key elements of the previous experiment were repeated using less tractable animals, that might be considered to be more representative of the industry. A treatment with SSF1® mid-transport was also included at the request of MLA/Livecorp.

4.9.2 Materials and Methods

Yearling *Bos indicus* steers (n = 56) (Figure 28) that were considered to be less tractable than those used in the previous experiment, were sourced from a collaborator's herd and allocated by stratified randomisation to seven groups as follows: 1) Control A (n = 8): Not transported – Held in a pen in the open for the duration of the study on *ad libitum* feed and water for 84 h (the total period of curfew and transportation of Groups 3 – 6); 2) Control B (n = 8): Not transported – Held in a pen in the open for the duration of the study and deprived of feed and water for 84 h (the total period of curfew and transportation of Groups 3 – 6); 3) Control C (n = 8): Locked off feed and water for 12 h (curfew) - transported by road for 72 h; 4) Treatment 1 (n = 8): Locked off feed and water for 12 h (curfew) – Dosed with SSF1® (2 g/kg BW) orally, immediately prior to being transported by road for 72 h; 5) Treatment 2 (n = 8): Locked off feed and water for 12 h (curfew) – Dosed with Mg Proteinate 1 gm/kg BW) orally, immediately prior to being transported by road for 72 h; 6) Treatment 3 (n = 8): Locked off feed and water for 12 h (curfew) – Dosed with SSF1® (2 gm/kg BW) and Mg Proteinate (1 gm/kg BW) orally, immediately prior to being transported by road for 72 h; 7) Treatment 4 (n = 8): Locked off feed and water for 12 h (curfew) – Dosed with SSF1® orally after 48 h of transport, prior to being transported by road for a further 24 h.

The experiment was completed in three separate journeys, each of 72 h duration. For the first and last journeys, the cattle crate on the truck was divided by a centre partition into front and rear compartments that each held 8 steers. For the second journey, the three sentinel steers for serial plasma cortisol concentration determination were held in individual compartments in the front of the cattle crate, and 8 steers were held in the rear compartment. Steers were allocated to compartments from each of the transported groups at random so that there were similar numbers of animals from each transported group, in any particular compartment. The transported cattle each travelled ≈4300 km virtually non-stop, over the period of transportation. All animals in the study were assessed for temperament using the Flight Time Test developed by CSIRO (Burrow and Corbet 2000).

In addition to the 56 animals in the experiment, three sentinel steers from the same cohort were used to monitor plasma cortisol concentrations of the animals during the 72 h of transportation. The animals were fitted with bilateral indwelling jugular catheters and maintained in individual pens on the truck, separate from the primary experimental animals. Blood samples were taken from the sentinel steers from a remote location (the truck cabin) every hour from 2 h before the start of 72 h of transportation until 4 h after transportation ceased. All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (Approval No. A730-02).

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4.9.3 Results and Discussion

Temperament

Mean flight time of all animals in the study was 0.79 ± 0.04 sec (mean ± SEM) ranging from 0.32 to 1.88 sec. Mean flight times did not differ between treatment groups, nor did they influence any of the parameters reported here. This flight time compares with 1. 00 ± 0.09 sec (mean ± SEM) ranging from 0.39 to 4.62 sec, for the *Fletcherview* steers used in the previous experiment

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Figure 28. *Bos indicus* steers used in this experiment.

Figure 29. Sentinal steers in individual pens. Note the black piping that carried the catheter lines into the cabin of the truck to facilitate blood sampling while mobile.

A mean plasma cortisol profile for the transported steers is presented in Figure 30. This cortisol profile reflects the more fractious temperament of the steers used in the study, and illustrates the diurnal pattern of secretion of cortisol and the adaptation of the steers to the transport process.

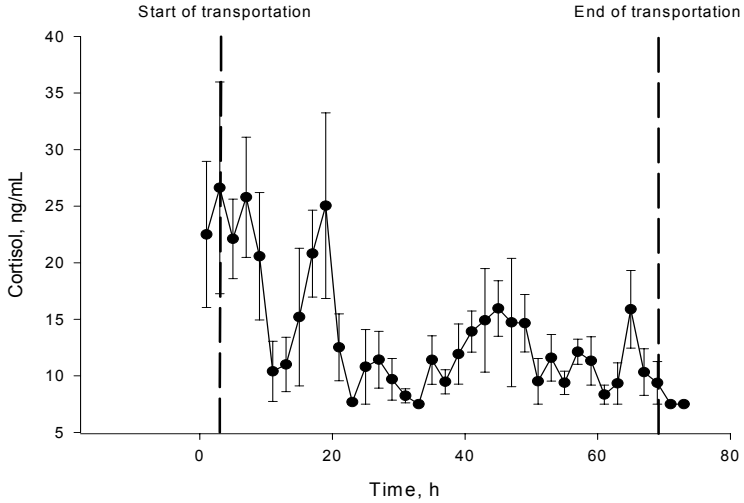

Figure 30. Mean (±SEM) concentrations of cortisol in plasma of *Bos indicus* steers transported for 72 h.

Haemoglobin

Haemoglobin levels were elevated ($P < 0.001$) in all of the treatment groups that were locked off water, reflecting the progressive dehydration of these animals over the course of the experiment.

Plasma pH

Plasma pH increased with time in all groups ($P = 0.002$) and differed between some treatment groups [Control B differed from Treatment 2 ($P = 0.013$) and Treatment 4 ($P = 0.23$); Control C differed from Treatment 2 ($P = 0.06$)], although it is doubtful that these differences were biologically significant.

Blood PCO_2

There were no time or treatment group effects on pCO_2, most probably reflecting a stabilization of the renal system from 48 h onwards.

Plasma electrolytes

Plasma concentrations of $^{-}HCO_3$ increased with time in transported animals. Plasma concentrations of Ca decreased with time, in feed and water deprived animals, reflecting the effect of feed deprivation. Transported animals had lower plasma concentrations of Ca than did non-transported animals. Interestingly, following 72 h of transportation, a number of transported animals showed clinical signs of hypocalcaemia (Figure 31), from which all recovered uneventfully (Radostits *et al*. 2000). In general, plasma concentrations of Na increased and plasma concentrations of K decreased with time in water-deprived animals, as would be expected in animals trying to conserve water.

Figure 31. Steer from Treatment Group 2, showing clinical signs of hypocalcaemia after 72 h of transportation.

White Blood Cell Counts

There were no significant differences between total white blood cell counts of the animals in any treatment group at 5 d post-transport (Table 11). This is most likely due to the increased variation in pre-transport white cell counts in this group of experimental animals, which ranged from 5.49 – 13.09 x 10^9/L, and the relatively small numbers of animals per group. However, if the data from penned animals (Control groups A and B, ie not transported) and those treated with SSF1® pre-transport (Treatment groups 1 and 3) are pooled to increase the *n*, then a trend emerges for transportation to depress white blood cell counts at 5 d post transport, an effect that was blocked by pre-transport treatment with SSF1®. This supports the data from the previous experiment. Interestingly, mean white cell counts on day 5 post-transport for steers in this study were 6.62±0.19 x 10^9/L compared with 8.83±0.41 x 10^9/L for the steers in the previous experiment. This suggests that the steers used in this study may not have fully recovered from the stress of being mustered and transported from Hughenden to Townsville (6 h), 11 days prior to the start of the experiment.

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Table 11. Total white blood cell counts (mean ± SEM, x 10^9/L) 5 days after transportation for Bos indicus steers either: **1) Control A** (n = 8); **2) Control B** (n = 8); **3) Control C** (n = 8); **4) Treatment 1** (n = 8); **5) Treatment 2** (n = 8); **6) Treatment 3** (n = 8); **7) Treatment 4** (n = 8).

	Control Groups			Treatment Groups			
	A	**B**	**C**	**1**	**2**	**3**	**4**
Total white cell count	6.33±0.28	6.61±0.66	5.41±0.16	6.48±0.43	7.53±0.51	6.47±0.24	7.50±0.64

Total Body Water

There were no differences between any of the transported groups for total body water at 72 h (Table 12). However, there were significant differences between steers left on water for 72 h and those that received Mg Proteinate and transported (P = 0.002), and those that received SSF1® @ 48 h while being transported. These effects of Mg Proteinate were not observed in the previous experiment, and along with the data from the SSF1® @ 48 h group are difficult to explain.

Overall, however, this data supports previously reported findings with *Fletcherview* steers described in the previous experiment. It would appear that long haul transportation (>48 h) resulted in body water loss regardless of the prophylactic treatments used. The prophylactic use of SSF1® in particular, in cattle being transported by road for 12 – 24 h warrants further investigation.

Liveweights and Feed Intakes

Liveweights for the three control and four treatment groups are presented in Table 2. The only significant differences in liveweights between the groups were due to the restricted intake of feed and water during the period of fasting and transport of Control groups A and B and the four Treatment groups. There were no differences between the groups in return to normal feed intakes post-transport.

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Table 12. Total Body water for *Bos indicus* steers subjected to 72 h of transportation (Treatments 1 to 4 and Control C) or penned for an equivalent period of time (Controls A and B).

Group	No.	Mean ± SEM
Treatment 1	8	51.49 ± 1.60
Treatment 2	8	46.55 ± 4.25
Treatment 3	6	51.72 ± 3.34
Treatment 4	8	49.59 ± 2.78
Control C	7	52.93 ± 2.01
Control A	8	62.67 ± 2.12
Control B	7	53.84 ± 1.12
Total	52	52.69 ± 1.18

Implications

The greatly increased variation in white cell counts in the steers used in this study compared with the steers used in the previous experiment was not anticipated. As a result of this variation, the size of the experimental groups (n = 8 per group) used was insufficient to demonstrate statistically significant differences between groups in a number of parameters of particular interest. However, the results presented here tend to confirm those reported for the previous experiment.

- Transportation of steers for 72 h subjected them to significant stress as indicated by the concentrations of cortisol in their plasma
- SSF1 treated steers did not show a decrease in white cell counts that was observed in non-treated steers 5 days after transportation
- Pre-transport treatment of steers with Mg Proteinate or SSF1® + Mg Proteinate had no significant or additional beneficial effect

Treatment of steers with SSF1® after 48 h of transportation appeared to have no significant beneficial effect.

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Table 13. Least squares means ± SEM for liveweight changes during transportation for 72 h and during the recovery period of 20 days post transit for *Bos indicus* steers.

	Control A	Control B	Control C	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Pre-fast	268.56±8.308	248.39±8.88	268.88±9.67	261.67±8.60	274.16±8.30	267.50±10.18	267.50±8.31
Fasted 12 h	263.83±9.32	239.44±9.96	252.11±10.86	248.96±9.64	262.58±9.32	260.50±11.42	254.42±9.32
24 h transit	268.00±8.60	224.13±9.19	228.03±10.02	226.42±8.90	238.06±8.60	235.08±10.53	233.17±8.60
48 h transit	263.78±10.80	225.72±11.55	216.56±12.58	215.96±11.18	225.47±10.80	222.92±13.23	221.72±10.81
72 h transit	259.08±8.31	210.06±8.88	209.08±9.67	208.79±8.60	218.36±8.31	214.38±10.17	214.19±8.31
5 d rest	271.30±9.56	251.56±10.22	258.06±11.14	256.13±9.90	279.64±9.56	271.04±11.71	266.69±9.56
10 d rest	273.92±10.22	256.72±10.92	267.13±11.90	265.25±10.57	286.03±10.22	275.63±12.51	272.89±10.22
15 d rest	280.86±10.36	260.97±11.07	271.09±12.07	270.50±10.72	285.52±10.36	279.46±12.69	274.44±10.36
20 d rest	270.61±10.17	252.97±10.88	265.72±11.85	260.54±10.53	277.11±10.17	267.33±12.46	264.61±10.67

5.0 GENERAL DISCUSSION

In Northern Australia, cattle are required to be transported over great 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 undertaken in this project have identified some of the physiological mechanisms involved when ruminants are placed under long-haul transportation and handling stress.

The measurement of water balance in ruminants is problematic under field conditions. The gold standard for measuring TBW is a TOH dilution, however, radioisotope contaminated animals are not able to subsequently enter the food chain, severely restricting the application of the technique. A further limitation on the use of TOH dilution is the fact that in ruminants, equilibrium occurs about 12 h post-injection. These studies demonstrate that measurement of the urea space within the animal also allows for the accurate determination of TBW, with equilibrium occurring within 15 min post-injection.

Initial pen studies, utilizing a stress model in sheep based on cortisol infusions, demonstrated that well hydrated ruminants placed under stressful conditions respond with a diuresis. Water deprivation has been touted as a significant stressor to ruminants. 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. In contrast, 60 h of water deprivation resulted in a significant increase in the concentration of cortisol in the plasma of *Bos indicus* steers.

Evidence from the studies described here demonstrates that plasma potassium consistently decreases in ruminants under stress. 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 by the HPA axis.

One transportation study showed no differences in the pH of arterial blood from transported or non-transported steers, confirming other data, albeit on venous blood, that transportation stress causes no differences in the acid-base status of transported versus non-transported ruminants. 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 towards lowering the PCO_2 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 of this, the AG and strong ion group calculations also showed no differences 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.

This data is important in that it challenges the current "Best Practice" management protocols for transported ruminants, in particular the efficacy of electrolyte solutions administered pre- or post-transportation to minimise transport stress. Furthermore, the experiments described here are the only studies to utilise arterial blood to assess acid-base balance in *Bos indicus* steers after long haul transportation and handling.

Hydration strategies involved with the transportation process rely on the replacement of lost TBW and electrolytes at the completion of the journey, ie after the welfare of the animals has been compromised. This project sought a novel approach to the problem of dehydration associated with transportation of *Bos indicus* steers. Prophylactic hyper-hydration of Bos indicus steers was achieved during the first 24 h of transportation, with the oral supplement SSF1*, allowing a delay in TBW loss. This finding has significant welfare and production implications in that a prophylactic dose of SSF1* could assist the animal to remain hydrated longer in the export process.

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Furthermore, hydration status governs the metabolic state of an animal, in as much as hydration has an anabolic effect, then hyperhydration with SSF1* may assist in delaying the catabolic effects of dehydration.

As an important fuel source for gluconeogenesis, SSF1* hypothetically may be a preferential supply of energy for *Bos indicus* cattle during times of transportation stress. In promoting gluconeogenesis and glycogen formation, SSF1* appears to have nitrogen-sparing effects in the ruminant, which may aid in the preservation of carcass protein and decrease dark cutting beef. Further to this, SSF1* 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 higher concentrations of plasma glucose may lead to greater steady state insulin concentrations, inhibiting muscle protein breakdown. More research is needed into the metabolic effects of SSF1* during periods of heightened gluconeogenesis such as starvation and transit stress. Further to this, genotype comparisons are needed to assist in understanding the phenomena of preferential fuel sources during times of stress.

Immunoprotective effects of the oral supplement SSF1* were identified in steers treated pre-transport. This ability of SSF1* treated steers to maintain white blood cell numbers in the face of significant stressors indicates a potential application for this treatment in limiting the physiological effects of stress by modulating the decrease in immunocompetence that is a feature of stress in cattle. Further research is warranted to better understand the underlying mechanism of this apparent effect.

In conclusion, the studies undertaken for this project were designed to increase the level of understanding of the physiological responses of *Bos indicus* steers subjected to long-haul transportation and handling stress. The physiological data obtained 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 on these animals have been tested and refuted. The strategic application of oral oral supplements administered pre-transportation has been investigated with promising results for the beef industry as a whole and the live export industry, in particular. 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 enhanced animal welfare.

* Provisional Patent Application No.: 2004902760

6.0 SUCCESS IN ACHIEVING OBJECTIVES

The objectives of the project were:

1) To enhance knowledge and understanding of the response of cattle to *stressors* typically associated with the collection and transportation for live export and their impacts on animal welfare and performance

• *The data generated by the experiments detailed above have added significantly to our knowledge and understanding of the physiological responses of cattle to the stress of transportation. This objective has been achieved.*

2) To determine the relationships between *stress* and dehydration, electrolyte and acid/base balance, plasma cortisol concentrations, a range of blood parameters, and cell mediated immunity

• *This objective has clearly been achieved. In particular, the data on electrolyte and acid/base balance generated by this project refute long held beliefs on the benefits of electrolyte supplementation of transported cattle.*

3) To develop and validate a simple (crush-side) test that reflects an individual animal's response to the stress of a live export protocol and associated transportation

• *As a result of the studies conducted under this project, we are confident that no simple test is available to determine an individual animal's response to the stress of handling and transportation. The best indication currently available would be to determine the pH of the blood (preferably arterial blood) of the animal in question. This information, when combined with the resting respiratory rate of the animal would give an indication of its acid-base balance. Haematocrit or packed cell volume (PCV) was found to be poorly correlated with hydration status in steers subjected to long-haul transportation. This objective has been achieved.*

4) To develop and test protocols to minimize production losses associated with *stress* in cattle for live export

• *This objective has been achieved, with three oral supplements being tested. The oral supplement SSF1*, in particular, showed promise as an effective prophylactic treatment to minimize the effects of stress on transported cattle. A provisional patent application (No.: 2004902760) has been filed for these applications of SSF1*.*

5) To develop and test treatments for cattle determined to be significantly stressed by the live export protocols

• *This objective was not pursued following a mid-term review of the project. It was felt that investigating protocols to minimize and manage the effects of stress would be a more productive approach.*

6) To provide high quality research training for a candidate enrolled in a formal graduate studies program

• The Research Fellow associated with the project's PhD thesis entitled "*Aspects of water, electrolyte and acid-base balance in transported Bos indicus steers*", has been passed by the committee of examiners. In addition, three research papers from this thesis have been published in the Journal of Animal Science, while a further two papers have been prepared for publication. This objective has been achieved.

* Provisional Patent Application No.: 2004902760

7.0 IMPACT ON MEAT AND LIVESTOCK INDUSTRY

There are two outcomes from this project that have the potential to significantly impact on the Meat and Livestock Industry.

- The data from this project challenges the current "Best Practice" management protocols for transported ruminants, in particular, the efficacy of electrolyte solutions administered pre- and(or) post-transportation to minimise the effect of transport stress. Electrolyte solutions fed post-transportation would provide little benefit in correcting an animal's acid-base balance, compared to water alone.

- Prophylactic pre-transport treatment of cattle with the oral supplement SSF1* could have a number of significant welfare and production benefits for cattle. Pre-transport hyperhydration with the oral supplement SSF1* may: 1) Reduce the relative loss of body water during transportation. 2) Assist in delaying the catabolic effects of dehydration. 3) Promote gluconeogenesis and glycogen formation thus aiding in the preservation of carcass protein and decreasing the incidence of dark cutting meat. 4) Provide immunoprotective effects by enhancing lymphocyte function in the post-transport period.

These outcomes have the potential to benefit not just the livestock export and feedlot industries, but the entire meat and livestock industry, whenever ruminants are transported.

* Provisional Patent Application No.: 2004902760

8.0 CONCLUSIONS AND RECOMMENDATIONS

This project sought to understand the physiological responses of cattle to the stress of transportation, and attempted to minimise those effects through the prophylactic application of novel oral supplementation, prior to transportation.

- The studies described here clearly demonstrate that offering electrolyte solutions to transported *Bos indicus* cattle is unlikely to reduce physiological stressors any more than water alone.

- The oral supplement SSF1* appears to have potential for reducing the effects of handling and transportation stress, and enhancing the well-being of transported cattle.

Further studies are required to confirm and clearly define the potential beneficial effects of SSF1* in cattle subjected to stress, including:

1) An additional study of the physiological responses of *Bos indicus* steers to oral supplementation with SSF1* prior to transportation of long duration (48 h), with increased experimental group sizes (ie n = 16). This study should include an evaluation of post-transport lymphocyte function.

2) Investigation of the effects of pre-transport treatment with SSF1* on the maintenance of body water and muscle glycogen levels in *Bos indicus* cattle subjected to transportation of shorter duration (ie 12 h).

3) Evaluation of the effects of oral supplementation with SSF1* on the carcass characteristics of *Bos indicus* cattle following transportation and slaughter.

4) Repeat of 1), 2) and 3), above, with *Bos taurus* genotype cattle.

5) Investigation of the oral supplementation with SSF1* on the physiological responses of *Bos taurus* genotype cattle to heat stress.

* Provisional Patent Application No.: 2004902760

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